ABSTRACT

Introduction: *Ralstonia solanacearum* IPO1609 (Rs IPO1609) is a gram-negative phytopathogenic bacteria that causes severe bacterial wilt disease in potatoes. Agricultural practices and agrochemicals are often ineffective solutions to control it. Identification of essential antibacterial targets in the phytopathogen could enable the design/development of suitable bactericides and eventually, control potato brown rot.

Objective: To reveal prospective antibacterial targets in Rs IPO1609 utilizing subtractive genomics strategy coupled with differential pathway analysis, subcellular localization, virulent prediction and drug bank database screening.

Methods: The study was designed to identify potential antibacterial targets in Rs IPO1609. Among the 4545 proteins present in the pathogen, non-orthologs cum non-paralogs were obtained and subjected to *in silico* comparative analysis against potato proteome to reveal non-homologous proteins present in the bacterium. Furthermore, the essentiality of these non-homologs for pathogen’s survival was determined using DEG Database. Metabolic pathways involvement of the short-listed essential proteins was implemented using KAAS and virulent proteins were determined using MP3 web server analysis. Intracellular localization of essential virulent proteins determined using CELLO2GO & PSORTb programs enable enlisting of plausible antibacterial targets.

Results: Subtractive genomics-based approach revealed that a list of 136 proteins of Rs IPO1609 were potato non-homologs and essential. A total of 55 targets are involved in the unique biochemical pathways of the pathogen. Of 55, 29 proteins were found virulent. Furthermore, based on intracellular localization, 3 virulent proteins were identified as promising therapeutic targets.

Conclusion: Among the 55 targets identified in this study, three proteins were found highly potential as antibacterial targets in Rs IPO1609 based on their metabolic pathway, virulence and intracellular localization properties. The outcome might be used as a design in genomics-based strategies to control bacterial phytopathogens.

Key Words: *Ralstonia solanacearum* IPO1609, Antimicrobial targets, Subtractive genomics, Essential genes, Potato pathogen, KEGG pathway

INTRODUCTION

Current strategies to manage bacterial plant diseases are becoming obsolete primarily due to deficiency in availability of suitable agrochemicals, resulting in an overall loss of twenty per cent (approx.) in plant productivity globally. Thus, forcing researchers to explore and identify novel agrochemicals to control bacterial phytopathogens. In today’s era, computational approaches have massively supported modern medicinal drug discovery and development processes. However, investigators express that *in silico* strategies are vital in exploring and understanding plant sciences too. Few even predict applying computational protocols to decode phytopathogens, their molecular virulence factors and discover effective therapeutics to control them.

Antimicrobial target discovery in today’s post-genomics era is achieved by ‘omics’ based approaches rather than by the traditional generic methods. This strategy is also being explored against phytopathogens. Genomic data of several bacterial phytopathogens (draft/completed) generated by cutting-edge sequencing technologies are publicly accessi-
Thus, allowing investigators to implement computational strategies (especially *in silico* comparative, subtractive, and functional genomics) on these collected data to discover novel antibacterial targets.\(^8\)\(^\text{-11}\)

Promising antimicrobial targets in a pathogen can be detected with an interesting approach known as ‘Differential genome display’ or ‘Subtractive Genomics’.\(^8\)\(^\text{-12}\) This approach filters gene(s) (or its protein products) essentially needed for the pathogen, but absent in the host organism (also termed as essential non-host homologous sequences) and thus, are regarded as worthy targets against the pathogen.\(^\text{13}\) Although experimental and computational strategies exists for essential gene-based target prediction, the latter is preferred as it involves less time, labour and economic.\(^\text{14}\)\(^\text{-15}\) Computational target identification in human bacterial pathogens has been successfully implemented by several investigators.\(^\text{16}\)\(^\text{-23}\) However, only a few researchers have followed this strategy to recognize antibacterial targets in phytopathogens.\(^\text{8}\)\(^\text{-10}\)

*Ralstonia solanacearum* (Rs) is a heterogeneous group of bacterial phytopathogen causing the most devastating wilt disease globally in more than 450 economically important plants that include banana, tomato, potato, eggplant, groundnut and tobacco. This soil bacterium is gram-negative, aerobic, non-spore-forming motile bacilli and belongs to the $\varepsilon$-proteobacteria family. More than 140 Rs strains recognized worldwide are classified into five races (1, 2, 3, 4 and 5), six biovars (1, 2, 3, 4, 5 and 6) and four phylotypes (I, II, III and IV) based on their ability to infect different hosts, biochemical properties and geographical distribution,\(^\text{24}\)\(^\text{-26}\) respectively. The phylotypes are further subgrouped into different sequevars.\(^\text{24}\)\(^\text{-25}\)\(^\text{27}\)

