Genome-wide analysis of bacterial determinants of plant growth promotion and induced systemic resistance by *Pseudomonas fluorescens*

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Originality-Significance Statement

Genome-wide analyses of the interaction between plant growth-promoting rhizobacterium *Pseudomonas fluorescens Pf*.SS101 and Arbidopsis suggest that modulation of auxin biosynthesis and transport, steroid biosynthesis, carbohydrate metabolism and sulfur assimilation in Arabidopsis are key mechanisms linked to growth promotion and induced systemic resistance. In particular sulfur assimilation was shown to be an important biological process modulated in Arabidopsis by *Pf*.SS101.

Summary

Pseudomonas fluorescens strain SS101 (*Pf.*SS101) promotes growth of *Arabidopsis thaliana*, enhances greening and lateral root formation, and induces systemic resistance (ISR) against the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*). Here, targeted and untargeted approaches were adopted to identify bacterial determinants and underlying mechanisms involved in plant growth promotion and ISR by *Pf.*SS101. Based on targeted analyses, no evidence was found for volatiles, lipopeptides and siderophores in plant growth promotion by *Pf.*SS101. Untargeted, genome-wide analyses of 7,488 random transposon mutants of *Pf.*SS101 led to the identification of 21 mutants defective in both plant growth promotion and ISR. Many of these mutants, however, were auxotrophic and impaired in root colonization. Genetic analysis of three mutants followed by site-directed mutagenesis, genetic complementation and plant bioassays revealed the involvement of the phosphogluconate dehydratase gene *edd*, the response regulator gene *colR* and the adenylsulfate reductase gene *cysH* in both plant growth promotion and ISR. Subsequent comparative plant transcriptomics analyses strongly suggest that modulation of sulfur assimilation, auxin biosynthesis and transport, steroid biosynthesis and carbohydrate metabolism in Arabidopsis are key mechanisms linked to growth promotion and ISR by *Pf.*SS101.

Key words: *Pseudomonas fluorescens*, Arabidopsis, plant growth promotion, induced systemic resistance, amino acids, sulfur assimilation

Introduction

Pseudomonas represents one of the most abundant bacterial genera in the plant rhizosphere (Pieterse et al. 2002; Loper and Gross, 2007; Raaijmakers et al., 2009, 2010; Mendes *et al.*, 2011; Raaijmakers and Mazzola, 2012; Zamioudis *et al.*, 2013; Mendes *et al.*, 2013; Philippot *et al.*, 2013; Chowdhury *et al.*, 2015). Certain *Pseudomonas* strains promote plant growth via enhancement of nutrient and iron acquisition or by protection against pathogen infection via competition, antibiosis or induction of systemic resistance (ISR) (van Loon *et al.*, 1998; Ryu *et al.*, 2003; Haas and Défago 2005; Berendsen *et al.*, 2012; Zamioudis *et al.*, 2013; Pieterse *et al.*, 2014, 2016; Chowdhury *et al.*, 2015). To date, several bacterial traits have been identified for their role in plant growth promotion and ISR by *Pseudomonas*: (1) production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that reduces ethylene levels in the root, thereby increasing root length and growth (Li *et al.*, 2000; Penrose and Glick, 2001); (2) production of hormones like indole acetic acid (IAA) (Patten and Glick, 2002), abscisic acid (ABA) (Dangar and Basu, 1987; Dobbelaere *et al.*, 2003), gibberellic acid (GA) and cytokinins (Dey *et al.*, 2004); (3) solubilization and mineralization of nutrients, particularly mineral phosphates (de Freitas *et al.*, 1997; Richardson, 2001); (4) production of vitamins including niacin, pantothenic acid, thiamine, riboflavine and biotin (Martinez-Toledo et al,. 1996; Sierra *et al.*, 1999; Revillas *et al.*, 2000); (5) cell-surface components including flagella and lipopolysaccharides (Peter *et al.*, 2007); (6) secondary metabolites including lipopeptides (Peter *et al.*, 2007; Audenaert *et al.*, 2002; Tran *et al.* 2007), 2,4-diacetylphloroglucinol (Iavivoli *et al.*, 2003; Weller *et al.* 2012), siderophores (Bakker *et al.*, 2007; Pieterse *et al.*, 2014), salicylic acid (Maurhofer *et al.*, 1994, 1998; Audenaert *et al.*, 2002; De Vleesschauwer *et al.*, 2014), and (7) volatile organic compounds (Blom *et al.*, 2011; Park *et al.*, 2015).

In this study, we conducted a genome-wide analysis to discover new bacterial genes and traits involved in plant growth promotion and ISR by the rhizobacterial strain *P. fluorescens* SS101 (*Pf.*SS101). *Pf.*SS101 was originally isolated from the wheat rhizosphere (de Souza *et al.*, 2003; de Bruijn *et al.*, 2008) and has biocontrol activities either directly or via ISR against *Pythium* root rot of flower bulb crops (de Souza *et al.*, 2003), tomato late blight caused by *Phytophthora infestans* (Tran *et al.*, 2007), the bacterial pathogen *P. syringae* pv. *tomato* (*Pst*) and the insect herbivore *Spodoptera exigua* on Arabidopsis (Van de Mortel *et al.* 2012). To investigate the underlying mechanisms and bacterial traits involved in plant growth promotion, induction of lateral root formation and ISR, several known mechanisms such as ACC deaminase activity, volatile and lipopeptide production were studied first. To identify other, potentially novel bacterial traits, a total of 7,488 random transposon mutants of *Pf.*SS101 were screened individually for lost or reduced ability to induce lateral root formation and/or ISR in Arabidopsis. Results of these high-throughput bioassays led to the selection of 21 mutants that did not induce lateral root formation nor were able to induce resistance in Arabidopsis against *P. syringae* pv. *tomato* (*Pst*). These 21 *Pf.*SS101 mutants were disrupted in genes involved in amino acid biosynthesis, glucose utilization, transcription, or sulfur assimilation. Site-directed mutagenesis, genetic complementation, phenotypic and plant transcriptional analyses were performed to further assess the functions of these genes in the *Pf.*SS101-Arabidopsis interaction in order to unravel the underlying mechanisms of growth promotion and ISR.

Results

*Pf.*SS101 promotes plant growth and changes root architecture

Introduction of *Pf.*SS101 onto roots of Arabidopsis seedlings grown in soil resulted in significant growth promotion with 1.7- and 2.9-fold increases in leaf and root biomass, respectively, relative to the nontreated control plants (Table 1). Comparable but more pronounced effects of *Pf.*SS101 on shoot and root biomass were observed for Arabidopsis seedlings grown under *in vitro* conditions on vertically oriented MS agar plates (Fig. 1a, Table 1). Next to the biomass increase, *Pf.*SS101-treated seedlings also showed altered shoot and root development, exemplified by enhanced greening, a two-fold reduction of primary root length and a three-fold increase in the number of lateral roots (Fig. 1b,c). Using a *gfp*-tagged Tn*7*-derivative of *Pf.*SS101, we found no evidence for endophytic colonization: *Pf.*SS101 was only found on the root surface and not detected inside the root tissue of Arabidopsis (data not shown). Microscopic analysis of *Pf.*SS101-treated seedlings showed an increased number of pericycle cells in the roots (Fig. 1d, e). Furthermore, the roots of *Pf.*SS101-treated plants appeared to switch earlier into secondary growth (Fig. 1i) than the roots of control plants (Fig. 1h). The enhanced formation of pericycle cells was visualized in the GAL4-GFP enhancer trap lines of Arabidopsis (J2351, J1922 and J0661) (Fig. 1f, g). GAL4 enhancer trap lines are useful markers to tag specific cell types and to reveal developmental transitions (Sabatini et al., 1999; Wysocka-Diller et al., 2000; Cary et al., 2002; Birnbaum et al., 2003; Laplaze et al., 2005).

When surface-sterilized Arabidopsis seeds were inoculated with a *Pf.*SS101-cell suspension, *Pf.*SS101 established a population density on the roots of approximately $1x10^6$ CFU mg^{-1} root fresh weight after 18 days of plant growth. In the *in vitro* plant growth assays on MS agar plates, root tip inoculation with cell suspensions of *Pf.*SS101 resulted in a density

of approximately $5x10^5$ CFU mg^{-1} root fresh weight after 18 days of plant growth (data not shown). In these latter *in vitro* assays, *Pf.*SS101 was not detected on or in the leaves.

Targeted identification of bacterial traits involved in plant growth promotion and ISR

When roots of Arabidopsis seedlings were treated with heat-killed cells of *Pf.*SS101, we did not observe the typical plant phenotypes induced by live *Pf.*SS101 cells, including growth promotion, enhanced lateral root formation and ISR (Fig. 2a; Table 2, EXP1). Next, we conducted a series of experiments to determine if specific bacterial traits, described previously for other *Pseudomonas* strains and other rhizobacterial genera, are involved in plant growth promotion and ISR by *Pf.*SS101. These traits include siderophore, lipopeptide (i.e. massetolide), ACC deaminase, and volatile production. To test the role of these bacterial traits, several approaches were adopted, including site-directed mutagenesis. To study the role of ACC deaminase in growth promotion, we first analyzed the *Pf.*SS101 genome but did not find the *acdS* gene involved in the biosynthesis of ACC deaminase. Also, spectrophotometric analysis (with *P. fluorescens* F113 as a positive control) revealed that *Pf.*SS101 did not exhibit ACC deaminase activity (Fig. S1). To determine the potential role of volatile organic compounds (VOCs) in plant growth promotion, a split-plate assay was used where *Pf.*SS101 was grown on MS agar medium on one side physically separated from the Arabidopsis seedlings on the other side of the plate. After 14 days of plant exposure to the bacterial VOCs, no enhancement of shoot biomass was observed (Fig. 2b).

For extracellular metabolites produced by *Pf.*SS101, the results showed that the siderophore and the lipopeptide massetolide A do not play a significant role in growth promotion of Arabidopsis. The siderophore-deficient mutant of *Pf.*SS101, generated in this study by plasposon (single inserted) mutagenesis of gene *Pflss101_3099* and designated mutant 61C8, enhanced root biomass and induced resistance to the same extent as wildtype

*Pf.*SS101 (Table 2, EXP4). Similar results were obtained for *Pf.*SS101 mutant *ΔmassA* (de Bruijn *et al.*, 2008) deficient in the production of the lipopeptide massetolide (Table 2, EXP2). To further investigate the potential role of massetolide in growth promotion and ISR, we also grew Arabidopsis seedlings on plates amended with different concentrations of massetolide. The results showed no effects of massetolide A on plant growth or ISR (Table 2, EXP3).

Untargeted identification of bacterial traits involved in plant growth promotion and ISR

A library of 7,488 random *Pf.*SS101 mutants was generated via plasposon mutagenesis and each of these mutants was tested individually in two different high-throughput (HTP) bioassays: the first was a plate assay for plant growth promotion and root architecture; the second HTP-assay was a 96-well plate assay for ISR (Fig. 3). We identified 21 potential mutants that were not able to promote plant growth, alter root architecture and induce systemic resistance to *Pst* (Table 3). The lack of effects on plant growth and ISR by these 21 mutants was confirmed independently in the 'regular' *in vitro* bioassay described above (Fig. 3). The results of these bioassays also showed that many of the 21 mutants established significantly lower cell densities on roots of Arabidopsis than wildtype *Pf.*SS101, suggesting they were significantly impaired in root colonization (Table 3). Only two mutants (20H12, 25C8) established rhizosphere population densities similar to that of wildtype *Pf.*SS101 (Table 3). These results suggest that for most mutants, except 20H12 and 25C8, the lack of effects on plant growth and ISR may be due, at least in part, to poor root colonization by these mutants.

