**Transcriptomic response of Ralstonia solanacearum to antimicrobial Pseudomonas fluorescens SN15-2 metabolites**

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**Pseudomonas fluorescens**

*SN15-2 metabolites*

**IC 50**

**Ralstonia solanacearum**
Transcriptomic response of *Ralstonia solanacearum* to antimicrobial *Pseudomonas fluorescens* SN15-2 metabolites


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ABSTRACT

To develop efficient biocontrol agents, it is essential to investigate the response of soil-borne plant pathogens to such agents. For example, the response of Ralstonia solanacearum, the tomato wilt pathogen, to antimicrobial metabolites of Pseudomonas fluorescens is unknown. Thus, we assessed the effects of P. fluorescens SN15-2 fermentation broth on R. solanacearum by transmission electron microscopy and transcriptome technology. RNA sequencing identified 109 and 155 genes that are significantly up-regulated and down-regulated, respectively, in response to P. fluorescens metabolites, many of which are associated with the cell membrane and cell wall, and with nucleotide acid metabolism, iron absorption, and response to oxidative stress. This study highlights the effectiveness of P. fluorescens metabolites against the tomato wilt pathogen, and helps clarify the underlying molecular mechanisms.

Keywords:
Pseudomonas fluorescens metabolites
Ralstonia solanacearum
R. solanacearum morphology
Transcriptome
Introduction

*Ralstonia solanacearum* is a devastating soil-borne pathogen that infects more than 200 different plant species in 53 taxonomic families (Hayward 1991). The pathogen colonizes the root and invades the xylem to rapidly migrate to the shoot. However, exopolysaccharides produced by *R. solanacearum* in the xylem block water flow, and eventually result in wilting and plant death.

Different approaches to control *R. solanacearum* wilt disease, including modification of cultural practices, use of resistant plant varieties, crop rotation, and application of agrochemicals, have limited success (Li et al. 2016; Raza et al. 2016b). Hence, environmentally friendly biological control strategies are an attractive alternative. Of known biocontrol agents, *Pseudomonas fluorescens* is one of the most important, and has been shown to be active against an array of soil-borne plant pathogens (Ganeshan and Manoj Kumar 2005). For example, three *P. fluorescens* strains were shown to prevent damping-off in alfalfa (Quagliotto et al. 2009). *P. fluorescens* Pf4 also inhibits *Aspergillus niger*, which causes wilting, yellowing, and plant death (Yunus et al. 2016). *P. fluorescens* was also shown to control bacterial wilt disease due to *R. solanacearum* (Rao et al. 2015).

However, understanding the underlying molecular mechanisms is essential to develop commercially successful biocontrol strategies against *R. solanacearum*. In *P. fluorescens*, biocontrol mechanisms include production of antibiotics and volatile organic compounds, competition for space and nutrients, effective root colonization, and induction of systemic resistance (Haas and Defago 2005; Raza et al. 2016a). Although the universal activities of *P. fluorescens* against soil-borne pathogens have been widely studied, specific mechanisms
against *R. solanacearum* are not as well-characterized. For example, the PhlF^- mutant of *P. fluorescens* J2, which produces 2,4-diacetylphloroglucinol (2,4-DAPG) more abundantly than wild type, is also more efficient against *R. solanacearum* (Zhou et al. 2014). Similarly, volatile organic compounds produced by *P. fluorescens* WR-1 significantly inhibit growth and virulence in *R. solanacearum* (Raza et al. 2016a). Of note, the antibacterial molecules in *P. fluorescens* Pf11, *P. fluorescens* Pf16 and *P. fluorescens* Pp23 against *R. solanacearum* are natural products of their own metabolism (Kheirandish and Harighi 2015). Nevertheless, the response of *R. solanacearum* to antimicrobial *P. fluorescens* metabolites is unknown.

Therefore, we analysed, by transmission electron microscopy and transcriptome technology, the effects of metabolites from *P. fluorescens* SN15-2 on the growth and metabolism of *R. solanacearum*. The data collected lay the foundation for effective application of *P. fluorescens* against *R. solanacearum*, and may promote wide agronomic use of biological control against soil-borne plant pathogens.