Rs IPO1609 strain is a race 3/biovar 2 isolate obtained from potato in Europe and has been recently classified as phylo-type IIB sequevar 1 (IIB1) strain. In addition to being highly destructive among known Rs strains affecting potato and ability to adapt to highland temperatures, IIB1 are reported as highly dangerous potato pathogens because they cause asymptomatic latent infections.\(^\text{28}\) A whole-genome sequence draft (20x) of Rs IPO1609 was established at Genoscope, France.\(^\text{29}\) During the sequencing process, only the final assembling step was not completed. Hence, these sequences were assembled to generate ten supercontigs (length of 4 to 3372 kb) and are deposited in NCBI GenBank (accession nos.: CU694431 - CU694438, CU914166 and CU914168). They are also accessible at iant.toulouse.inra.fr/bacteria/annotation/cgi/ralso.cgi. Over 99% of the 5.313 Mb genome sequence is covered by 6 supercontigs (each having >10 kb length) alone. The genome seems to possess a high G+C content (average 60%).\(^\text{29}\)

The present work aims to utilize a subtractive genomics strategy on *Ralstonia solanacearum* IPO1609 (a potato brown rot causing pathogen) to reveal its prospective antimicrobial target candidates. Furthermore, employing differential pathway analysis, subcellular localization, virulent prediction and drug bank database screening on the plausible targets will identify novel therapeutic targets among them. Eventually, facilitating the discovery of suitable therapeutics to use against this important bacterial phytopathogen.

**MATERIALS AND METHODS**

**Data Collection**

All protein sequences of *Ralstonia solanacearum* IPO1609 were retrieved individually in Fasta format from http://iant.toulouse.inra.fr/bacteria/annotation/cgi/ralso.cgi. The whole Proteome of *Solanum tuberosum* (UP000011115) was obtained from UniProtKB database (http://www.uniprot.org/taxonomy/complete-proteomes). All essential genes associated with prokaryotes were downloaded from the Database of Essential Genes (DEG) version 15.2 (http://tubic.tju.edu.cn/deg/).\(^\text{30}\)

**Mining Essential Proteins of Rs IPO1609**

**Exclusion of Paralogs and Orthologs**

CD HIT server analysis (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=cd-hit)\(^\text{31}\) at 80% identity threshold\(^\text{21}\) was employed to screen duplicate proteins or paralogs present in the proteome of Rs IPO1609. Likewise, orthologs (i.e. protein sequences with <=100 amino acids) among the protein sequences of Rs IPO1609 were also examined.\(^\text{16}\)\(^\text{-22}\)\(^\text{-23}\) These identified paralogs and orthologs were excluded from Rs IPO1609 proteome dataset.

**Screening of Non-Host Homologs**

All the non-paralog cum non-ortholog proteins of Rs IPO1609 resulting from the previous step were analysed using BLASTP tool\(^\text{12}\) to detect their similarity with the host i.e. potato (*Solanum tuberosum*) proteins. A 0.01 random e-value (expectation value) threshold was fixed. Pathogen proteins showing ‘no hits found’ during BLAST analysis form the resultant dataset and are termed as ‘Non-Host Homologs’.

**Extraction of Non-Host Homolog Essential Proteins**

Extraction of essential proteins from non-host homologs of Rs IPO1609 was achieved by BLAST search (e-value <= (1/10^9)\(^\text{9}\) and 30% or more sequence identity\(^\text{23}\) against all prokaryotic sequences of the DEG database. The resulting protein hits from Non-Host Homolog Essential Proteins of Rs IPO1609.

All BLAST searches utilized Stand-alone BLAST (version 2.6.0+) and the necessary parameters such as e-value, sequence identity, etc. was provided using command-line
options. An in-house developed PERL script was employed to parse protein sequences from the respective BLAST results.