Genetic characterization of *Pf.*SS101 mutants

For all 21 mutants, the regions flanking the plasposon insertion were cloned and sequenced. In 19 of the 21 mutants, the plasposon insertion was located in genes involved in

biosynthesis of different amino acids, including arginine (40H11; 44D8), cysteine (42B9, 20H12), glutamate (18F11), histidine (13E4; 13H6; 24A12; 32H11), tryptophan (24B12; 24D10; 71H9; 74F8), methionine (22G5; 51G1) and valine, leucine, isoleucine (7H2; 9F8; 59B6; 76G8) (Table 3). For the other two mutants, the plasposon was inserted in the genes coding for the DNA-binding response regulator ColR (16G6) and for phosphogluconate dehydratase (25C8), respectively (Table 3). All mutants were able to grow in KB broth to the same density as *Pf.*SS101, but only 16G6 and 25C8 were able to grow in minimal medium (SSM) to final densities alike wildtype *Pf.*SS101 (Table 3). The growth deficiency of the mutants in minimal medium was restored by supplementing the amino acid whose biosynthesis was disrupted by the plasposon mutation (Table 4). These results indicate that most mutants, except 25C8 and 16G6, were auxotrophic.

Southern-blot hybridization showed that 19 mutants had a single plasposon insertion except the two mutants 20H12 and 42B9 where two insertions were found. To confirm the role of *cysH* (20H12) or *cysM* (42B9) in plant growth promotion and ISR, site-directed mutagenesis of each of these genes was performed to obtain single knockout mutants for *cysH* and *cysM*. The location of the gentamycin resistance cassette and the absence of the tetracycline resistance cassette in these mutants was confirmed by PCR using primers targeting each of these two cassettes and genes flanking the targeted genes. Consistent with the phenotype of the random mutants, also these site-directed mutants lacked the ability to induce lateral root formation and ISR against *Pst*. The site-directed mutants for *cysH* and *cysM* were used for further experiments described below. The *in vitro* bioassay also confirmed that mutants 16G6 and 25C8 did not promote plant growth, alter root architecture nor induced systemic resistance against *Pst* (Fig. 4b). Mutants 16G6 (*colR*, PflSS101_4370), 25C8 (*edd*, PflSS101_4354), 20H12 (*cysH*, PflSS101_3982) and 42B9 (*cysM*, PflSS101_3837) were selected for further functional analysis. For each of these four mutants, genetic

complementation with the respective gene restored plant growth promotion, alteration of root architecture to the same level as observed for *Pf.*SS101; also ISR was restored although not entirely to the level as observed for *Pf.*SS101 (Fig. 4b). Next, we studied if the genes mutated in these 4 mutants were expressed in wildtype *Pf.*SS101 when colonizing Arabidopsis roots. Over a course of 7-18 days of plant growth, the genes *edd* (PflSS101_4354) and *cysM* (PflSS101_3837) were indeed expressed in *Pf.*SS101 on roots of Arabidopsis; also *cysH* (PflSS101_3982) and *colR* (PflSS101_4370) showed higher expression in *Pf.*SS101 on Arabidopsis roots after 7, 10 and 14 days but not at 18 days (Fig. 4d-f).

Role of sulfur assimilation in plant growth promotion and ISR by *Pf.*SS101

The *cysH* and *cysM* genes are essential in sulfur assimilation and the biosynthesis of the amino acids cysteine and methionine (Fig. 5). More specifically, *cysH* in *Pf.*SS101 encodes a predicted protein with the conserved (KRT)ECG(LS)H signature of the APS/PAPS reductase families and the critical two cysteine pairs found in APS reductases (Fig. S2). The final step of sulfur assimilation into cysteine is the synthesis of L-cysteine from O-acetyl-L-serine and sulfide catalyzed by O-acetyl-L-serine(thiol)-lyase encoded by *cysM*. A putative *cysE* gene was also detected in the *Pf.*SS101 genome, embedded in a gene cluster predicted to be involved in the formation of Fe-S clusters (Table 5, Fig. 5). Cysteine serves as the main source of sulfur for the biosynthesis of Fe-S centers. Based on these genomic analyses, we hypothesized that modulation of the plant's sulfur metabolism is one of the mechanisms underlying growth promotion and/or ISR by *Pf.*SS101.

To experimentally provide support for this hypothesis, we conducted a genome-wide transcriptome analysis of Arabidopsis seedlings treated with *Pf.*SS101 or the *cysH* mutant (20H12). To explore the expression pattern of Arabidopsis genes that were altered by *Pf.*SS101 or the *cysH* mutant, the expression of all 22,850 genes present on the ATH1 genome

array were subjected to one-way ANOVA; for exploratory purposes, this analysis was initially done without false discovery rate (FDR) correction. A total of 6,308 genes showed differential regulation (P<0.05) between Arabidopsis plants treated with *Pf.*SS101, the *cysH* mutant or the non-treated control. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed with these 6,308 differential genes to explore the pattern of their expression and amount of total variation in expression attributed to *Pf.*SS101 or the *cysH* mutant, respectively (Fig. 6). In the HCA, six major clusters were found that explain the total variation in gene expression in the different treatments. These clusters represent genes induced or repressed in plants treated with *Pf.*SS101 or the *cysH* mutant (Fig. 6). Clusters II and V represent Arabidopsis genes induced or repressed by *Pf.*SS101, respectively. Similarly, clusters VI and III represent Arabidopsis genes induced or repressed by the *cysH* mutant, respectively. The remaining clusters I and IV correspond to Arabidopsis genes induced or repressed by both *Pf.*SS101 and the *cysH* mutant, respectively (Fig. 6). In the PCA, the first principal component (PC1) explained 41% of the total variation in gene expression and is attributed to the unique clusters of genes whose expression was altered in plants treated with *Pf.*SS101 as compared to control plants or to plants treated with the *cysH* mutant (Fig. 6, clusters II, III, V and VI). The second principal component (PC2) explained 30% of the total variation and is attributed to clusters of genes that were altered in plants treated by *Pf.*SS101 and by the *cysH* mutant as compared to the control plants (Fig. 6, clusters I and IV).

To understand the major growth and defence related biological processes (BPs) that are altered in Arabidopsis by *Pf.*SS101, we performed gene set enrichment analysis (GSEA) specifically on genes in Cluster II of the HCA, representing genes in Arabidopsis whose expression was significantly induced by *Pf.*SS101 (Fig. 6b). Prior to performing the GSEA, we selected the genes in this cluster and computed independent t-tests by comparing the mean expression value for each of the genes in *Pf.*SS101-treated plants with the genes in the *cysH*-

treated plants. Cluster II contains a total of 967 genes of which 547 genes were significantly different ($P < 0.05$, with FDR correction) between plants treated with *Pf*.SS101 and plants treated with the *cysH* mutant. The GSEA on these 547 genes revealed 246 significantly enriched BPs. However, these long lists of BPs were largely redundant and reduced to 68 BPs by performing HCA on the gene X GO matrix, an output from the GSEA (Table 6). These 68 BPs fall into the following major categories: biosynthesis, transport, catabolism, response to stimulus and growth. From the processes associated with biosynthesis, sulfur compounds and specifically serine, cysteine and glucosinolate biosynthetic processes were the most significantly enriched (Table 6). The BPs involved in plant growth, such as indole acetic acid biosynthetic process and auxin transport, steroid biosynthesis and isopentenyl diphosphate biosynthesis, also showed significant enrichment in this cluster. Another significantly enriched BP was carbohydrate biosynthetic processes, specifically the biosynthesis of starch. In line with this, also glucose catabolic processes were significantly enriched (Table 6). In Cluster VI (fig. 6b), 831 genes were significantly upregulated ($P < 0.05$, with FDR correction) in plants treated with the *cysH* mutant as compared to plants treated with wild type *Pf.SS101*. The GSEA on these 831 genes revealed 276 significantly enriched BPs. Following similar procedures as stated above, redundant BPs were reduced to 67 representative BPs (Table 7). The majority of these 67 BPs fall into BPs that are induced during incompatible plant-microbe interactions while BPs associated with sulfate reduction were suppressed (Fig. 6b, cluster VI, Fig. 6c, d (see, sulfur reduction) and Table 7).

Discussion

In the present study, we showed that *Pf*.SS101 enhances Arabidopsis growth, alters root architecture and induces systemic resistance against the leaf pathogen *P. syringae* pv. *tomato* (*Pst*). In line with these phenotypes, the genome-wide plant transcriptome profiling

performed showed significant enrichment of biological processes that play a critical role in plant growth, including processes related to auxin biosynthesis, auxin polar transport and steroid biosynthesis. Other studies have shown that different rhizobacterial genera enhance plant growth and induce systemic resistance via the production of phytohormones, siderophores, lipopeptides or volatiles (VOCs) (Ryu *et al.*, 2003; Tran *et al.* 2007; Raaijmakers *et al.*, 2010; Van de Mortel *et al.* 2012; Bakker *et al.*, 2013). Our results indicate that the siderophore and the lipopeptide massetolide produced by *Pf.*SS101 do not significantly contribute to growth promotion and ISR in Arabidopsis under the experimental *in vitro* conditions used here. In the rhizosphere of plants grown in more complex substrates, however, siderophores and lipopeptides are commonly involved in interspecific bacterial competition contributing to rhizosphere colonization. Hence, these metabolites may be more relevant for plant growth promotion and ISR by *Pf.*SS101 in a natural soil-plant context. Also VOCs produced by *Pf.*SS101 do not seem to play a role in growth promotion and ISR of Arabidopsis. This is in contrast to earlier work with tobacco seedlings where *Pf.*SS101 promoted plant growth via the production of specific VOCs, in particular, 13-Tetradecadien-1-ol, 2-butanone and 2-Methyl-n-1-tridecene (Park *et al.,* 2015). A major difference between this former study and the work presented here is that the growth medium used in the tobacco assay was a rich medium. In the present study, a poor agar medium (0.5x MS) was used which does not support excessive growth of *Pf.*SS101 which in turn may have had qualitative and quantitative effects on the VOCs produced. Whether other or higher concentrations of VOCs are produced by *Pf.*SS101 when colonizing the roots of Arabidopsis seedlings remains to be investigated.

Results from the genome-wide screening of 7,488 *Pf.*SS101 random mutants led to the selection of 21 mutants deficient in both growth promotion and ISR. This result seems to be in contrast to the results of Zamioudis *et al.* (2013), who showed that plant growth promotion

and ISR by *Pf*.WCS417 are mediated by different pathways. Given the complexity of the genetic and molecular basis of both plant phenotypes (growth promotion, ISR), it was surprising that only 21 *Pf*.SS101 mutants were found out of a total of 7,488. This may be explained, at least in part, by the high stringency used in the plant screens, where we only selected those *Pf*.SS101 mutants with a strongly reduced ability to induce resistance or to alter root architecture and plant growth. Hence, we may have overlooked a number of mutants that affect these plant phenotypes in a more subtle and differential manner. Furthermore, the fact that all 21 *Pf*.SS101 mutants affected both plant phenotypes is, for many of these mutants, most likely due to their poor root colonizing abilities not reaching the required threshold densities to induce these phenotypes. Most of the 21 mutants were deficient in the biosynthesis of specific amino acids. The role of amino acids in rhizobacteria-plant interactions is not well studied, although some amino acids such as methionine and tryptophan may act in soil as precursors for the biosynthesis of the phytohormones ethylene and indole-3-acetic acid, respectively (Murcia *et al.*, 1997; González-López *et al.*, 2005). What the role is of these and other amino acids (histidine, valine, leucine and isoleucine) in root colonization and *Pf*.SS101-Arabidopsis interactions is yet unknown.

The *cysH* and *cysM* genes identified in our *Pf*.SS101 mutant screens are essential in sulfur assimilation and the biosynthesis of the amino acids cysteine and methionine. Results from *in vitro* assays with Arabidopsis grown on MS agar medium supplemented with different concentrations of these two amino acids showed that both cysteine and methionine induced lateral root formation in Arabidopsis in a concentration dependent manner (Fig. S3a). Moreover, cysteine at relatively high concentrations induced disease resistance against *Pst* in Arabidopsis (Fig. S3b). These results confirm and extend observations that cysteine homeostasis is important for plant immunity (Alvarez et al. 2012). The plant responses observed here may not be typical for cysteine and methionine only as several studies have

shown effects of exogenous amino acids on root growth (Walch-Liu et al. 2006) and disease resistance (Hijwegen, 1963; Kadotani et al., 2016). Analyses of the temporal *in situ* production levels of amino acids by *Pf*.SS101 on roots of Arabidopsis should be conducted to further disentangle the role of these amino acids in the observed plant responses. In a more indirect way, however, our transcriptome data did reveal that biosynthetic processes associated with sulfur compounds and specifically serine, cysteine and glucosinolates, were the most significantly enriched in seedlings treated with *Pf*.SS101 as compared to the control plants and plants treated with the *cysH* mutant. These results indicate that *Pf*.SS101 modulates sulfur metabolism in Arabidiopsis, particularly processes related with sulfur reduction (Fig. 6c and d). These results extend findings in previous studies by Meldau *et al.* (2013) and Aziz et al. (2016) who attributed modulation of sulfur metabolism as a mechanism of growth promotion and induction of lateral roots of tobacco and Arabidopsis by different *Bacillus* strains. Meldau et al. (2013) further showed that the growth-promoting effects on tobacco were mediated by the production of the VOC dimethyl disulphide.