**Materials and methods**

**Bacterial strains and culture conditions**

*P. fluorescens* SN15-2 was isolated from tomato root, and cultured in King's B (KB) medium (King et al. 1954) at 28 °C. *R. solanacearum* 3-1 was obtained from Dr. Tao Li (Institute of Vegetables, Guangdong Academy of Agricultural Sciences), and cultured in nutrient agar (NA) medium (Ge et al. 2017) at 30 °C.

**Calculation of half maximal inhibitory concentration**
Half maximal inhibitory concentration was calculated according to previous methodology (Yang et al. 2016). \textit{P. fluorescens} SN15-2 in logarithmic phase (optical density ~1 at 600 nm) was inoculated at 1 % v/v into 50 mL fresh KB medium, and grown in 250 ml flask at 28 °C on a rotary shaker set at 200 rpm for 48 h, at which point the fermentation broth was collected and sterile-filtered. \textit{R. solanacearum} in logarithmic phase (optical density ~0.8 at 600 nm) was inoculated at 1 % v/v into 50 mL fresh NA medium containing 0 % (control), 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, and 40 % v/v sterile fermentation broth from \textit{P. fluorescens} SN15-2, and grown in 250 ml flask at 30 °C on a rotary shaker set at 200 rpm for 12 h, at which time the optical density was measured. Inhibitory rate (%) was calculated as \((A_0 - A_1)/A_0 \times 100\), where \(A_0\) represents the corrected optical density of the control culture at 600 nm and \(A_1\) represents the corrected optical density of test cultures at 600 nm. Inhibitory rates at different concentrations of sterile \textit{P. fluorescens} fermentation broth are listed in Table S1, and plotted in Fig. 1. Data were fit with \(R^2\) 0.9534 to a linear function of equation \(Y = 1.3917X + 17.4\), and the half-maximal inhibitory concentration was calculated to be 23.4 %.

\textit{Effect of P. fluorescens SN15-2 metabolites on \textit{R. solanacearum} morphology}

\textit{R. solanacearum} in logarithmic phase (optical density ~0.8 at 600 nm) was inoculated at 1 % v/v into 50 mL fresh NA medium containing 0 % (control) or 23.4 % v/v \textit{P. fluorescens} SN15-2 48 h sterile fermentation broth, and grown in 250 ml flask at 30 °C on a rotary shaker set at 200 rpm for 12 h. Ultrathin sectioning sample preparation of \textit{R. solanacearum} was then implemented according to previous method (Dinh et al. 2015). Bacterial morphology was examined on a transmission electron microscope.
RNA isolation and sequencing

*R. solanacearum* in logarithmic phase (optical density ~0.8 at 600 nm) was inoculated at 1 % v/v into 50 mL fresh NA medium containing 0 % (control) or 23.4 % v/v *P. fluorescens* SN15-2 48 h sterile fermentation broth, and grown in 250ml flask at 30 °C on a rotary shaker set at 200 rpm for 12 h. Cultures were then harvested by centrifugation, and total RNA was extracted using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA quality and integrity were assessed on a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). For mRNA sequencing, samples were processed with Ribol-zerO rRNA Removal Kit (Illumina, San Diego, CA, USA), and reverse transcribed to first-strand cDNA using random oligonucleotides and SuperScript III. Second-strand cDNA was then synthesized using DNA Polymerase I and RNase H. Remaining overhangs were blunted using T4 DNA polymerase with exonuclease activity, which was then removed by agarose gel electrophoresis. After adenylation of 3’ ends, DNA fragments were ligated to Illumina PE adapter oligonucleotides. The library was then purified using AMPure XP system (Beckman Coulter, Beverly, CA, USA) to select cDNA fragments of the preferred 300 bp length. Finally, DNA fragments with adapter molecules at both ends were selectively enriched over 15 cycles of PCR with Illumina PCR Primer Cocktail, purified (AMPure XP system), quantified by Agilent High-Sensitivity DNA Assay on a Bioanalyzer 2100 system (Agilent), and sequenced on an Illumina Hiseq platform at Shanghai Personal Biotechnology Co. Ltd.
Analysis of transcriptome data