**Metabolic Pathway Studies**

Each Non-Host Homolog Essential Protein of Rs IPO1609 was submitted as a query sequence to KAAS (KEGG Automatic Annotation Server) at KEGG (http://www.genome.jp/tools/kaas). Functional annotation of each query protein is identified by searching against KEGG GENES database and the results are reported as KEGG Orthology (KO) assignments and KEGG Pathways. Thirty-three unique pathways of Rs IPO1609 are revealed by manual comparison of pathogen and potato metabolic pathways listed in KAAS results. As the remaining pathways are part of only host cells or common to both (host and pathogen), hence, can be considered insignificant. So, they are not taken up further in our study. Thus, essential proteins associated with unique pathways of the pathogen alone were enlisted as possible therapeutic targets.

**Prediction of Virulent Proteins**

The disease-causing ability of a microbe is dependent on its virulent factors, especially proteins produced by them. Thus, predicting the virulence nature of a protein will enable us to classify virulent ones as better therapeutic targets. So, each putative therapeutic target protein identified was subjected to MP3 web server analysis. This online tool integrates Support Vector Machine and Hidden Markov Model approach for fast, accurate and sensitive prediction of bacterial virulent proteins.

**Subcellular Localization Prediction**

Intracellular localization of target proteins in the pathogen was predicted using PSORTb tool (version 3.0) and CELLO2GO server (http://cello.life.nctu.edu.tw/cello2go). Results from both the web tools have higher accuracy. Thus, each protein in the dataset was segregated based on its subcellular localization.

The overall workflow involved in genomic subtraction based antibacterial target identification in *Ralstonia solanacearum* IPO1609 is depicted in Figure 1.

**RESULTS**

**Identification of Non-Host Homolog Essential Proteins in Rs IPO1609**

A total of 4545 proteins encoded by Rs IPO1609 genome were individually retrieved. 322 orthologs and 127 paralogs of the pathogen were identified and excluded. Thus, 9.87% of the pathogen’s proteins were removed from the dataset. The remaining 4096 proteins of the bacterium BLASTed against 53,105 proteins of the potato (host) proteome reveals 2530 ‘Non-Host Homologous Proteins’ of the pathogen. Thus, 34.4% of bacterial proteins homologous to the host were eliminated from further analysis. BLAST Alignment of Non-Host Homologs against DEG’s prokaryotic protein sequences (18,835 sequences) recognizes 136 essential proteins of the pathogen (2.9%) as Non-Host Homolog Essential Proteins vital for Rs IPO1609 survival. Table 1 displays results obtained during different stages of subtractive and metabolic pathway analyses performed in this study.

**Unique Metabolic Pathway(s) Involvement of Rs IPO1609 Essential Proteins**

The role of shortlisted essential proteins in different biochemical pathways were determined using the KAAS server. Among 136 proteins analyzed, 126 were involved in different biochemical pathways of the pathogen. Several proteins were involved in primary metabolic pathways such as oxidative phosphorylation, amino acid biosynthesis, citric acid cycle, etc. Some proteins were associated with peptidoglycan biosynthesis, LPS biosynthesis, bacterial chemotaxis, flagellar assembly, protein export and secretion systems. A total of 16, 13 and 7 proteins were identified to be involved in the two-component system, biosynthesis of secondary metabolites and ABC transporters mechanisms, respectively. However, KO details of ten essential proteins couldn’t be established and so, were excluded from further analysis.

The involvement of each chosen essential protein in unique metabolic pathways of Rs IPO1609 was also determined. A total of 59 biochemical pathways were identified in the pathogen. Of 59 pathways, 20 (i.e. 33.8%) were characterized as unique. Similarly, among 136 essential proteins, 55 were found participating in several unique pathways of the bacterium. Surprisingly, among 55, 22 essential proteins reveal their role in only one pathway of the pathogen. Whereas, remaining 33 proteins were associated with more than one unique pathway associated with the pathogen (Table 2). Because of their commonness to both pathogen and host, the remaining 39 pathways were not considered for further investigation.

**Virulence and Subcellular Localization Prediction Analyses**

The virulent/Avirulent property of the 55 essential proteins selected was predicted with an MP3 webserver. Our results reveal 29 virulent proteins among the 55 proteins studied (Table 3).

Figure 2 displays the distribution of Rs IPO1609 essential proteins at various subcellular localizations. Table 3 provides consensus results of subcellular localization prediction obtained from PSORTb and CELLO2GO programs. Results
reveal 49% of query proteins localize in the cytoplasmic membrane and 36% localize in the cytoplasm. Only 7% of proteins were found to localize in the outer membrane region of the cell. An equal number of proteins (i.e. 2%) was predicted as molecules associated with extracellular and periplasm zones. However, the subcellular localization of 4% proteins could not be determined.