In plants, sulfur is important in various stress responses (Bloem *et al.*, 2005; Kertesz *et al.*, 2007). Elemental sulfur itself can be used directly by plants, via deposition in the xylem parenchyma (Cooper and Williams, 2004). The metal-chelating properties of sulfur in phytochelatins help alleviate heavy metal stress and sulfur is also important to the plant in responding to pathogen attack, since many defense compounds contain sulfur, in particular the glucosinolates (Brader *et al.*, 2006). Cysteine biosynthesis in plants involves the incorporation of the carbon backbone from serine with reduced inorganic sulfur (Neuenschwander *et al.*, 1991; Saito *et al.*, 1994; Bonner *et al.*, 2005). Cysteine might enter into the glucosinolate biosynthesis pathway by three routes. The first route involves direct donation of reduced sulfur to glucosinolate biosynthesis. The second route involves the incorporation of cysteine into methionine and through a series of side chain elongation, S-glycosilation and other

secondary modification, it ends up in the glucosinolate pool. The third route could involve the conjugation of cysteine, glutamate and glycine to form glutathione (GSH) (Meister, 1995). (Geu-Flores et al., 2011) showed that GSH acts as a sulfur donor for glucosinolate biosynthesis. In our previous study, we have shown that *Pf.*SS101 enhances glucosinolate levels in roots and shoots of Arabidopsis seedlings (Van de Mortel *et al*. 2012). In line with this, metabolic processes related to all the aforementioned amino acids showed significant enrichment among the genes induced by *Pf.*SS101, indicating that the second route is the most probable means of reduced sulfur channelling mechanism into the glucosinolate pool. Bacteria that are able to successfully establish beneficial relationship with plants typically circumvent or suppress the induction of the host immune system (Zamioudis and Pieterse, 2012). Interestingly, treatment of Arabidopsis with the *cysH* mutant led, in contrast to wild type *Pf.*SS101, to the induction of biological processes (BPs) that are associated with incompatible plant-microbe interaction. This suggests that a mutation in the *cysH* gene may have compromised the ability of the bacteria to circumvent or suppress the host immune responses, resulting in recognition of the *cysH* mutant by the plant as a harmful invader. In line with this, *Sinorhizobium meliloti* mutants that lack sulfation of Nod factors are strongly impaired in their ability to nodulate their host alfalfa (Roche et al., 1991). In this context, we speculate that the *cysH* mutation in *Pf.*SS101 affects sulfation of yet unknown bacterial traits involved in modulation of the plant immune system.

Carbohydrate biosynthetic processes in general and starch biosynthetic processes in particular were highly induced by *Pf.*SS101 and these processes are critically important for biomass formation. The recycling of glucose is the primary step before its incorporation into starch through the enzymes of the glycolytic, glucogenic and pentose phosphate pathways (Glawischnig *et al.*, 2002). Interestingly, the transcriptome data showed that genes involved in these biological processes are also significantly enriched in *Pf.*SS101-treated seedlings.

In addition to the *cysH* and *cysM* mutants, two other *Pf.*SS101 mutants with mutations in the *colR* and *edd* genes were identified in this study. The ColR-ColS pathway was first characterized in *P. fluorescens* for its role in competitive colonization of plant roots (Dekkers *et al.*, 1998). Subsequent studies have shown that mutations in the ColR-ColS two-component system lead to several other defects in different *Pseudomonas* strains (Hõrak *et al.*, 2004; Kivistik *et al.*, 2006). De Weert and colleagues (2006) showed that a putative methyltransferase/wapQ (*inaA*) operon is located downstream of ColR-ColS in *P. fluorescens* WCS365 and regulated by ColR-ColS. Since *wapQ* (*inaA*) encodes a putative lipopolysaccharide (LPS) phosphatase, the possibility was studied that the integrity of the outer membrane of *P. fluorescens* WCS365 mutant PCL1210 was altered. PCL1210 was identified as a colonization mutant with an insertion in the ColR-ColS two-component system (Dekkers *et al.*, 1998). Mutants in the methyltransferase/*wapQ* operon were also altered in their outer membrane permeability and defective in competitive tomato root tip colonization (De Weert *et al.*, 2006). In *Pf.*SS101, we also identified a putative methyltransferase/*inaA* (*wapQ*) operon downstream of ColR-ColS but its exact role and underlying mechanisms in plant growth promotion and ISR are not yet known.

The *edd* gene codes for 6-phosphogluconate dehydratase, an enzyme that catalyzes the first step in the Entner-Doudoroff (ED) pathway (Wanken *et al.*, 2003) which comprises the dehydration of 6-phospho-D-gluconate into 6-phospho-2-dehydro-3-deoxy-D-gluconate (Peekhaus and Conway, 1998; Kim *et al.*, 2007). Many bacteria possess genes for the ED pathway (Kim *et al.*, 2007). For *P. chlororaphis* O6, Kim *et al.* (2007) showed that the *edd* gene contributes to root colonization and ISR. They concluded that metabolism of sugars through the ED pathway in *P. chlororaphis* O6 may be important as it may facilitate the production of effectors involved in ISR (Kim *et al.*, 2007). In our study, we showed that the *edd* gene was significantly higher expressed in *Pf.*SS101 in the rhizosphere of Arabidopsis

compared to *Pf.*SS101 grown KB medium only and that *edd* mutant 25C8 showed no induction of lateral root formation and systemic resistance in Arabidopsis, similar to what was shown for *P. chlororaphis* O6 (Kim *et al.*, 2007). In contrast to *P. chlororaphis* O6, however, elimination of the *edd* gene in *Pf.*SS101 had no effect on colonization of Arabidopsis seedlings grown *in vitro*.

In conclusion, modulation of auxin biosynthesis and transport, steroid biosynthesis, carbohydrate metabolism and sulfur assimilation in Arabidopsis appear to be key mechanisms linked to growth promotion and ISR by *Pf.*SS101. In particular sulfur assimilation was shown to be an important biological process modulated by *Pf.*SS101 in Arabidopsis. The molecular signals and sulfur-containing compounds involved have not yet been identified and need further investigation. Also identification of the bacterial traits associated with the ColR-ColS two-component system and the ED pathway in *Pf.*SS101 as well as the plant transcriptional and metabolic responses to these two *Pf.*SS101 mutants will be required to shed more light on the other mechanisms of plant growth promotion and ISR.

Experimental Procedures

Bacterial strains and culture conditions

Pseudomonas fluorescens SS101 (*Pf.*SS101) was cultured in liquid King's B medium (KB) at 25 °C for 24 h. Bacterial cells were collected by centrifugation, washed three times with 10mM $MgSO_4$ and resuspended in 10 mM $MgSO_4$ to a final density of 10^9 CFU ml^{-1} (OD_{600} = 1.0). *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) was cultured in KB broth supplemented with rifampicin (50 µg ml^{-1}) at 25 °C for 24h. *Escherichia coli* strain DH5α was used as a host for the plasmids for site-directed mutagenesis and complementation. *E. coli* strains were grown on Luria-Bertani (LB) plates or in LB broth amended with the appropriate antibiotics. The random plasposon mutants of *Pf.*SS101 were obtained by biparental mating with *E. coli* strain S17 λ *pir* harboring the TnModOKm element in plasmid (Dennis and Zylstra, 1998), according to protocols described by Sambrook and Russel (Sambrook *et al.*, 2001). Transformants were selected on KB agar plates supplemented with rifampin (100 µg ml^{-1}) and kanamycin (100 µg ml^{-1}).

Auxotrophy of selected plasposon mutants were tested by growing these mutants O/N in 5ml KB supplemented with the appropriate antibiotics and shaken at 220 rpm at 25 °C. O/N cultures were washed three times with 10 mM $MgSO_4$ and set to OD_{600} = 1.0. Then a starting culture was inoculated at a concentration of 0.5% (v/v) in 200 µl KB or in 200 µl minimal medium (SSM) in a 96-well plate. SSM medium composition (per liter): 7.5g K_2HPO_4*$3H_2O$, 3g KH_2PO_4, 1g $(NH_4)_2SO_4$, 0.2g $MgSO_4$*$7H_2O$, 4g Succinic Acid (Bernstein Saure). Growth was determined in a Bio-Rad 680 microplate reader at 600 nm with settings at 25 °C, high shaking, 1min mixing and OD-measurements every 2 min over a period of 24 hours. ACC deaminase activity of *Pf.*SS101 was measured according to methods described by Penrose and Glick (2003).

Site-directed mutagenesis

Site-directed mutagenesis of the genes *cysH* and *cysM* was performed based on the method described by Choi and Schweizer (2005). The primers used for amplification are described in supporting information Table S1. The FRT-Gm-FRT cassette was amplified with pPS854-GM, a derivative of pPS854, and FRT-F and FRT-R were used as primers (Supporting information Table S1). The first-round PCR was performed with KOD polymerase (Novagen), according to the manufacturer's protocol. PCR reactions were carried out under the following conditions: an initial denaturation step for 2 min at 95 °C followed by denaturation for 15 s at 95 °C, annealing for 20 s at 58 °C and extension for 30 min at 72 °C for 30 cycles, followed by a final elongation step at 72 °C for 5min. All fragments were run on a 1% (w/v) agarose gel and purified with illustra^{TM}GFX^{TM}PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences). The overlap extension PCR was performed with Verbatim High Fidelity DNA polymerase (Thermoscientific) according to the manufacturer's protocol by addition of equimolar amounts of the 5-end fragment, FRT-Gm-FRT, and 3-end fragment. PCR reactions were carried out under the following conditions: an initial denaturation step for 2 min at 95 °C followed by denaturation for 20 s at 98 °C, annealing for 15 s at 58 °C and extension for 2 min at 72 °C for 30 cycles, followed by a final elongation step at 72 °C for 5 min and the PCR fragments were purified as described above. The fragments were digested with BamHI and cloned into BamHI-digested plasmid pEX18Tc and transformed colonies were selected on LB medium supplemented with 25 μg ml^{-1} gentamicin (Sigma). Integration of the inserts was verified by PCR analysis with pEX18Tc primers (Supporting information Table S1) and by restriction analysis of isolated plasmids. The pEX18Tc-*cysH* and pEX18Tc-*cysM* constructs were subsequently transformed to *Pf*.SS101. Competent cells were obtained by washing the cells three times with 300 mM sucrose from a 6-ml overnight culture and finally dissolving the cells in 100 μl of 300 mM

sucrose. Electroporation occurred at 2.4 kV and 200 F and after incubation in SOC medium for 2 h at 25 °C cells were plated on KB supplemented with gentamicin (40 µg ml^{-1}) and rifampicin (50 µg ml^{-1}). Six obtained colonies were grown in LB for 2-3 h at 25 °C than diluted 10 times and plated on LB supplemented with gentamicin (40 µg ml^{-1}) and 5% sucrose to accomplish the double crossover. The plates were incubated at 25 °C for at least 48 h and colonies were re-streaked on LB supplemented with gentamicin and 5% sucrose. Twelve colonies per transformation were transferred to KB plates supplemented with tetracycline (25 µg ml^{-1}) and KB plates with gentamycin and rifampicin. Colonies that grew on LB with gentamicin and rifampicin but not on LB with tetracycline were selected and subjected to colony PCR to confirm the presence of the gentamicin resistance cassette and the absence of the tetracycline resistance cassette. Positive colonies were confirmed by sequencing the PCR fragments obtained with the Up forward and Dn reverse primers (Supporting information Table S1). The mutants obtained were tested for induction of lateral root formation in the *in vitro* assay with Arabidopsis.