Raw sequencing data are deposited in the National Center for Biotechnology Information Sequence Read Archive under accession number SRP124795. Raw reads were filtered to remove low-quality and chimeric sequences. Paired-end clean reads of high quality were then aligned to the *R. solanacearum* reference genome (GMI1000) in Bowtie 2. Transcript levels were estimated as RPKM (Mortazavi et al. 2008). Differential expression from exposure to *P. fluorescens* SN15-2 fermentation broth was assessed in DESeq (Anders and Huber 2010). *P* values were adjusted by Benjamini and Hochberg’s method for controlling false discovery rate (FDR) (Yan et al. 2017). Genes with \(|\log_2 \text{fold change}| > 1\), adjusted *P* value < 0.05, and FDR < 0.05 were considered differentially expressed.

qRT-PCR

Differential expression of six genes were confirmed by qRT-PCR using gene-specific primers designed in Primer 5 and listed in Table S2. Targets were amplified over one cycle at 95 °C for 5 min and 40 cycles at 95 °C for 15 s and 60 °C for 30 s, followed by high-resolution melting curve analysis. Targets were normalised to DNA gyrase subunit B (gyrB), and analysed using the comparative critical threshold (CCT) method (Zhang et al. 2014). The qRT-PCR experiments were performed in biological triplicate with three technical replicates.

Determination of nucleotide acid content

*R. solanacearum* in logarithmic phase (optical density ~0.8 at 600 nm) was inoculated at
1 % v/v into 50 mL fresh NA medium containing 0 % (control) or 23.4 % v/v *P. fluorescens*
SN15-2 48 h sterile fermentation broth, and grown in 250ml flask at 30 °C on a rotary shaker
set at 200 rpm for 12 h. Cultures were then harvested by centrifugation, and optical density
values at 600 nm were adjusted to 1 by phosphate buffer solution. 1ml *R. solanacearum* cells
(optical density =1 at 600 nm) were used to extract DNA or RNA. DNA was extracted using
genomic DNA extraction kit (Axygen, Inc., USA), and total RNA was extracted using Trizol
Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The DNA and RNA
concentrations were determined by biospectrometer (eppendorf, Hamburg, Germany).

**Results**

**Effect of *P. fluorescens* SN15-2 fermentation broth on *R. solanacearum* morphology**

Ultrathin section transmission electron microscopy was utilized to detect effect of *P.
fluorescens* SN15-2 fermentation broth on *R. solanacearum* cells ultrastructure. On
transmission electron microscopy, untreated *R. solanacearum* cells presented intact cell walls
and membranes and plump cytoplasm ([Fig. 2](#)). In contrast, cells exposed to *P. fluorescens*
SN15-2 fermentation broth were deformed, and presented incomplete cell walls and
membranes and uneven cytoplasm. These results indicate that *P. fluorescens* SN15-2
metabolites destroyed the *R. solanacearum* cell walls and membranes and caused loss of the *R.
solanacearum* cytoplasm.

**Differentially expressed genes**

Transcriptomic analyses were utilized to investigate the global effect of *P. fluorescens*
SN15-2 metabolites on *R. solanacearum* at the molecular level. Transcriptome sequencing produced total 89 million clean reads for untreated samples (three biological replicates) and total 86 million clean reads for samples exposed to *P. fluorescens* SN15-2 fermentation broth (three biological replicates). RNA-Seq correlation analysis of all samples was shown in Fig. S1. RNA sequencing identified 264 genes that are significantly different expression after treatment with *P. fluorescens* SN15-2 fermentation broth at the half-maximal inhibitory concentration for 12 h.

A volcano plot, shown in Fig. 3, was constructed to visualize the relationship between q-value (ordinate) and log₂ fold change (abscissa) in expression of *R. solanacearum* genes following exposure to *P. fluorescens* SN15-2 fermentation broth. Fold change represents gene expression difference multiple; q-value represents adjusted *P*-value, and larger q-value indicates a more significant difference. Genes with |log₂ fold change| > 1 and q-value < 0.05 are considered differentially expressed. In this plot, genes in blue are differentially expressed in response to *P. fluorescens* metabolites, while genes in red are not. Blue points on the right are significantly up-regulated, while blue points on the left are significantly down-regulated. In total, 109 and 155 *R. solanacearum* genes were significantly up-regulated and down-regulated, respectively, in response to *P. fluorescens* SN15-2 metabolites.