Analyzing results of virulence and subcellular localization prediction renders only three proteins viz. one extracellular (Id: RS IPO_04212) and two cytoplasms (Id: gspE-RS IPO_02864, RS IPO_04405) localizing as highly plausible therapeutic targets among the studied 55 essential proteins.

**DISCUSSION**

In the drug discovery phenomenon, antimicrobial target identification is a primary step that is both sensitive and critical. In this post-genomics era, investigating “omics” data combined with advanced computational protocols has enabled easier and improved identification of protein targets in several pathogens. One such popular approach referred to as ‘subtractive genomics’ has been employed in effective target predictions in human pathogens. Over the last couple of decades, ‘omics’ data on bacterial phytopathogens obtained from various sequencing projects has generated renewed interest to apply in silico research in identifying antibacterial targets. Nevertheless, only a few investigators have utilized in silico essential-gene based target identification in phytopathogenic bacteria. To the best of our knowledge, no literature is available on the computational identification of novel antimicrobial targets in potato brown rot causing bacterial pathogen Rs IPO1609. Thus, the current study applies a subtractive genomics strategy on the Rs IPO1609 proteome to reveal its’ plausible targets.

The suitability of antimicrobial targets identified by genome subtraction technique is weighed upon two fundamental criteria viz. ‘Selectivity’ and ‘Essentiality’. Any protein exclusively present in a pathogen as well as is necessary for its basic survival makes an effective antimicrobial target. Hence, an initial step in this investigation focused on identifying proteins that are both specific and essential to Rs IPO1609 strain. Among the 4545 proteins of the pathogen, 4096 non-paralogs cum non-orthologs were identified. Since paralogs are redundant as antimicrobial targets and orthologs have little chances of being essential to the pathogen, both were eliminated from the study. A total of 2530 proteins of the pathogen were identified as ‘Non-Host Homologs’ following BLAST analysis against potato host proteome. Previously, only 158 proteins in *Pseudomonas syringae pv phaseolicola* were recognized as non-homologs. Similarly, only 406 and 152 sequences were non-homologs in two different phytopathogenic strains of *Xanthomonas oryzae* studied. Several earlier literature report between a few hundred and a few thousand proteins identified as non-homologs in several gram-negative bacterial members.

According to Luo and coworkers, essential proteins of microbes are regarded as interesting antimicrobial targets. Thus, 136 essential proteins among the ‘Non-Host Homologs’ of the pathogen were identified by BLAST Alignment against the DEG database. In 2004, 300 – 400 genes were identified as essential in *Pseudomonas aeruginosa*. Whereas, only 137 essential proteins were reported in *Pseudomonas syringae pv phaseolicola*. However, less than 40 proteins were found essential in two different strains of *Xanthomonas oryzae*. Several researchers document varying essential protein numbers identified by them in gram-negative bacteria. Number of Non-Homologs, as well as essential proteins identified by different investigators, seems to vary due to the differences in the computational protocols followed as well as experimental parameters employed by them.

According to Barh and coworkers, Essential Non-Host Homologs identified during in silico subtractive/comparative genomics analysis has been the major criterion in establishing therapeutic targets. However, advancements in insilico resources of the preceding decade have facilitated researchers to opt for additional factors in determining the suitability of antimicrobial targets. Thus, essential proteins that are associated with pathogen-specific biochemical pathways, their subcellular localization, virulence and druggable properties were employed as additional factors by previous investigators. Hence, these characters for all the enlisted essential proteins were determined.

KAAS analyses of the 136 essential proteins reveal the involvement of 126 proteins in different biochemical pathways of the pathogen; many engaged in primary metabolic pathways and few in other pathways. These processes have a direct or indirect link to motility, virulence factor, pathogenesis and nutrient mobilization/uptake mechanisms of the bacterium.