Construction of pME6031-based vectors for genetic complementation

A fragment of approximately 2 kb containing the *cysH* or *cysM* gene, including the promoter and terminator, was obtained by PCR with specific primers (Table S1) and Phusion DNA polymerase (Finnzymes) according to the manufacturers protocols. The PCR fragments were isolated from gel with Illustra^{TM}GFX^{TM}PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences) and digested with *HindIII* and cloned into the shuttle vector pME6031 (Heeb *et al.*, 2000). *E. coli* DH5α was transformed with the constructs by heat shock transformation (Inoue *et al.*, 1990) and transformed colonies were selected on LB agar plates supplemented with tetracycline (25 µg ml^{-1}). Correct integration of the fragments was verified by PCR analysis and restriction analysis of the isolated plasmids. The pME6031-

cysH, pME6031-*cysM* constructs were subsequently transformed into the *cysH* or *cysM* plasposon mutant. Transformed cells were plated on KB supplemented with tetracycline (25 µg ml^{-1}) and the presence of pME6031-*cysH* or pME6031-*cysM* was verified by PCR analysis with primers specific for pME6031. The complemented mutants obtained were tested for their ability to induce lateral root formation in the *in vitro* assay with Arabidopsis.

Plant Material and Growth Conditions

Seeds of *Arabidopsis thaliana* Columbia-0 (Arabidopsis) were surface sterilized for three hours by placing seeds in opened Eppendorf tubes in a desiccator jar. Two 100-ml beakers each containing 50 ml commercial bleach was placed inside and 1.5 ml concentrated HCl was added to each beaker. The desiccator jar was closed and the seeds were sterilized by chlorine gas. After 4 h, seeds were transferred on water-saturated filter paper in petri dishes followed by a 3-day treatment at 4 °C. Thereafter, 10-12 seeds were sown on plates containing 50 ml half-strength Murashige Skoog (MS) medium (Murashige and Skoog, 1962). One-week-old Arabidopsis seedlings were inoculated at the root tip with 2 µl *Pf.*SS101 cell suspensions (10^9 CFU ml^{-1}); in the control treatment, seedlings were inoculated with 2 µl of 10 mM $MgSO_4$. After an additional three days of plant growth, the 10-day-old seedlings were transferred to 60 ml PVC pots containing a sand-potting soil mixture that was autoclaved twice for 20 min with a 24 h interval. Once a week, plants were supplied with modified half-strength Hoagland nutrient solution (Hoagland and Arnon, 1938).

In the *in vitro* assays with half-strength MS medium, *Pf.*SS101 was applied to the seeds or to the root tips. For both treatments, seeds were sterilized and sown on plates as described above. For the *Pf.*SS101 seed treatment, a cell suspension (10^9 CFU ml^{-1}) of *Pf.*SS101 was added to the sterilized seeds in a Petri dish and incubated for 30 minutes at room temperature. For the control, seeds were incubated for 30min with sterile 10mM

$MgSO_4$. For the root tip treatment, 2 µl *Pf.*SS101 (10^9 CFU ml^{-1}) was applied to the root tips of one-week-old seedlings. Control plants were inoculated with 2 µl of 10 mM $MgSO_4$. The challenge with *Pst* was performed by inoculation of 2 µl cell suspension (10^9 CFU ml^{-1}) in the center of the leaf rosette of 14-day-old plants. Five to seven days after challenge inoculation, disease incidence was assessed by determination of the percentage of diseased leaves per plant. Leaves were scored as diseased when they exhibited necrotic or water-soaked lesions surrounded by chlorotic tissue. From the number of diseased and non-diseased leaves, the disease incidence was calculated for each plant (20-30 plants per treatment). The experiment was performed at least twice.

Plant Microscopic analysis

Seeds of Arabidopsis were pre-treated with *Pf.*SS101 and grown for 18 days vertically in plates containing half-strength MS medium. Then longitudinal and cross sections of the roots were made. Parts of the tip and base of the roots were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 h at room temperature. Tissues were dehydrated in ethanol and propylene oxide and embedded in Spurr's resin. Sections of 1 µm were stained with 0.1% toludine blue in 1% borax. Sections were viewed and photographed with a Leitz Orthoplan microscope, equipped with a Leica camera DFC 420C. The GAL4-GFP enhancer trap lines of Arabidopsis, J2351, J1922 and J0661, were viewed with a Nikon Eclipse 90i epifluorescence microscope at 10x magnification. The ND filter 8 and FGP(R)-LP filter set were used in combination with the NIS-Elements imaging software version 2.3.

Bacterial gene expression in the Arabidopsis rhizosphere

Arabidopsis was grown with *Pf.*SS101 on half-strength MS medium for 18 days. Cells of *Pf.*SS101 were collected at day 0, 7, 10, 14 and 18 by collecting Arabidopsis roots in 1 ml

10 mM $MgSO_4$, vortexing and spinning down. The *Pf*.SS101 cells were frozen in liquid N_2 and stored at –80 °C. For the RNA isolations and cDNA synthesis, four biological replicates were used for each time point. RNA was isolated from the frozen bacterial cells with Trizol reagent (Invitrogen) followed by DNase I (GE Healthcare) treatment. One microgram of RNA was used for cDNA synthesis with Superscript III (Invitrogen) according to the manufacturer's protocol. For the Q-PCR, conducted with the 7300SDS system from Applied Biosystems, the SensiMix™ SYBR kit (Bioline) with a final concentration of 3.0 mM $MgCl_2$ was used according to the manufacturer's protocol. The concentrations of the primers were optimized (400 nM final concentration for all) and a dissociation curve was performed to check the specificity of the primers. The primers used for the Q-PCR are listed in Supporting Information Table S2. To correct for small differences in template concentration, *rpoD* was used as the housekeeping gene. The cycle where the SYBR green fluorescence crosses, a manually set threshold cycle (C_T) was used to determine transcript levels. For each gene the threshold was fixed based on the exponential segment of the PCR curve. The C_T value for the gene of interest was corrected for the housekeeping gene as follows: $\Delta C_T = C_{T(gene)} - C_{T(rpoD)}$. The relative quantification (RQ) values were calculated by the formula $RQ = 2^{-[\Delta CT(\text{day 7, 10, 4 or 18}) - \Delta CT(\text{day 0})]}$. If there was no difference in transcript level between day 7, 10, 14 or 18 and day 0, then RQ = 1 (2^0) and log RQ = 0. Q-PCR analysis was performed on four independent RNA isolations (biological replicates). Statistically significant differences were determined for log-transformed RQ values by analysis of variance ($P < 0.05$) followed by the Bonferroni and Dunnet post hoc multiple comparisons.

Transcriptome analysis of Arabidopsis exposed to *Pf*.SS101 and the *cysH* mutant

Total RNA was extracted from shoots of untreated, *Pf*.SS101-treated and *cysH* mutant-treated plants after 18 d of growth. Four biological replicates with 30 plants per

replicate were used for each treatment. RNA was isolated from the frozen tissues with Trizol reagent (Invitrogen). The RNA samples were further purified using the NucleoSpin RNA II kit (Macherey-Nagel). For the Affymetrix Arabidopsis genome GeneChip array analysis (ServiceXS), amplification and labelling of the RNA samples as well as hybridization, staining, and scanning were performed according to the manufacturer's specifications. The raw array data (CEL files) were normalized using the RMA probe summarization algorithm in R programme using Bioconductor package; the processed data were used for further analysis and can be found at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103117. ANOVA without false discovery rate (FDR) correction was performed to identify significantly altered transcripts between Arabidopsis plants treated with *Pf.*SS101, the *cysH* mutant (20H12) and non-treated control plants. Using the transcripts that were significantly altered ($P < 0.05$, without FDR correction) between the treatments, discriminant function analysis (DFA) and hierarchical cluster analysis (HCA) were performed in Genemaths XT software (Applied Maths, Inc. Austin, TX, USA). For HCA, Pearson's correlation coefficients were used to calculate the distance or similarity between two entries and the resulting clusters were summarized using a complete linkage algorithm. To compare the expression values, the raw values of each sample were auto-scaled by the use of the average as an offset and the standard deviation as scale (raw value-average (offset)/SD (scale)). Clusters of genes that showed altered expression patterns between the contrasting treatments in the HCA were selected. Independent t-Test was performed to compare the expression of these genes in *Pf.*SS101-treated plants with gene expression in plants treated with either the *cysH* mutant or control plants. Genes that showed significant alteration in their expression in plants treated with *Pf.*SS101 were further investigated by gene set enrichment analysis (GSEA) using the web-based Plant GeneSet Enrichment Analysis toolkit (http://structuralbiology.cau.edu.cn/PlantGSEA/) with the standard settings. The gene X GO

matrix was used to perform HCA to reduce the number of redundant biological processes described by a group of genes as described by Etalo et al. (2013). The aliphatic glucosinolate biosynthesis pathway shown in Figure 6d was reconstructed based on KEGG and the overview by Sønderby et al. (Sønderby *et al.,* 2010); genes for which experimental verification is lacking (such as *GGP1*, which has recently been shown not to be coexpressed with the rest of the pathway, and may be in fact unrelated to it (Wisecaver *et al.,* 2017)) were purposefully left out.

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Table and figure legends

Table 1 Effects of *Pf.*SS101 on growth of Arabidopsis cultivated *in vitro* or in soil. Plant fresh weights were quantified and expressed in milligrams. For the *in vitro* assays, the "±" represents the standard error of the mean of 4 replicates with 15-20 plants per replicate; for the soil assay, the "±" represents the standard error of the mean of at least 35 Arabidopsis plants. Means followed by different letters are statistically different (P<0.05).

Table 2 Analysis of known bacterial traits in growth promotion and ISR of Arabidopsis by *Pf.*SS101. Effects on plant growth and ISR by *Pf.*SS101 and heat-killed *Pf.*SS101 cells (EXP1), by the *massA* mutant (EXP2), by different concentrations of the lipopeptide massetolide A (EXP3), and by the siderophore mutant (61C8, Pflss101_3099) (EXP4). For each experiment, different letters indicate statistically significant differences between the treatments (P<0.05). The asterisk indicates different concentrations of masssetolide A (µg ml^{-1}) supplemented to the 0.5x MS agar medium. The "±" represents the standard error of the mean of 4 replicates with 10 plants per replicate.

Table 3 Phenotypic and genetic characterization of *Pf.*SS101 and mutants deficient in plant growth promotion and ISR. [a]COG Functional annotation. C = Energy production and conversion; E = Amino acid transport and metabolism; T = Signal transduction mechanisms. Cell densities of *Pf.*SS101 and the mutants grown in KB and SSM media at 25°C were measured spectrophotometrically (OD_{600nm}): + = growth; - = no growth.

Table 4 Growth of *Pf.*SS101 and 10 mutants in SSM medium supplemented with different L-/D-amino acids (up to 15mM) at 25°C; cell density was measured spectrophotometrically (OD_{600nm}) for six replicates. + = growth; - = no growth.

Table 5 Proposed pathway and genes for sulfur transport and assimilation in *Pf.*SS101.

[α] Functional annotation. C = Energy production and conversion; E = Amino acid transport and metabolism; H = Coenzyme metabolism; J = Translation, ribosomal structure and biogenesis;

K = Transcription; O = Post-translational modification, protein turnover, chaperones; P = Inorganic ion transport and metabolism; R = General function prediction only; S = Function unknown.

Table 6 Gene set enrichment analysis (GSEA) representing significantly enriched biological processes (BPs) in *A.thaliana* treated with *Pf.*SS101. The asterisk represents the number of Arabidopsis genes belonging to the indicated biological process. The dollar sign represents the number of genes in the selected cluster (see also cluster II, Fig.6) belonging to the indicated biological process. GO stands for gene ontology.