An MA plot, depicting the relationship between transcript levels (abscissa) and differential gene expression (ordinate), is shown in Fig. 4. As in Fig. 3, blue points are genes that are differentially expressed in response to *P. fluorescens* SN15-2 fermentation broth, and red points are not. The plot shows that differential expression did not correlate with transcript levels.
qRT-PCR validation

To confirm differential expression, six genes sensitive to *P. fluorescens* SN15-2 metabolites were analysed by qRT-PCR. As shown in Fig. 5, qRT-PCR results were consistent with that of RNA sequencing, implying that results from the latter are reliable.

Determination of nucleotide acid content

The DNA and RNA concentrations of *R. solanacearum* cells exposed to *P. fluorescens* SN15-2 fermentation broth were lower than untreated *R. solanacearum* cells (Fig. 6). These results indicate that *P. fluorescens* SN15-2 metabolites can inhibit DNA and RNA of *R. solanacearum* synthesis.

Discussion

In this study, the half-maximal inhibitory concentration of metabolites from *P. fluorescens* SN15-2 cultured in KB medium at 28 °C for 48 h was determined to be 23.4 % v/v against *R. solanacearum*. *P. fluorescens* metabolites appear to damage the *R. solanacearum* cell wall, as assessed by transmission electron microscopy. Underlying molecular mechanisms were then investigated by RNA sequencing. Accordingly, 264 *R. solanacearum* genes were found to be sensitive to *P. fluorescens* metabolites, of which 18 (Table 1) were selected for further discussion based on their association with *R. solanacearum* survival and replication.
The cell membrane is a critical foundation of cellular structure. Cell membrane not only stabilizes a stable physical and chemical environment within cells, but also mediates transport, energy conversion, and signal transduction between cells or between cells and the environment. In *R. solanacearum* exposed to *P. fluorescens* metabolites, RSc0803, which encodes a putative membrane-bound serine protease of the Clpp class, was significantly upregulated. It is possible that this up-regulation is due to oxidative damage of the cell membrane, since erythrocyte membrane proteins were found to be vulnerable to degradation by membrane-bound serine protease after oxidative membrane damage (Fujino et al. 1998). RSc2727, which encodes a multidrug resistance transmembrane protein, was similarly up-regulated. Multidrug resistance transmembrane proteins are integral membrane proteins (Rockwell 2013) that function as efflux pumps to confer resistance against toxic substances (Keppler 2011). In addition, it is possible that up-regulation of these proteins compensates for the loss of other integral membrane proteins such as RSc0233, which was significantly down-regulated. Of note, roughly 30% of all bacterial genes are integral membrane proteins (Musatov and Sedlak 2017), and function as transporters, receptors, channels, and enzymes that regulate various cellular processes, including signal transduction and cell-cell and cell-environment interactions (Vit and Petrak 2017). Thus, downregulation of integral membrane proteins may perturb normal physiology, and may directly or indirectly affect cell growth and even cause cell death.
The cell wall is a thick, tough outer layer that maintains cell shape, protects against injury, and contains murein in gram-negative bacteria. Murein, also known as peptidoglycan, is the main component of bacterial cell wall. Notably, RSc2669, which encodes a putative transmembrane effector of murein hydrolase, was significantly up-regulated. Higher expression level will have higher activity for murein hydrolase. Murein hydrolases are potentially autolytic enzymes that elicit bacteriolysis (Höltje 1995). In contrast, RSc0773, which encodes a putative outer membrane biogenesis transmembrane protein, was significantly down-regulated. The outer membrane is the outermost layer of the cell wall, and contains lipopolysaccharides, phospholipids, and various proteins. The outer membrane is unique to gram-negative bacteria, forms a permeability barrier against harsh environments, and enables bacteria to adapt to different conditions (Dong et al. 2017). Therefore, suppression of outer membrane biogenesis may damage cell wall integrity and lower bacterial viability. RSc1987, which encodes aspartate-semialdehyde dehydrogenase, was also significantly down-regulated. Aspartate-semialdehyde dehydrogenase is a key enzyme in the biosynthesis of lysine, threonine, methionine, and diaminopimelic acid, which is the main component of the gram-negative cell wall. Indeed, mutation of the enzyme abrogates synthesis of diaminopimelic acid, resulting in cell lysis and death (Schleifer and Kandler 1972).