Essential proteins engaged in unique metabolic pathways of Rs IPO1609 were determined, as they are considered good antibacterial targets. A total of 55 essential proteins participate as metabolites in 20 unique metabolic pathways of the pathogen. Since these molecules are part of pathways indispensable for bacterial life, they form interesting targets. Less than 50 essential proteins have been reported to be involved in exclusive pathways of several gram-negative bacteria. In phytopathogen *Pseudomonas syringae pv phaseolicola*, 22 essential proteins with a role in pivotal metabolic pathways were documented. Analogous to earlier reports, several essential proteins associated with pathways of both i.e. host and pathogen (called common host-pathogen pathways) were also revealed. These proteins, if chosen
as targets, may also damage host cells, so, were eliminated from the dataset.

A protein of the pathogen can find an active role in single or multiple pathways and more than one protein can involve in one or several metabolic pathways. Results in our investigation revealed 22 proteins participating in only one pathway. However, the remaining 33 proteins were associated with multiple pathways of the pathogen and can be regarded as significant antimicrobial targets.22

A two-component system in a bacterium is a signal transduction machinery that mounts a suitable response identifying changes in a cell’s exterior or interior.48 7 Rs IPO1609 proteins associated with a two-component system were identified and may be regarded as superior antimicrobial targets as they are essential for bacterial survival. Similarly, 12 chemotaxis proteins necessary to sense chemical gradients in their environment and facilitate movement towards favourable conditions were recognized as potential targets in Rs IPO1609. Additionally, PhosphoTransferase System (PTS) and Bacterial Secretion System in Rs IPO1609 are among its identified unique metabolic pathways. As these pathways are pivotal for the growth and survival of a bacterium in extreme conditions, they form significant antibacterial targets. Our results are in concurrence with earlier reports.16,20,22,49

Peptidoglycan in the cell wall of a bacterium helps the cell to maintain its structure as well as resists osmosis. Being an important virulence factor of the bacterium, compounds inhibiting the proteins participating in peptidoglycan biosynthesis can be effective antimicrobials.16,17,22,38 Our results prioritize two unique proteins associated with Rs IPO1609 peptidoglycan biosynthesis pathways and might be recognized as plausible targets.

In structural terms, LipoPolySaccharides (LPS) consists of a core oligosaccharide linked to Lipid A and O-antigen molecules on either ends and is responsible for gram-negative bacteria outer membrane stability.50 Our results list three proteins as key metabolites in the LPS biosynthesis pathway of Rs IPO1609. Thus, these proteins may have opted as significant targets for therapeutics discovery. Antimicrobials blocking the permeability of solutes across the membrane results in cell death. Results obtained in our study are in parallel to earlier literature reports.16,20,22,38

Autoinducers help communication among bacterial cells and their concentration is directly proportional to bacterial proliferation. Once a threshold is reached, increased virulence occurs resulting in biofilm formation.51 Chemicals targeting quorum sensing (QS) prevents in vivo QS activation, eventually leading to a decrease in virulence. Since bacterial growth is not directly controlled by QS, blocking them does not compel the emergence of antimicrobial resistance in target organisms.52,53 Achieving QS inhibition (aka Quorum quenching) by blocking receptors using antagonistic molecules, blocking autoinducer synthesis and degradation of autoinducers with hydrolytic enzymes, eventually affects the formation of biofilms.53 In this study, five proteins of Rs IPO1609 recognized as metabolites of the QS mechanism might be regarded as significant antimicrobial targets. Recently, Pseudomonas aeruginosa QS proteins were suggested as attractive antibacterial targets.18

Cationic antimicrobial peptide (CAMP) resistance and beta-lactam resistance-conferring proteins occurring in Rs IPO1609 suggest that the pathogen possesses a resistance mechanism towards antimicrobial molecules. Despite CAMP’s ability to weaken bacterial cell membrane integrity, many bacteria have evolved alternative pathways to resist CAMP molecules.54 In this computational study, four CAMP resistant proteins with potentiality as therapeutic targets have been identified. Recently, Prabha and team19 reported CAMP as possible targets in a rice pathogen. Beta-lactam proteins are considered as antibiotics targeting cross-linkage in the peptidoglycan layer of a bacterial cell wall. Thus, beta-lactam resistance conferred by proteins elaborated by a bacterium is a vital virulence factor. Proteins of these pathways can form as good targets for influencing resistance and thus, rendering the bacterium susceptible. Three proteins responsible for beta-lactam resistance in Rs IPO1609 represent plausible targets in the pathogen. Few other researchers have recorded similar results in bacterial pathogen of humans38 and plants.10