Table 7 Gene set enrichment analysis (GSEA) representing significantly enriched biological processes (BPs) in *A.thaliana* treated with CysH mutant 20H12. The asterisk represents the number of Arabidopsis genes belonging to the indicated biological process. The dollar sign represents number of genes in the selected cluster (cluster VI, Fig.6b) belonging to the indicated biological process. GO stands for gene ontology.

Figure 1 Growth promotion of Arabidopsis by *Pf.*SS101 on MS agar medium. (a) Primary root length of mock- and *Pf.*SS101-treated Arabidopsis grown for 14 days; (b) Average number of lateral roots of mock- and *Pf.*SS101-treated Arabidopsis grown for 14 days; (c) Longitudinal root sections of Arabidopsis seedlings grown on half-strength MS medium for 18 days without (d, f, h) or with *Pf.*SS101 (e, g, i); root section images were captured by light-microscopy (d, e, h, i) or GFP fluorescence (GAL4-GFP enhancer trap) (f, g). Black bars represent mock-inoculated plants and white bars represent *Pf.*SS101-treated plants. Bars represent the mean of 4 replicates (± standard error) with 15-20 plants per replicate. Bars with asterisk are significantly different (One-way ANOVA, Tukey $P<0.05$).

Figure 2 Effect of heat-killed and live *Pf.*SS101 cells on Arabidopsis growth.

Figure 3 Assays for the screening 7,488 *Pf.*SS101 random plasposon mutants for induction of lateral root formation (a) or for disease resistance (b+c). Bacterial suspensions were

inoculated on Arabidopsis seeds after sowing on plates carrying 0.5x MS. *P. syringae* pv. *tomato* (*Pst*) was inoculated in the centre of the rosset of the Arabidopsis seedlings.

Figure 4 Effects of *Pf.*SS101, mutants 20H12, 25C8, 16G6, and 42B9, and their complemented strains on lateral root formation (a) and ISR (b). Bars represent the average disease incidence (%, ± standard deviation) of 4 replicates with 10-15 Arabidopsis seedlings per replicate. Q-PCR analysis of genes involved in plant growth promotion, lateral root formation and pathogen resistance by *Pf.*SS101: (c) expression of *ColR* (*PflSS101_4370*); (d) expression of *edd* (*PflSS101_4354*); (e) expression of *cysH* (*PflSS101_3982*); (f) expression of *cysM* (*PflSS101_3837*). Gene expression levels were measured in wild-type strain *Pf.*SS101 after 0, 7, 10, 14 and 18 days of *Arabidopsis thaliana* Col-0 growth. The transcript level of each gene was corrected for the transcript level of the household gene *rpoD* and is presented relative to the level at day 0 (logRQ). Black bars represent, for each time point, the mean values of gene expressions of four biological replicates. Experiments were performed at least twice and representative results are shown. The asterisk indicates statistically significant differences relative to day 0 and letters represent a significant difference of the means according to One-way ANOVA (Tukey, $P<0.05$).

Figure 5 Proposed metabolic model for the uptake and assimilation of sulfur in *Pf.*SS101. Candidate genes are shown in bold.

Figure 6 Genome wide transcriptome analysis of Arabidopsis treated with cell suspensions of wild type *Pf.*SS101, the *cysH* mutant (20H12) or not treated (control). (**a**) Principal component analysis (PCA) based on 6308 Arabidopsis genes that showed differential regulation (P<0.05, without FDR correction) between *Pf.*SS101, the *cysH* mutant and control. In the PCA, the first principal component (PC1) explained 41% of the total variation in gene expression and is attributed to the unique clusters of genes whose expression was altered in plants treated with *Pf.*SS101 as compared to control plants or to plants treated with the *cysH*

mutant (clusters II, III, V and VI). The second principal component (PC2) explained 30% of the total variation and is attributed to clusters of genes that were altered in plants treated by *Pf*.SS101 and by the *cysH* mutant as compared to the control plants (clusters I and IV). (**b**) Hierarchical cluster analysis (HCA) performed on the same 6308 genes to explore the pattern of their expression in Arabidopsis treated with *Pf*.SS101 or *cysH*. Six major clusters were formed, which explain the total variation that corresponds to the above mentioned principal components. These clusters represent genes induced or repressed in the three treatments. (**c**) Expression profile of selected genes involved in aliphatic glucosinolate biosynthesis in Arabidopsis not treated (control), treated with *Pf*.SS101 or the *cysH* mutant. The scale is based on z-scores. Genes marked bold are significantly differentially expressed between plants treated with *Pf*.SS101 and the *cysH* mutant. (**d**) Methionine-derived aliphatic glucosinolate biosynthesis pathway. Colored boxes corresponding to each genes are significantly differentially expressed ($p < 0.05$, with FDR correction) when their mean expression value is compared between plants treated with *Pf*.SS101 and the *cysH* mutant. Red and blue colored boxes represent upregulation and downregulation of genes involved in the biosynthetic pathway, respectively.

Supplementary table and figure legends

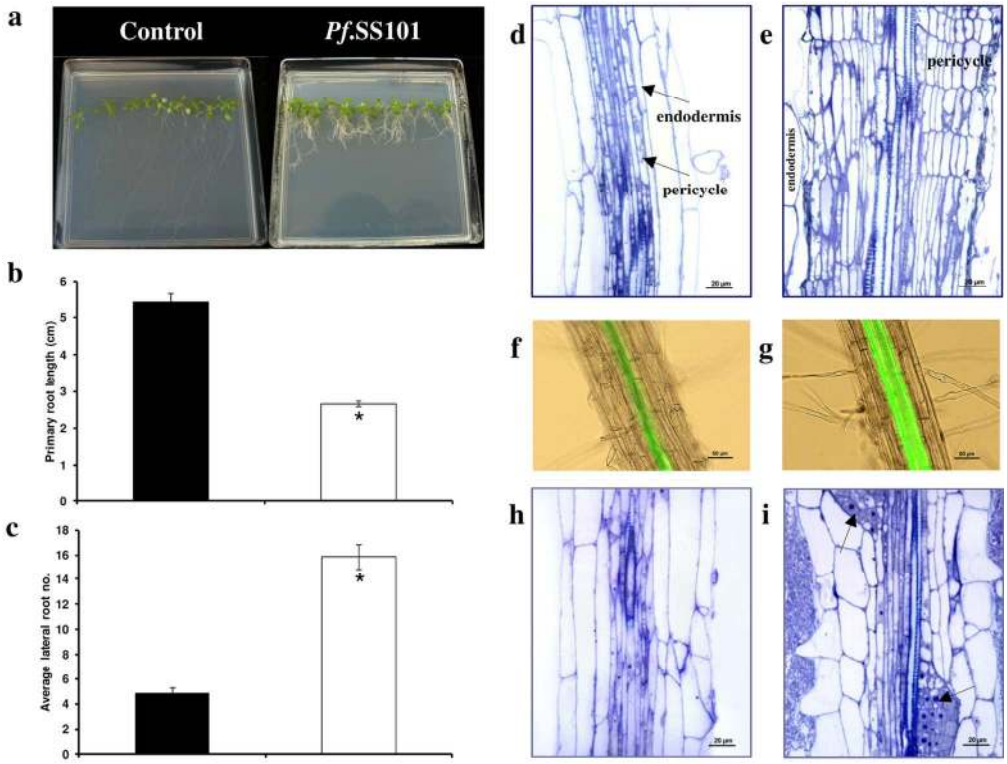
Table S1 Strains, plasmids and primers used in this study for site-directed mutagenesis and genetic complementation.

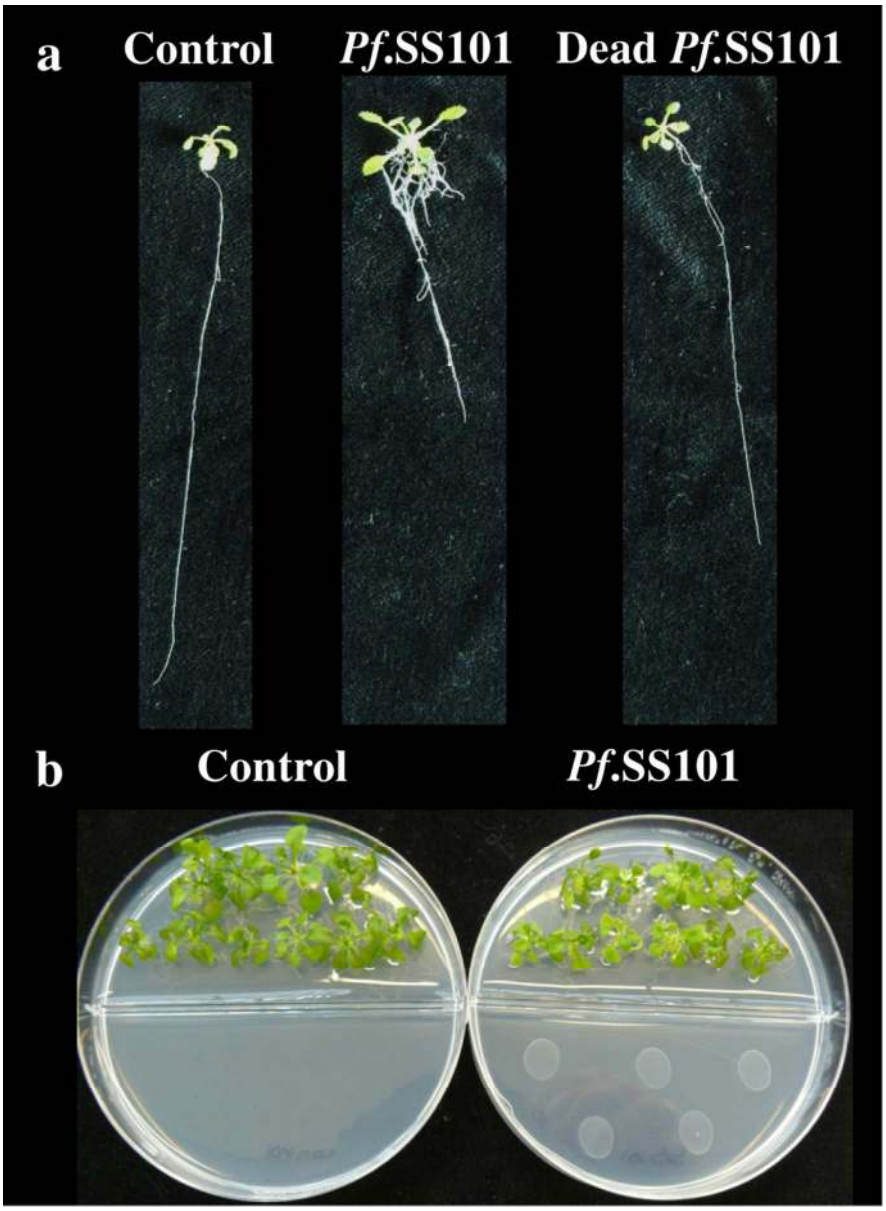
Table S2 Primers used in Q-PCR analysis.

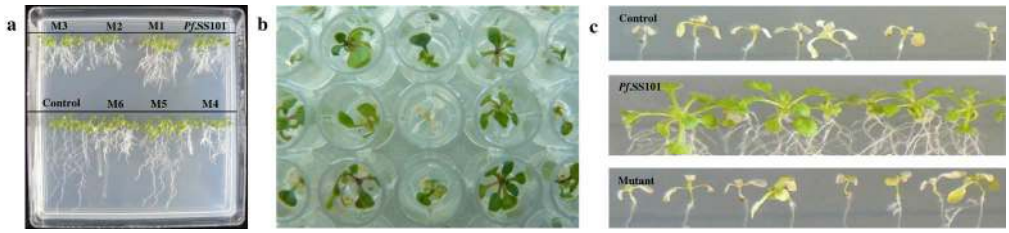
Figure S1 ACC-deaminase activity in *Pf.*SS101. *Pf.*F113 was used as a positive control and *P. protegens* CHA0 was used as a negative control. α-Ketobutyrate production by strains F113, CHAO and SS101 was measured after incubation of their cell extracts with ACC. Averages of 4 replicates are given. Different letters indicate significant differences (P<0.05).