Genes encoding proteins associated with nucleotide acid metabolism

Nucleotides are essential components of DNA and RNA, of which the latter is the basis for protein synthesis, while the former is necessary for cell growth and division. Of note,
RSc1451, which encodes adenosine deaminase, was significantly up-regulated. The enzyme is essential for adenine catabolism. On the other hand, RSc1722 and RSc2454, which encode phosphoribosylformylglycinamidine synthase and phosphoribosylglycinamide formyltransferase, respectively, were significantly down-regulated. Both enzymes are required to synthesize of purine nucleotides. Collectively, enhanced adenine catabolism and repressed purine nucleotide anabolism may suppress cell proliferation by inhibiting DNA and RNA synthesis which is consistent with result of nucleotide acid content determination. Indeed, genistein was previously reported to suppress *Vibrio harveyi* by inhibiting DNA and RNA synthesis (Ulanowska et al. 2006).

**Genes encoding proteins associated with iron absorption**

Bacteria have evolved numerous mechanisms to compete for and acquire iron, an essential micronutrient for most life forms (Ellermann and Arthur 2017). For example, *P. fluorescens* expresses a siderophore, a small high-affinity iron chelator (Zhang et al. 2017a). Accordingly, siderophore secreted by *P. fluorescens* SN15-2 may impact iron absorption in *R. solanacearum* by chelating Fe$^{3+}$. In response, *R. solanacearum* up-regulates RSp0100, a ferrisiderophore receptor, as well as RSp0417, which encodes the putative siderophore biosynthesis protein SbnA. These responses may help relieve iron stress in *R. solanacearum* exposed to *P. fluorescens* metabolites.

**Genes encoding proteins associated with heat shock**

The stress response in most bacteria is believed to depend on small heat-shock proteins...
such as RSc0200, which was significantly up-regulated. RSc0374, which encodes RNA polymerase factor sigma-32, was also significantly up-regulated, presumably because this RNA polymerase subunit controls the heat shock response (Arsène et al. 2000). For example, up-regulation of RNA polymerase factor sigma-32 may also up-regulate small heat-shock protein. As small heat shock protein was reported to protect the cell membrane against stress (Balogi et al. 2008), its upregulation may prevent cell membrane damage in *R. solanacearum*.

*Genes encoding proteins associated with universal stress response*

Bacterial pathogens have also evolved highly sophisticated mechanisms to sense and respond to external stresses by altering gene expression patterns (Chowdhury et al. 1996). For example, most cells express universal stress proteins to survive environmental stress (O'Connor and McClean 2017). However, RSc1359 and RSc1504, which encode putative universal stress proteins, were significantly down-regulated, possibly due to extreme toxicity from *P. fluorescens* SN15-2. Indeed, extreme stress was reported to suppress production of universal stress proteins (Bangera et al. 2015).

*Genes encoding proteins associated with response to oxidative stress*

Stressors such as antibiotics, acid, or heat may cause cell death via a general mechanism mediated by secondary oxidative stresses (Kohanski et al. 2007; Mols and Abee 2011; Mols et al. 2010). For instance, oxidants may cause damage to DNA, lipids, and proteins (Akaike et al. 1992). Cell membrane lipids are also major targets of peroxidation (Oliveira et...
al. 2006), a process that thus degrades the integrity of the cell membrane and of
membrane-bound proteins (Richter 1987). Hence, bacteria have evolved several complex
mechanisms to respond to oxidative stress (Oliveira et al. 2006). Accordingly, organic
hydroperoxide resistance protein, encoded by RSp0326, and DNA protection during
starvation or oxidative stress transcription regulator, encoded by RSc2687, were significantly
up-regulated. The former reduces peroxides, while the latter protects DNA from DNase and
oxidative damage (Poole 2005). In addition, putative choline dehydrogenase and related
flavoprotein oxidoreductase, encoded by RSc0123, was significantly upregulated, presumably
to enhance synthesis of glycine betaine, a very common osmoprotectant (Boncompagniet al.
1999). Indeed, glycine betaine was reported to enhance oxidative stress tolerance in
_Pichi caribbica_ (Zhang et al. 2017b), and may also do so in _R. solanacearum_.