All the 55 essential proteins short-listed through the current study can be regarded as novel potential targets. However, the virulent nature, as well as intracellular localization of these proteins, might provide significant details for the drug design and development process. Our results identified 29 virulent proteins among the 55 plausible protein targets. Earlier, Keshri and co-workers9 reported three virulent proteins in Xanthomonas oryzae pv oryzae PXO99A. Similarly, four proteins were found virulent in Xanthomonas oryzae pv oryzae KACC10331 in a recent investigation.10

Extracellular space, outer membrane, periplasmic, cytoplasmic (inner) membrane and cytoplasm are the five probable localizing zones in gram-negative bacteria.36 Our analysis revealed 25 and 2 virulent proteins localizing in membranous and cytoplasm region of the bacterium respectively. However, 1 virulent protein was predicted secreting extracellularly and 1 virulent protein’s localization could not be determined. According to Barh et al.,22 bacterial proteins secreted extracellularly or localizing in the cytoplasm are favourable targets. Thus, three virulent proteins viz. RS IPO_04212 (extracellular), gspE-RS IPO_02864 and RS IPO_04405 (cytoplasm) are plausible antibacterial targets identified in Rs IPO1609. RS IPO_02864 (gspE) is a well-known protein involved in type 2 secretion systems of a bacterium and RS IPO_04405
has been computationally predicted as a signal transduction protein. However, RSIPO_04212 is computationally predicted to be a haemagglutinin/hemolysin related protein. In 2012, Katara and co-workers have reported intracellular localization of certain proteins in *Pseudomonas syringae*. But, their reports lack details on either the proteins involved or their intracellular localization. No other literature regarding localization prediction of bacterial phytopathogen proteins using computational approaches is currently documented. Nevertheless, investigators utilize *in silico* strategies to predict the localization of proteins in numerous gram-negative human bacterial pathogens. For obtaining more reliable results, researchers have opted for two or more tools during their computational prediction analysis.

## CONCLUSION

The subtractive genomics strategy attempted in this research work can act as a preparatory step in the discovery of suitable agrichemicals against the potato pathogen *Rs IPO1609*. Since the 55 proteins (Table 2) are essential and unique to *Rs IPO1609* strain, they can be considered as significant antibacterial targets. Based on virulence and intracellular localization properties, three proteins viz. gspE-RSIPO_02864, RSIPO_04405 and RSIPO_04212 are reported as promising targets. Bactericides targeting these proteins can help to chemically control wilt disease of potato caused by *Rs IPO1609*. However, validating these targets through experimental studies is required. Our methodology and the results generated can be considered significant to the researchers actively involved in the bacterial genomics-based study for control of phytopathogens.

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## Conflicts of Interest

None

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## Author’s Contribution

SG conceived, designed and performed the experiments. Both the authors analyzed the results, provided critical feedback, contributed to discussion and in shaping the final manuscript.

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Table 1: Results of Subtractive Analysis and Metabolic Pathway Studies of R. solanacearum IPO1609

| Analysis Stage | Number of Proteins |
|----------------|--------------------|
| Retrieved Proteome | 4545 |
| Non-paralogs | 4418 |
| Non-orthologs | 4096 |
| Non-Host Homologs (E-value 10\(^{-5}\)) | 2530 |
| Essential Proteins after DEG analysis (E-value 10\(^{-10}\)) | 136 |
| Total No. of Metabolic Pathways Identified | 59 |
| No. of Unique Metabolic Pathways Identified | 20 |
| Essential Proteins in Unique Pathways | 55 |

Table 2: List of Rs IPO1609 Essential Proteins in Unique Metabolic Pathways with its corresponding KEGG Orthology (KO)