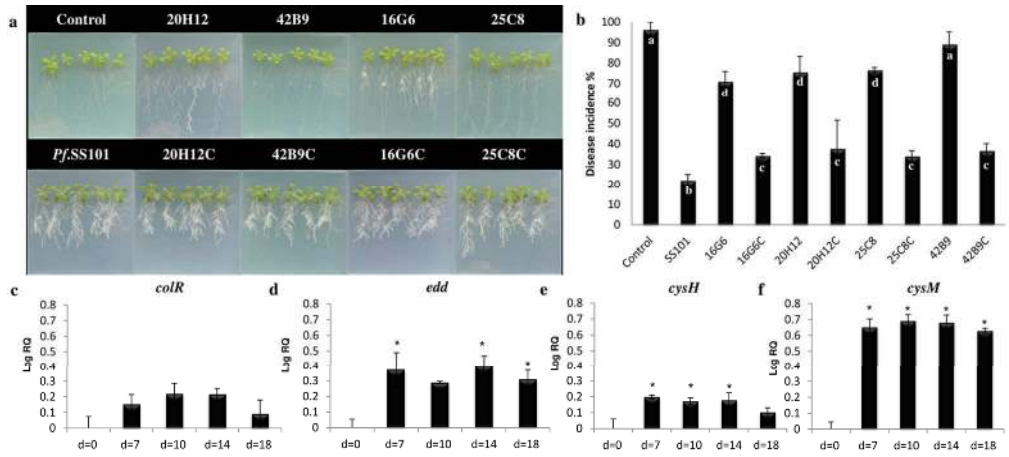
Figure S2 Comparison of amino acid sequences of the APS reductase from *Pf.*SS101 with the APS reductase from *P. putida*, *P. entomophila*, *P. syringae*, *Pf.*SBW25, *Pf*01, *Pf*5 and *P. aeruginosa*. The sequences were aligned with Clustal W. Asterisks depict identical residues, and boxes mark the additional cysteine residues in the APS reductases. The conserved APS reductase signature is underlined.

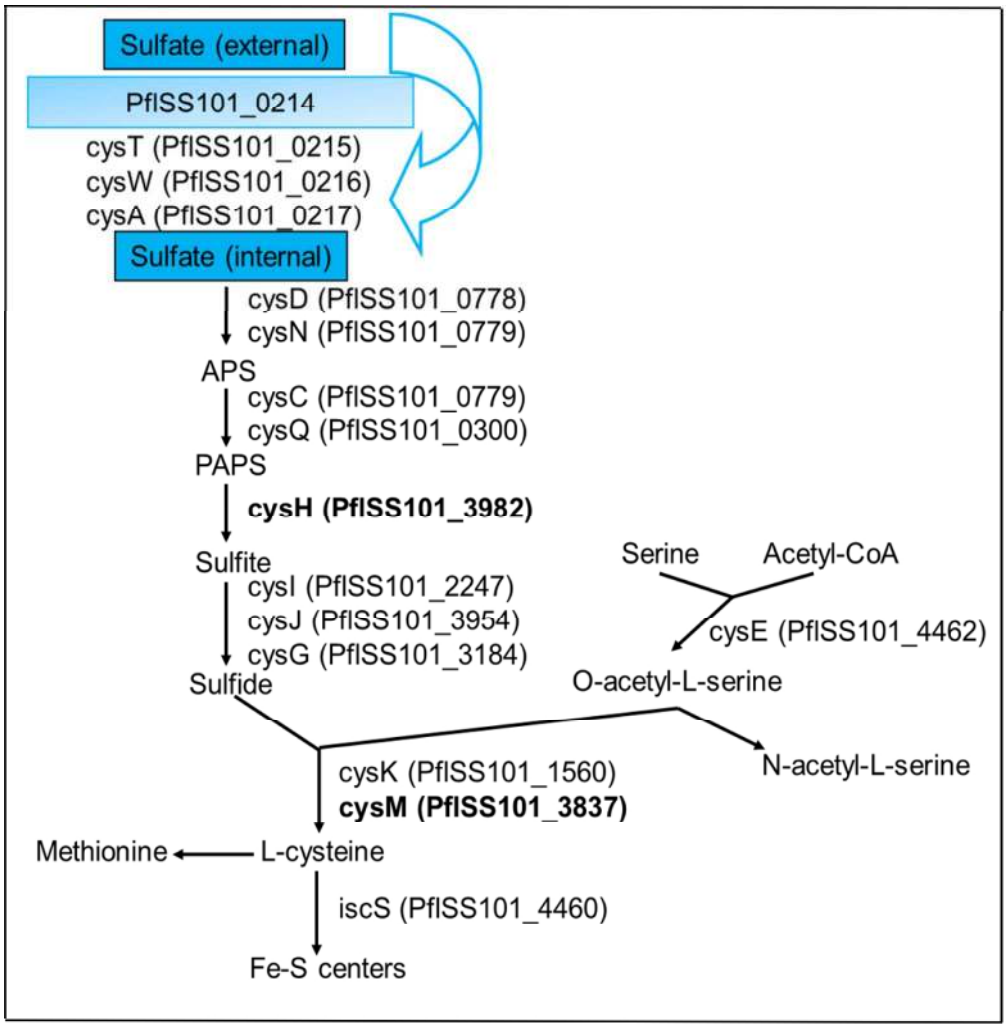
Figure S3 Effects of exogenous cysteine and methionine on Arabidopsis growth. (**a**) Effects of L-/D-cysteine and methionine (0.5, 1.0, 1.5 and 2.0mM) on root growth of Arabidopsis; (b) effects of methionine and cysteine (100, 250 and 500μM) on ISR of Arabidopsis against *Pst*. Bars represent the mean disease incidence (%, ± standard deviation) of 4 biological replicates with 10-15 Arabidopsis seedlings per replicate. Bars with asterisk are significantly different from each other (One-way ANOVA, Tukey, P<0.05)**.**

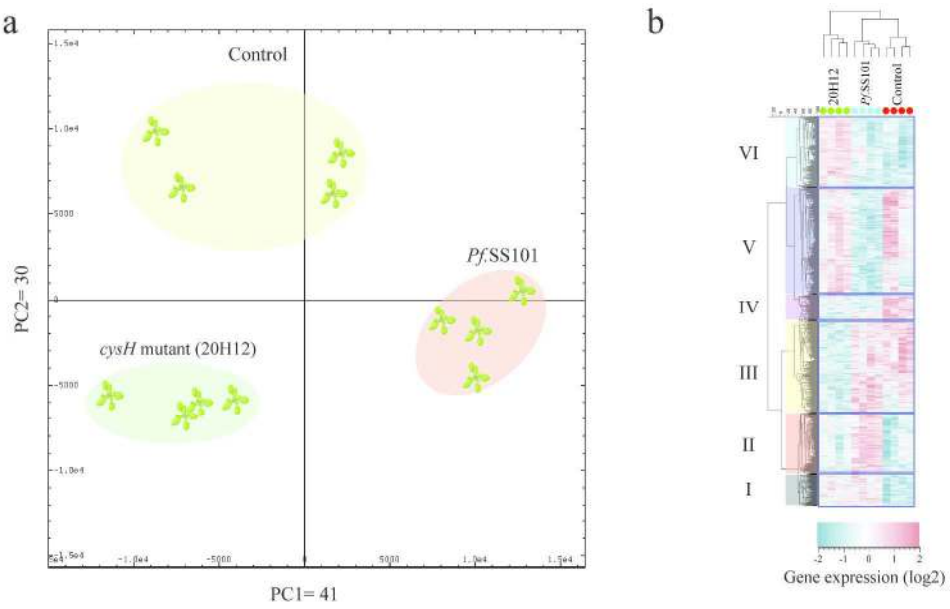












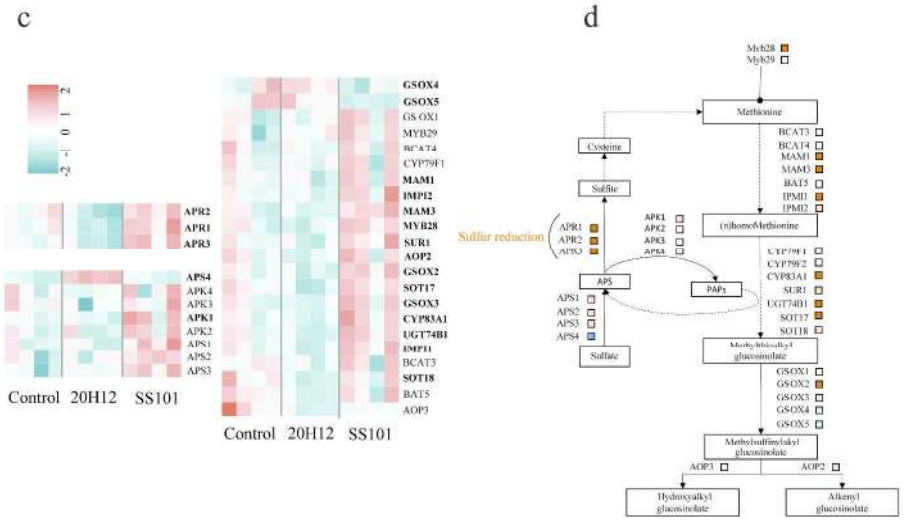


Table 1 Growth promotion of Arabidopsis by *Pf* .SS101.

Conditions	Treatment	Leaves (mg)*	Roots (mg)*
Soil	Control	1120 ± 120[a]	940 ± 200[a]
	SS101	1940 ± 200[b]	2730 ± 530[c]
in vitro	Control	3.95 ± 0.32[d]	7.3 ± 0.00[e]
	SS101	14.6 ± 1.07[f]	41.6 ± 5.70[g]

*Plant growth is expressed as milligrams of plant fresh weight.

± standard error of the mean of at least 35 Arabidopsis seedlings per treatment

Letters represent a significant difference between the means according to One-way ANOVA (Tukey, $P < 0.05$).

Table 2 Arabidopsis tested with known *Pf*.SS101 bacterial traits

		Plant growth promotion		ISR
	Treatment	**Leaves (mg)**	**Roots (mg)**	**Chlorotic leaves (%)**
EXP1	Control	4,0±0,3[a]	7,3± 0.0[a]	79,4 ± 9,7[a]
	Pf.SS101	14,6±1,1[b]	41,6± 5.7[b]	7,8 ± 2.4[b]
	Dead cells	4,5±0,5[a]	8,6 ± 0.9[a]	83,9 ± 4.9[a]
EXP2	Control	4,3 ± 0,7[a]	1,2 ± 0,5[a]	93.7 ± 1,8[a]
	Pf.SS101	7,3 ± 2,1[b]	2,9 ± 0,1[b]	0.0 ± 0,0[b]
	*massA*Δ	7,1 ± 1,2[b]	3,3 ± 1.0[b]	3.3 ± 1,2[b]
EXP3	massA0 *	7,4 ± 1,2[b]	2,5 ± 0,4[c]	89.7 ± 2.0[a]
	massA10*	5,8 ± 1,1[a]	2,5 ± 0,6[c]	94.1 ± 2,4[a]
	massA25*	5,9 ± 0,4[a]	2,1 ± 0,3[d]	85.1 ± 3,6[a]
	massA50*	5,3 ± 0,2[a]	2,5 ± 0,6[c]	84.8 ± 5,3[a]
EXP4	Control	10,6 ± 0,8[a]	3,6 ± 0,5[a]	79,4 ± 9,7[a]
	Pf.SS101	12,3 ± 0,1[a]	5,1 ± 0,5[b]	7,8 ± 2.4[b]
	61C8	10,9 ± 0,4[a]	5,1 ± 0,6[b]	9,4 ± 3.9[b]

Means with different letters indicate significant differences among the treatments according
One way ANOVA analysis (P <0.05).

* indicates different concentrations of the lipopeptide massetolide A in µg $ml^{-1.}$

"±" represents the standard error of the mean of 4 replicates with 10 plants per replicate.

Table 3 Phenotypic and genetic characterization of *Pf.* SS101 and mutants deficient in plant growth promotion and ISR.