**Conclusion**

In this study, we investigated the molecular mechanisms underlying the inhibitory
activity of _P. fluorescens_ SN15-2 against _R. solanacearum_. Transcriptome sequencing
revealed that 264 genes in the latter were differentially expressed in response to the former.
These changes in expression suggest that _P. fluorescens_ metabolites damage the cell
membrane and cell wall in _R. solanacearum_, inhibit biosynthesis of the cell membrane, cell
wall, and nucleic acids, but stimulate catabolism of nucleic acids. On the other hand, genes
encoding small heat shock protein, organic hydroperoxide resistance protein, DNA protection
during starvation or oxidative stress transcription regulator, and putative choline
dehydrogenase and related flavoproteins oxidoreductase were up-regulated, presumably to
mitigate damage to the cell membrane and cell wall. Gene encoding multidrug resistance transmembrane protein was also up-regulated, presumably to promote efflux of harmful substances.

In general, *P. fluorescens* SN15-2 not only destroyed *R. solanacearum* cell walls and membranes, but suppressed *R. solanacearum* by inhibiting DNA and RNA synthesis though some resistance mechanisms of *R. solanacearum* were stimulated. Our study provides new insight into the underlying mechanisms of *P. fluorescens* against *R. solanacearum*.

**Author contributions**

Wei Wang, Haibo Lou, and Xiaobing Wang designed experiments. Haibo Lou performed experiments. Haibo Lou, Bozhi Wang and Xiaobing Wang analysed data. Wei Wang, Haibo Lou, and Jun Chen prepared the manuscript.

**Acknowledgments**

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**Compliance with ethical standards**

**Conflict of interest**

The authors declared that they have no conflicts of interest related to this work.
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Table 1

*R. solanacearum* genes associated with the cell membrane and cell wall, and with nucleotide acid metabolism, iron absorption, heat shock, universal stress response, and response to oxidative stress, and that are differentially expressed following exposure to *P. fluorescens*

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<td></td>
<td>RSc2727</td>
<td>Multidrug resistance transmembrane protein</td>
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<td>Involved in cell wall</td>
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<td></td>
<td>RSc0773</td>
<td>Putative outer membrane biogenesis transmembrane protein</td>
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<td></td>
<td>RSc1987</td>
<td>Aspartate-semialdehyde dehydrogenase</td>
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<td>Involved in nucleotide acid metabolism</td>
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<td>RSc2454</td>
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<td>RSc0123</td>
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Fig. 1. Linear regression analysis of the activity of *P. fluorescens* SN15-2 48 h sterile fermentation broth against *R. solanacearum*. X is *P. fluorescens* SN15-2 48 h sterile fermentation broth concentration (V/V) %, and Y is growth inhibitory rate of *R. solanacearum* %.

Fig. 2. Effect of *P. fluorescens* SN15-2 metabolites on *R. solanacearum* morphology. Left, control; right, cells exposed to *P. fluorescens* SN15-2 metabolites at the half-maximal inhibitory concentration of 23.4 % v/v. Red arrows indicate damaged *R. solanacearum* cell wall and membrane exposed to *P. fluorescens* SN15-2 metabolites.

Fig. 3. A volcano plot of the relationship between q-value and log2 fold change. Red points are genes expressed at the same level in *R. solanacearum* cultured in the presence or absence of *P. fluorescens* metabolites. Blue points are genes differentially expressed following exposure to *P. fluorescens* SN15-2 fermentation broth.

Fig. 4. MA plot depicting the relationship between transcript levels and differential expression. Blue points are genes differentially expressed following exposure to *P. fluorescens* SN15-2 fermentation broth, while red points are not.