| S. No. | Protein ID(s) | KEGG Orthology (KO) No. | Involved Pathway(s) |
|--------|---------------|-------------------------|---------------------|
| 1 | acrA-RSIPO_00080 | K03585 | Beta-lactam resistance, CAMP resistance |
| 2 | aroG2-RSIPO_02256 | K01626 | Quorum sensing |
| 3 | cheD1-RSIPO_03688 | K03406 | Two-component system, Bacterial Chemotaxis |
| 4 | cheD2-RSIPO_04485 | K03406 | Two-component system, Bacterial Chemotaxis |
| 5 | cheD4-RSIPO_00028 | K03406 | Two-component system, Bacterial Chemotaxis |
| 6 | cheR-RSIPO_03340 | K00575 | Bacterial Chemotaxis |
| 7 | cysD-RSIPO_00793 | K00957 | Selenocompound Metabolism |
| 8 | dapF-RSIPO_00194 | K01778 | Lysine Biosynthesis |
| 9 | dctA1-RSIPO_03355 | K11103 | Two-component system |
| 10 | dctA2-RSIPO_02972 | K1103 | Two-component system |
| 11 | dnaA-RSIPO_00094 | K02313 | Two-component system, Cell cycle |
| 12 | dnaB-RSIPO_01511 | K02314 | Cell cycle |
| 13 | epsB-RSIPO_04042 | K06692 | Two-component system |
| 14 | epsC-RSIPO_04041 | K01791 | Two-component system, Biofilm formation |
| 15 | epsC2-RSIPO_03691 | K01791 | Two-component system, Biofilm formation |
| 16 | fbaA-RSIPO_00617 | K01624 | Methane Metabolism, Microbial metabolism in diverse environments |
| 17 | fruA-RSIPO_02614 | K02770 | Phosphotransferase System, Microbial metabolism in diverse environments |
| 18 | ftsA-RSIPO_02589 | K03590 | Cell cycle |
| 19 | ftsI-RSIPO_02599 | K03587 | Peptidoglycan Biosynthesis, Beta-lactam resistance |
| 20 | ftsW-RSIPO_02594 | K03588 | Cell cycle |
| 21 | glnD-RSIPO_0145 | K00990 | Two-component system |
| 22 | gltK-RSIPO_00517 | K0002 | Two-component system |
| 23 | gspE-RSIPO_02864 | K02454 | Bacterial Secretion System, Biofilm formation |
| 24 | motA-RSIPO_03333 | K02556 | Two-component system, Bacterial chemotaxis, Flagellar Assembly |
| S. No. | Protein ID(s) | KEGG Orthology (KO) No. | Involved Pathway(s) |
|--------|---------------|-------------------------|---------------------|
| 25     | narH-RSIPO_03620 | K00371 | Two-component system, Microbial Metabolism in diverse environments |
| 26     | oprM-RSIPO_04477  | K08139 | Quorum sensing, Beta-Lactum resistance |
| 27     | oxyR-RSIPO_02448  | K04761 | Biofilm formation |
| 28     | pilJ-RSIPO_02329  | K02660 | Two-component system, Biofilm formation |
| 29     | pmrK-RSIPO_01498  | K02764 | LPS biosynthesis, CAMP resistance |
| 30     | pstSs-RSIPO_01085  | K02040 | Two-component system, Tuberculosis |
| 31     | rfaC1-RSIPO_02308  | K02841 | LPS biosynthesis |
| 32     | rfaF-RSIPO_00609  | K02843 | LPS biosynthesis |
| 33     | rfbA-RSIPO_02316  | K00973 | Polyketide sugar unit Biosynthesis, Streptomycin Biosynthesis, Acarbose and Validamycin Biosynthesis |
| 34     | rpoN2-RSIPO_03239  | K03092 | Two-component system, Biofilm Formation |
| 35     | RSIPO_00082       | K08139 | Quorum sensing, Beta-Lactam resistance |
| 36     | RSIPO_00120       | K05515 | Peptidoglycan biosynthesis, Beta-Lactam resistance |
| 37     | RSIPO_00550       | K21023 | Biofilm formation |
| 38     | RSIPO_00653       | K03406 | Two-component system, Bacterial Chemotaxis |
| 39     | RSIPO_01033       | K03406 | Two-component system, Bacterial Chemotaxis |
| 40     | RSIPO_01355       | K03406 | Two-component system, Bacterial Chemotaxis |
| 41     | RSIPO_01504       | K13014 | CAMP resistance |
| 42     | RSIPO_01549       | K00404 | Two-component system |
| 43     | RSIPO_01682       | K03406 | Two-component system, Bacterial Chemotaxis |
| 44     | RSIPO_01683       | K03406 | Two-component system, Bacterial Chemotaxis |
| 45     | RSIPO_02545       | K03406 | Two-component system, Bacterial Chemotaxis |
| 46     | RSIPO_03589       | K14444 | Two-component system, Biofilm formation |
| 47     | RSIPO_03872       | K08139 | Quorum sensing, Beta-Lactum resistance |
| 48     | RSIPO_03874       | K03585 | Beta-lactam resistance, CAMP resistance |
| 49     | RSIPO_04169       | K03406 | Two-component system, Bacterial Chemotaxis |
| 50     | RSIPO_04212       | K20276 | Quorum sensing |
| 51     | RSIPO_04429       | K07789 | Two-component system |
| 52     | RSIPO_04402       | K14444 | Two-component system, Biofilm formation |
| 53     | RSIPO_04405       | K14444 | Two-component system, Biofilm formation |
| 54     | secD-RSIPO_02473  | K03072 | Bacterial Secretion System |
| 55     | thrC-RSIPO_0494    | K01733 | Microbial Metabolism in diverse environments |