Strains	Colonisation Log (cfu mg^{-1}) roots	Locus_tag	Gene	Product	COG[1]	Growth curve	
						KB	SSM
Pf .SS101	5.54 ± 0.04[a]					+	+
7H2; 76G8	4.16 ± 0.03[b]	PflSS101_4580	*ilvC*	ketol-acid reductoisomerase	E (valine, leucine, isoleucine)	+	-
44D8	3.55 ± 0.00[c]	PflSS101_4908	*argC*	N-acetyl-gamma-glutamyl-phosphate reductase	E (arginine)	+	-
24D10; 24B12	4.31 ± 0.07[b]	PflSS101_0919	*hisD*	histidinol dehydrogenase	E (histidine)	+	-
22G5	3.95 ± 0.12[b]	PflSS101_5114	*metX*	homoserine O-acetyltransferase	E (methionine)	+	-
71H9; 74F8	873 ± 114[d]	PflSS101_0035	*trpB*	tryptophan synthase, beta subunit	E (tryptophan)	+	-
13E4; 13H6	2.93 ± 0.00[e]	PflSS101_0355	*hisA*	1-(5-phosphoribosyl)-5-[(5- phosphoribosylamino)methylideneamino]imidazole-4- carboxamide isomerase	E (histidine)	+	-
18F11	4.17 ± 0.09[b]	PflSS101_0437	*gltB*	glutamate synthase, large subunit	E (glutamate)	+	-
24A12; 32H11	4.09 ± 0.30[b]	PflSS101_4919	*trpD*	anthranilate phosphoribosyltransferase	E (tryptophan)	+	-
40H11	4.94 ± 0.06[e]	PflSS101_1161	*argG*	argininosuccinate synthase	E (arginine)	+	-
51G1	4.20 ± 0.15[b]	PflSS101_3512	*metZ*	O-succinylhomoserine sulfhydrylase	E (methionine)	+	-
59B6	3.08 ± 0.10[d]	PflSS101_3523	*leuB*	3-isopropylmalate dehydrogenase	C (valine, leucine, isoleucine)	+	-
9F8	4.36 ± 0.09[b]	PflSS101_3526	*leuC*	3-isopropylmalate dehydratase, large subunit	E (valine, leucine, isoleucine)	+	-
42B9	4.52 ± 0.06[b]	PflSS101_3837	*cysM*	cysteine synthase B	E (cysteine)/sulfur metabolism	+	-
20H12	5.63 ± 0.02[a]	PflSS101_3982	*cysH*	adenylylsulfate reductase, thioredoxin dependent	E (cysteine)/sulfur metabolism	+	-
25C8	5.60 ± 0.03[a]	PflSS101_4354	*edd*	phosphogluconate dehydratase	E (glucose utilization)	+	+
16G6	3.73 ± 0.10[c]	PflSS101_4370	*colR*	DNA-binding response regulator ColR	T	+	+

[1] COG Functional annotation. C = Energy production and conversion; E = Amino acid transport and metabolism; T = Signal transduction mechanisms.(+) = growth; (-) = no growth.
Letters indicate significant differences among the treatments according to One way ANOVA analysis (P<0.05).

Table 4 Growth test of *Pf*. SS101 mutants with plasposon insertion in different amino acid biosynthesis genes

Strains	Locus_tags	Mutated genes	Supplementation of amino acids		
			Amino acids supplemented in cultures	L-	D-
Pf.SS101				+	+
44D8	PflSS101_4908	*argC*	15mM	+	+
24B12	PflSS101_0919	*hisD*	15mM	+	+
71H9	PflSS101_0035	*trpB*	10mM	+	+
13E4	PflSS101_0355	*hisA*	15mM	+	+
18F11	PflSS101_0437	*gltB*	15mM	+	+
24A12	PflSS101_4919	*trpD*	15mM	+	+
40H11	PflSS101_1161	*argG*	15mM	+	+
59B6	PflSS101_3523	*leuB*	15mM	+	+
42B9	PflSS101_3837	*cysM*	0.1mM	+	+
20H12	PflSS101_3982	*cysH*	0.1mM	+	+

Table 5 *Pf.* SS101 genes involved in sulfur transport and assimilation

Locus_tag	Gene	Product	COG[a]
PflSS101_0214	*sbp*	sulfate ABC transporter, periplasmic sulfate-binding protein	P
PflSS101_1778	*sbp2/cysP*	periplasmic sulfate-binding protein	P
PflSS101_0215	*cysT*	sulfate ABC transporter, permease protein CysT	O
PflSS101_0216	*cysW*	sulfate ABC transporter, permease protein CysW	P
PflSS101_0217	*cysA*	sulfate ABC transporter, ATP-binding protein CysA	P
PflSS101_0778	*cysD*	sulfate adenylyltransferase, small subunit	E
PflSS101_0779	*cysC/cysN*	putative sulfate adenylyltransferase, large subunit/adenylylsulfate kinase	P
PflSS101_0300	*cysQ*	3'(2'),5'-bisphosphate nucleotidase	P
PflSS101_2247	*cysI*	sulfite reductase (NADPH) hemoprotein, beta-component	P
PflSS101_3954	*cysJ*	Molybdopterin oxidoreductase	R
PflSS101_3184	*cysG*	siroheme synthase	H
PflSS101_4462	*cysE*	serine O-acetyltransferase	E
PflSS101_3837	*cysM*	cysteine synthase B	E
PflSS101_1560	*cysK*	cysteine synthase A	E
PflSS101_1716	*cysB*	HTH-type transcriptional regulator CysB	K
PflSS101_4454	*iscX*	FeS assembly protein IscX	S
PflSS101_4455	*fdx*	ferredoxin, 2Fe-2S type, ISC system	C
PflSS101_4456	*hscA*	Fe-S protein assembly chaperone HscA	O
PflSS101_4457	*hscB*	Fe-S protein assembly co-chaperone HscB	O
PflSS101_4458	*iscA*	iron-sulfur cluster assembly protein IscA	S
PflSS101_4459	*iscU*	FeS cluster assembly scaffold IscU	C
PflSS101_4460	*iscS*	cysteine desulfurase IscS	E
PflSS101_4461	*iscR*	iron-sulfur cluster assembly transcription factor IscR	K
PflSS101_4462	*cysE*	serine O-acetyltransferase	E
PflSS101_4463	*trmJ*	tRNA (cytidine/uridine-2'-O-)-methyltransferase TrmJ	J

[a] Functional annotation. C = Energy production and conversion; E = Amino acid transport and metabolism;
H = Coenzyme metabolism; J = Translation, ribosomal structure and biogenesis; K = Transcription;
O = Posttranslational modification, protein turnover, chaperones; P = Inorganic ion transport and metabolism
R = General function prediction only; S = Function unknown

Table 6 Gene set enrichment analysis representing significantly enriched biological processes in A.thaliana treated with *Pf* .SS101

Gene Set Name (#*)	Gene orthology (GO)	NO. Genes in Overlap (k)[$]	p value	FDR
CYSTEINE_BIOSYNTHETIC_PROCESS(210)	GO:0019344	34	1.44E-19	1.54E-16
SERINE_FAMILY_AMINO_ACID_BIOSYNTHETIC_PROCESS(222)	GO:0009070	34	6.70E-19	5.72E-16
CARBOHYDRATE_BIOSYNTHETIC_PROCESS(1069)	GO:0016051	71	1.84E-18	1.47E-15
CELLULAR_AMINO_ACID_BIOSYNTHETIC_PROCESS(501)	GO:0008652	48	1.99E-18	1.50E-15
PRIMARY_METABOLIC_PROCESS(11672)	GO:0044238	321	1.91E-15	7.63E-13
SECONDARY_METABOLIC_PROCESS(1241)	GO:0019748	70	8.62E-15	3.35E-12
CARBOXYLIC_ACID_BIOSYNTHETIC_PROCESS(1113)	GO:0046394	65	1.90E-14	6.77E-12
RESPONSE_TO_SALT_STRESS(780)	GO:0009651	52	8.27E-14	2.72E-11
GLYCERALDEHYDE-3-PHOSPHATE_METABOLIC_PROCESS(232)	GO:0019682	28	1.85E-13	5.78E-11
ISOPENTENYL_DIPHOSPHATE_BIOSYNTHETIC_PROCESS,_MEVALONATE-INDEPENDENT_PATHWAY(229)	GO:0019288	27	8.27E-13	2.36E-10
LIPID_BIOSYNTHETIC_PROCESS(1156)	GO:0008610	62	2.65E-12	7.07E-10
PHOSPHOLIPID_BIOSYNTHETIC_PROCESS(403)	GO:0008654	34	5.70E-12	1.46E-09
CATABOLIC_PROCESS(2175)	GO:0009056	91	1.03E-11	2.58E-09
RESPONSE_TO_STRESS(4037)	GO:0006950	139	1.56E-11	3.85E-09
COENZYME_METABOLIC_PROCESS(507)	GO:0006732	37	3.36E-11	7.84E-09
SMALL_MOLECULE_CATABOLIC_PROCESS(931)	GO:0044282	52	4.40E-11	9.41E-09
MONOCARBOXYLIC_ACID_METABOLIC_PROCESS(1480)	GO:0032787	69	5.85E-11	1.23E-08
GLUCOSINOLATE_BIOSYNTHETIC_PROCESS(172)	GO:0019761	21	1.63E-10	3.11E-08
GLUCOSE_CATABOLIC_PROCESS(474)	GO:0006007	34	3.27E-10	5.91E-08
STARCH_BIOSYNTHETIC_PROCESS(191)	GO:0019252	21	9.28E-10	1.51E-07
REGULATION_OF_BIOLOGICAL_QUALITY(1637)	GO:0065008	68	7.85E-09	1.09E-06
PHOTORESPIRATION(161)	GO:0009853	18	1.16E-08	1.57E-06
HETEROCYCLE_METABOLIC_PROCESS(1020)	GO:0046483	49	1.78E-08	2.31E-06
NUCLEOTIDE_METABOLIC_PROCESS(685)	GO:0009117	38	2.39E-08	3.00E-06
TRANSPORT(3558)	GO:0006810	115	4.79E-08	5.68E-06
PENTOSE-PHOSPHATE_SHUNT(200)	GO:0006098	19	5.06E-08	5.86E-06
CALCIUM_ION_TRANSPORT(121)	GO:0006816	15	5.61E-08	6.36E-06
LOCALIZATION(3799)	GO:0051179	119	1.37E-07	1.45E-05
RESPONSE_TO_ORGANIC_SUBSTANCE(2739)	GO:0010033	91	5.29E-07	5.47E-05
ORGANELLE_ORGANIZATION(2037)	GO:0006996	73	6.06E-07	6.22E-05
JASMONIC_ACID_BIOSYNTHETIC_PROCESS(135)	GO:0009695	13	5.68E-06	5.24E-04
GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY(730)	GO:0006091	33	1.34E-05	1.15E-03
CELLULAR_CATION_HOMEOSTASIS(172)	GO:0030003	14	1.49E-05	1.26E-03
STEROID_BIOSYNTHETIC_PROCESS(221)	GO:0006694	16	1.47E-05	1.26E-03
HORMONE_BIOSYNTHETIC_PROCESS(277)	GO:0042446	18	1.74E-05	1.46E-03
GLYCINE_CATABOLIC_PROCESS(53)	GO:0006546	8	2.03E-05	1.69E-03
BIOLOGICAL_REGULATION(6172)	GO:0065007	162	2.32E-05	1.90E-03
RESPONSE_TO_DESICCATION(39)	GO:0009269	7	2.50E-05	2.03E-03
SERINE_FAMILY_AMINO_ACID_CATABOLIC_PROCESS(55)	GO:0009071	8	2.58E-05	2.08E-03
GLUCONEOGENESIS(169)	GO:0006094	13	5.15E-05	3.86E-03
RESPONSE_TO_NEMATODE(82)	GO:0009624	9	6.17E-05	4.55E-03
MICROTUBULE_NUCLEATION(66)	GO:0007020	8	8.37E-05	5.96E-03