Fig. 5. qRT-PCR validation of differential expression. The relative expression level is average value of biological triplicate with three technical replicates. Blue column represents untreated *R. solanacearum*, and red column represents *R. solanacearum* exposure to *P. fluorescens*.
SN15-2 fermentation broth.*, $P < 0.05$; **, $P < 0.01$.

**Fig. 6.** Effect of *P. fluorescens* SN15-2 metabolites on *R. solanacearum* DNA and RNA synthesis. Left, DNA; right, RNA. CG represents untreated *R. solanacearum*; EG represents *R. solanacearum* exposed to *P. fluorescens* SN15-2 metabolites.
Table 1

*R. solanacearum* genes associated with the cell membrane and cell wall, and with nucleotide acid metabolism, iron absorption, heat shock, universal stress response, and response to oxidative stress, and that are differentially expressed following exposure to *P. fluorescens* metabolites.

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Locus tag</th>
<th>Annotation</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involved in cell membrane</td>
<td>RSc0803</td>
<td>Putative membrane-bound serine protease (Clpp class)</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>RSc0233</td>
<td>Integral membrane protein</td>
<td>-90.17</td>
</tr>
<tr>
<td></td>
<td>RSc2727</td>
<td>Multidrug resistance transmembrane protein</td>
<td>4.30</td>
</tr>
<tr>
<td>Involved in cell wall</td>
<td>RSc2669</td>
<td>Putative transmembrane effector of murein hydrolase</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td>RSe0773</td>
<td>Putative outer membrane biogenesis transmembrane protein</td>
<td>-2.00</td>
</tr>
<tr>
<td></td>
<td>RSc1987</td>
<td>Aspartate-semialdehyde dehydrogenase</td>
<td>-2.27</td>
</tr>
<tr>
<td>Involved in nucleotide acid metabolism</td>
<td>RSc1451</td>
<td>Adenosine deaminase</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>RSc1722</td>
<td>Phosphoribosylformylglycinamidine synthase</td>
<td>-8.15</td>
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<tr>
<td></td>
<td>RSc2454</td>
<td>Phosphoribosylglycinamideformyltransferase</td>
<td>-3.11</td>
</tr>
<tr>
<td>Involved in iron absorption</td>
<td>RSp0100</td>
<td>Ferrisiderophore receptor</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>RSp0417</td>
<td>Putative siderophore biosynthesis protein SbnA</td>
<td>2.09</td>
</tr>
<tr>
<td>Involved in heat shock</td>
<td>RSc0200</td>
<td>Small heat-shock protein</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>RSe0374</td>
<td>RNA polymerase factor sigma-32</td>
<td>2.28</td>
</tr>
<tr>
<td>Involved in universal stress response</td>
<td>RSc1359</td>
<td>Putative universal stress protein uspa and related nucleotide-binding proteins</td>
<td>-2.76</td>
</tr>
<tr>
<td></td>
<td>RSc1504</td>
<td>Universal stress protein</td>
<td>-4.89</td>
</tr>
<tr>
<td>Involved in response to oxidative stress</td>
<td>RSp0326</td>
<td>Organic hydroperoxide resistance protein</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>RSc2687</td>
<td>DNA protection during starvation or oxidative stress transcription regulator</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>RSc0123</td>
<td>Putative choline dehydrogenase and related flavoproteinoxidoreductase</td>
<td>2.34</td>
</tr>
</tbody>
</table>
Table S1
Dose-dependent inhibitory activity of 48 h fermentation broth from *Pseudomonas fluorescens* SN15-2.

<table>
<thead>
<tr>
<th>Broth concentration, % v/v</th>
<th>Inhibitory rate, %</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>18.1</td>
</tr>
<tr>
<td>10</td>
<td>30.9</td>
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<tr>
<td>15</td>
<td>43.3</td>
</tr>
<tr>
<td>20</td>
<td>48.5</td>
</tr>
<tr>
<td>25</td>
<td>55.4</td>
</tr>
<tr>
<td>30</td>
<td>59.5</td>
</tr>
<tr>
<td>35</td>
<td>63.9</td>
</tr>
<tr>
<td>40</td>
<td>70.1</td>
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Table S2
Forward and reverse primers used in real time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences (5'-3')</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GyrB-F</td>
<td>GTGAGAAACTCGTGTTGGCTTG</td>
<td>DNA gyrase subunit B</td>
</tr>
<tr>
<td>GyrB-R</td>
<td>CCGGTCCCATAGTGTTTCC</td>
<td></td>
</tr>
<tr>
<td>RSc1088-F</td>
<td>TTCAAGCTCATCCCCGTCC</td>
<td>Probable ferredoxin subunit of a ring-hydroxylating dioxygenase oxidoreductase</td>
</tr>
<tr>
<td>RSc1088-R</td>
<td>CATTCGATCTCGGCGGTC</td>
<td></td>
</tr>
<tr>
<td>RSc0773-F</td>
<td>GCCAAGCCCTATCTCAACGA</td>
<td>Putative outer membrane biogenesis transmembrane protein</td>
</tr>
<tr>
<td>RSc0773-R</td>
<td>ATTCGCCTTCGGGCTTTTTC</td>
<td></td>
</tr>
<tr>
<td>RSc1359-F</td>
<td>GGAATATCCGTACAGCCT</td>
<td>Putative universal stress protein uspa and related nucleotide-binding proteins</td>
</tr>
<tr>
<td>RSc1359-R</td>
<td>CCATGAAGATGACGTGGCAG</td>
<td></td>
</tr>
<tr>
<td>RSc1987-F</td>
<td>AGGTAGGTCTCGGTGGTTTGG</td>
<td>Aspartate-semialdehyde dehydrogenase</td>
</tr>
<tr>
<td>RSc1987-R</td>
<td>GGTGATGATGGTGTGCGA</td>
<td></td>
</tr>
<tr>
<td>RSc0233-F</td>
<td>TCGTGTTCCGAGCGGTGGTC</td>
<td>Integral membrane protein</td>
</tr>
<tr>
<td>RSc0233-R</td>
<td>CGACCCCACTCATCGCA</td>
<td></td>
</tr>
<tr>
<td>RSc1504-F</td>
<td>AGAGGTCGAGGCACTGTTTG</td>
<td>Universal stress protein</td>
</tr>
<tr>
<td>RSc1504-R</td>
<td>GCGACGACGTTTTCATC</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Linear regression analysis of the activity of fermentation broth against % of *Solanacearum*.
Linear regression analysis of the activity of *P. fluorescens* SN15-2 48 h sterile broth against *R. solanacearum*. X is *P. fluorescens* SN15-2 48 h sterile concentration (V/V) %, and Y is growth inhibitory rate of *R.*
Fig. 3. A volcano plot of the relationship between genes at the same level in *R. solanacearum* cultured in the presence or absence of EG vs. CG. The points are genes differentially expressed.
A plot of the relationship between q-value and log₂ fold change. Red points are genes expressed in the presence or absence of *P. fluorescens* metabolites. Blue points are genes differentially expressed following exposure to *P. fluorescens* SN15-2 fermentation broth.
Fig. 4. MA plot depicting the relationship between transcript levels and differential expression.

genes differentially expressed following exposure to not.
MA Plot (EG vs. CG)

MA plot depicting the relationship between transcript levels and differential expression. Blue points are genes differentially expressed following exposure to *P. fluorescens* SN15-2 fermentation broth, while red points are genes with no differential expression.
Fig. 5. qRT-PCR validation of differential expression value of biological triplicate with *R. solanacearum*, and red column SN15-2 fermentation broth. *, $P < 0.05$; **, $P < 0.01$. 
The relative expression level is average of three technical replicates. Blue column represents untreated, and red column represents *R. solanacearum* exposure to *P. fluorescens*. *P* < 0.05; **, *P* < 0.01.
**Fig. 6.** Effect of *P. fluorescens* SN15-2 metabolites on DNA concentration in CG and EG. CG represents untreated *R. solanacearum*; EG represents treated *R. solanacearum*.
metabolites on *R. solanacearum* DNA and RNA synthesis. Left, DNA; right, RNA.  
EG represents *R. solanacearum* exposed to *P. fluorescens* SN15-2 metabolites.