RESPONSE_TO_GLUCOSE_STIMULUS(86)	GO:0009749	9	8.65E-05	6.13E-03
INDOLEACETIC_ACID_BIOSYNTHETIC_PROCESS(108)	GO:0009684	10	9.15E-05	6.41E-03
PROTEIN_COMPLEX_ASSEMBLY(577)	GO:0006461	26	1.15E-04	7.90E-03
PROTEIN_COMPLEX_BIOGENESIS(577)	GO:0070271	26	1.15E-04	7.90E-03
UNSATURATED_FATTY_ACID_BIOSYNTHETIC_PROCESS(70)	GO:0006636	8	1.22E-04	8.35E-03
ANTHOCYANIN_ACCUMULATION_IN_TISSUES_IN_RESPONSE_TO_UV_LIGHT(113)	GO:0043481	10	1.29E-04	8.59E-03
CHLOROPHYLL_METABOLIC_PROCESS(188)	GO:0015994	13	1.41E-04	9.26E-03
GLUTAMATE_METABOLIC_PROCESS(22)	GO:0006536	5	1.42E-04	9.29E-03
PROTEOLYSIS(940)	GO:0006508	36	1.48E-04	9.60E-03
RESPONSE_TO_BIOTIC_STIMULUS(1675)	GO:0009607	55	1.56E-04	1.01E-02
RESPONSE_TO_LIGHT_STIMULUS(1182)	GO:0009416	42	2.02E-04	1.29E-02
FLAVONOID_BIOSYNTHETIC_PROCESS(225)	GO:0009813	14	2.23E-04	1.41E-02
CELL_WALL_ORGANIZATION(609)	GO:0071555	26	2.57E-04	1.56E-02
ORGAN_DEVELOPMENT(2037)	GO:0048513	63	2.56E-04	1.56E-02
TRYPTOPHAN_CATABOLIC_PROCESS(79)	GO:0006569	8	2.61E-04	1.57E-02
PROTEASOME_CORE_COMPLEX_ASSEMBLY(126)	GO:0080129	10	2.94E-04	1.75E-02
CELLULAR_RESPONSE_TO_STIMULUS(2302)	GO:0051716	69	3.06E-04	1.80E-02
DEVELOPMENTAL_GROWTH(781)	GO:0048589	30	4.94E-04	2.78E-02
GLYCOLYSIS(219)	GO:0006096	13	5.63E-04	3.14E-02
RESPONSE_TO_CADMIUM_ION(470)	GO:0046686	21	5.63E-04	3.14E-02
ORGANIC_ANION_TRANSPORT(17)	GO:0015711	4	6.07E-04	3.34E-02
THYLAKOID_MEMBRANE_ORGANIZATION(198)	GO:0010027	12	7.65E-04	4.10E-02
RESPONSE_TO_COLD(622)	GO:0009409	25	7.74E-04	4.14E-02
AUXIN_POLAR_TRANSPORT(95)	GO:0009926	8	8.16E-04	4.34E-02
MERISTEM_GROWTH(173)	GO:0035266	11	8.67E-04	4.57E-02
PROTON_TRANSPORT(147)	GO:0015992	10	9.09E-04	4.76E-02

* represents numberber of genes in Arabidopsis genome belonging to the indicated biological process.

$ represents number of genes in the selected cluster (cluster II, Fig.6) belonging to the indicated biological process.

Table 7 Gene set enrichment analysis representing significantly enriched biological processes in A.thaliana treated with *CysH* mutant 20H12

Gene Set Name (#*)	Gene orthology (GO)	NO. Genes in Overlap (k)[$]	p value	FDR
RESPONSE_TO_CHITIN(421)	GO:0010200	168	2.00E-119	2.60E-115
INNATE_IMMUNE_RESPONSE(926)	GO:0045087	174	8.70E-79	2.23E-75
RESPONSE_TO_STRESS(4037)	GO:0006950	326	3.79E-65	5.40E-62
RESPONSE_TO_CHEMICAL_STIMULUS(3953)	GO:0042221	317	2.85E-62	3.66E-59
REGULATION_OF_DEFENSE_RESPONSE(529)	GO:0031347	119	2.00E-60	2.32E-57
HOST_PROGRAMMED_CELL_DEATH_INDUCED_BY_SYMBIONT(402)	GO:0034050	100	4.13E-54	3.53E-51
CELLULAR_RESPONSE_TO_CHEMICAL_STIMULUS(1403)	GO:0070887	168	9.89E-51	4.53E-48
RESPONSE_TO_OTHER_ORGANISM(1411)	GO:0051707	160	2.04E-45	8.42E-43
RESPIRATORY_BURST_INVOLVED_IN_DEFENSE_RESPONSE(121)	GO:0002679	58	1.98E-44	7.70E-42
SIGNAL_TRANSDUCTION(1659)	GO:0007165	170	4.86E-43	1.78E-40
SALICYLIC_ACID_MEDIATED_SIGNALING_PATHWAY(349)	GO:0009863	81	6.21E-42	2.15E-39
ENDOPLASMIC_RETICULUM_UNFOLDED_PROTEIN_RESPONSE(184)	GO:0030968	61	3.75E-39	1.12E-36
NEGATIVE_REGULATION_OF_PROGRAMMED_CELL_DEATH(170)	GO:0043069	59	8.18E-39	2.18E-36
DEFENSE_RESPONSE_TO_FUNGUS(342)	GO:0050832	75	2.01E-37	4.87E-35
SALICYLIC_ACID_BIOSYNTHETIC_PROCESS(209)	GO:0009697	61	1.63E-36	3.80E-34
NEGATIVE_REGULATION_OF_DEFENSE_RESPONSE(273)	GO:0031348	67	4.54E-36	1.00E-33
SYSTEMIC_ACQUIRED_RESISTANCE(444)	GO:0009627	81	2.43E-35	5.28E-33
RESPONSE_TO_WOUNDING(340)	GO:0009611	69	1.12E-32	2.32E-30
REGULATION_OF_CELLULAR_PROCESS(4571)	GO:0050794	279	2.47E-32	4.95E-30
JASMONIC_ACID_MEDIATED_SIGNALING_PATHWAY(282)	GO:0009867	60	1.72E-29	3.06E-27
INTRACELLULAR_TRANSPORT(1422)	GO:0046907	132	3.61E-29	6.34E-27
INTRACELLULAR_PROTEIN_TRANSPORT(1043)	GO:0006886	109	6.62E-28	1.06E-25
CELLULAR_MEMBRANE_FUSION(275)	GO:0006944	57	1.45E-27	2.24E-25
MAPK_CASCADE(209)	GO:0000165	49	6.79E-26	9.66E-24
HORMONE-MEDIATED_SIGNALING_PATHWAY(589)	GO:0009755	75	5.89E-24	7.86E-22
DEFENSE_RESPONSE_BY_CALLOSE_DEPOSITION(62)	GO:0052542	30	1.17E-23	1.55E-21
ABSCISIC_ACID_MEDIATED_SIGNALING_PATHWAY(252)	GO:0009738	50	1.39E-23	1.82E-21
PROTEIN_PHOSPHORYLATION(1135)	GO:0006468	105	4.41E-23	5.60E-21
RESPONSE_TO_HORMONE_STIMULUS(1364)	GO:0009725	115	3.31E-22	4.08E-20
CELLULAR_PROTEIN_MODIFICATION_PROCESS(2764)	GO:0006464	174	2.15E-20	2.51E-18
CARBOXYLIC_ACID_BIOSYNTHETIC_PROCESS(1113)	GO:0046394	98	3.53E-20	4.04E-18

CELLULAR_MODIFIED_AMINO_ACID_BIOSYNTHETIC_PROCESS(532)	GO:0042398	62	2.96E-18	2.99E-16
DEFENSE_RESPONSE_TO_BACTERIUM(390)	GO:0042742	50	1.94E-16	1.85E-14
REGULATION_OF_MULTI-ORGANISM_PROCESS(117)	GO:0043900	27	1.02E-14	9.36E-13
OXYGEN_AND_REACTIVE_OXYGEN_SPECIES_METABOLIC_PROCESS(347)	GO:0006800	42	2.94E-13	2.67E-11
METHIONINE_METABOLIC_PROCESS(236)	GO:0006555	33	3.27E-12	2.89E-10
PRIMARY_METABOLIC_PROCESS(11672)	GO:0044238	447	1.23E-11	1.08E-09
POSITIVE_REGULATION_OF_FLAVONOID_BIOSYNTHETIC_PROCESS(103)	GO:0009963	21	6.98E-11	6.04E-09
RESPONSE_TO_COLD(622)	GO:0009409	52	1.92E-10	1.63E-08
HYPEROSMOTIC_SALINITY_RESPONSE(161)	GO:0042538	25	1.96E-10	1.65E-08
RESPONSE_TO_SALT_STRESS(780)	GO:0009651	59	4.24E-10	3.50E-08
JASMONIC_ACID_BIOSYNTHETIC_PROCESS(135)	GO:0009695	22	1.07E-09	8.73E-08
RESPONSE_TO_ABSENCE_OF_LIGHT(34)	GO:0009646	12	5.06E-09	4.03E-07
RESPONSE_TO_WATER_DEPRIVATION(413)	GO:0009414	37	1.58E-08	1.22E-06
RESPONSE_TO_AUXIN_STIMULUS(425)	GO:0009733	37	3.14E-08	2.38E-06
ETHYLENE_MEDIATED_SIGNALING_PATHWAY(125)	GO:0009873	19	3.94E-08	2.97E-06
FATTY_ACID_BIOSYNTHETIC_PROCESS(303)	GO:0006633	30	4.90E-08	3.67E-06
CELLULAR_BIOSYNTHETIC_PROCESS(7714)	GO:0044249	296	6.34E-07	4.51E-05
GOLGI_VESICLE_TRANSPORT(327)	GO:0048193	29	6.77E-07	4.79E-05
REGULATION_OF_PRIMARY_METABOLIC_PROCESS(2751)	GO:0080090	126	1.64E-06	1.11E-04
PROTEIN_LIPIDATION(550)	GO:0006497	39	1.79E-06	1.19E-04
CARBOXYLIC_ACID_TRANSPORT(283)	GO:0046942	24	1.21E-05	7.65E-04
RESPONSE_TO_HEAT(303)	GO:0009408	25	1.24E-05	7.80E-04
INDOLE_GLUCOSINOLATE_METABOLIC_PROCESS(14)	GO:0042343	6	1.58E-05	9.86E-04
TRANSMEMBRANE_RECEPTOR_PROTEIN_TYROSINE_KINASE_SIGNALING_PATHWAY(130)	GO:0007169	15	2.16E-05	1.31E-03
POSITIVE_REGULATION_OF_CELLULAR_BIOSYNTHETIC_PROCESS(595)	GO:0031328	36	1.02E-04	5.94E-03
ER_TO_GOLGI_VESICLE-MEDIATED_TRANSPORT(105)	GO:0006888	12	1.53E-04	8.73E-03
PROLINE_TRANSPORT(74)	GO:0015824	10	1.57E-04	8.89E-03
PROTEIN_DEPHOSPHORYLATION(215)	GO:0006470	18	1.67E-04	9.41E-03
VESICLE_DOCKING_INVOLVED_IN_EXOCYTOSIS(35)	GO:0006904	7	1.86E-04	1.03E-02
PURINE_RIBONUCLEOSIDE_TRIPHOSPHATE_CATABOLIC_PROCESS(96)	GO:0009207	11	2.79E-04	1.46E-02
AMMONIUM_TRANSPORT(29)	GO:0015696	6	4.58E-04	2.32E-02
REGULATION_OF_RNA_METABOLIC_PROCESS(2384)	GO:0051252	100	4.92E-04	2.47E-02
CELLULAR_AMINO_ACID_METABOLIC_PROCESS(880)	GO:0006520	45	5.45E-04	2.71E-02

PURINE_NUCLEOSIDE_TRIPHOSPHATE_METABOLIC_PROCESS(163)	GO:0009144	14	6.77E-04	3.31E-02
CELLULAR_DEFENSE_RESPONSE(4)	GO:0006968	3	7.76E-04	3.71E-02
MACROMOLECULE_METABOLIC_PROCESS(9200)	GO:0043170	319	8.23E-04	3.89E-02

* represents numberber of genes in Arabidopsis genome belonging to the indicated biological process.

$ represents number of genes in the selected cluster (cluster VI, Fig.6b) belonging to the indicated biological process.