Table 3: Results of Virulence Prediction and Subcellular Localization of Rs IPO1609 Essential Proteins

| Protein ID(s) | Pathogenic (P) / Non-Pathogenic (NP) | Subcellular Localization |
|---------------|-------------------------------------|--------------------------|
| gspE-RSIPO_02864 | P                                   | Cytoplasm                |
| RSIPO_04405    | P                                   | Cytoplasm                |
| acrA-RSIPO_00080 | P                              | Cytoplasmic Membrane     |
| cheD2-RSIPO_04485 | P                               | Cytoplasmic Membrane     |
| cheD4-RSIPO_00028 | P                               | Cytoplasmic Membrane     |
| fruA-RSIPO_02614 | P                                   | Cytoplasmic Membrane     |
| Protein ID(s)          | Pathogenic (P) / Non-Pathogenic (NP) | Subcellular Localization |
|-----------------------|--------------------------------------|--------------------------|
| ftsI-RS IPO_02599     | P                                    | Cytoplasmic Membrane     |
| gltK-RS IPO_00517     | P                                    | Cytoplasmic Membrane     |
| motA-RS IPO_03333     | P                                    | Cytoplasmic Membrane     |
| RS IPO_00120          | P                                    | Cytoplasmic Membrane     |
| RS IPO_00550          | P                                    | Cytoplasmic Membrane     |
| RS IPO_00653          | P                                    | Cytoplasmic Membrane     |
| RS IPO_01033          | P                                    | Cytoplasmic Membrane     |
| RS IPO_01355          | P                                    | Cytoplasmic Membrane     |
| RS IPO_01549          | P                                    | Cytoplasmic Membrane     |
| RS IPO_01682          | P                                    | Cytoplasmic Membrane     |
| RS IPO_01683          | P                                    | Cytoplasmic Membrane     |
| RS IPO_02545          | P                                    | Cytoplasmic Membrane     |
| RS IPO_03874          | P                                    | Cytoplasmic Membrane     |
| RS IPO_04169          | P                                    | Cytoplasmic Membrane     |
| RS IPO_04209          | P                                    | Cytoplasmic Membrane     |
| secD-RS IPO_02473     | P                                    | Cytoplasmic Membrane     |
| RS IPO_04212          | P                                    | Extracellular            |
| oprM-RS IPO_04477     | P                                    | Outer Membrane           |
| pilJ-RS IPO_02329     | P                                    | Outer Membrane           |
| RS IPO_00082          | P                                    | Outer Membrane           |
| RS IPO_03872          | P                                    | Outer Membrane           |
| pstS-RS IPO_01085     | P                                    | Periplasm                |
| RS IPO_03589          | P                                    | Unknown                  |
| aroG2-RS IPO_02256    | NP                                   | Cytoplasm                |
| cheR-RS IPO_03340     | NP                                   | Cytoplasm                |
| cysD-RS IPO_00793     | NP                                   | Cytoplasm                |
| dapF-RS IPO_00194     | NP                                   | Cytoplasm                |
| dnaA-RS IPO_00094     | NP                                   | Cytoplasm                |
| dnaB-RS IPO_01511     | NP                                   | Cytoplasm                |
| epsC-RS IPO_04041     | NP                                   | Cytoplasm                |
| epsC2-RS IPO_03691    | NP                                   | Cytoplasm                |
| fbaA-RS IPO_00617     | NP                                   | Cytoplasm                |
| ftsA-RS IPO_02589     | NP                                   | Cytoplasm                |
| glnD-RS IPO_01415     | NP                                   | Cytoplasm                |
| oxyR-RS IPO_02448     | NP                                   | Cytoplasm                |
| rfaC-RS IPO_02308     | NP                                   | Cytoplasm                |
| rfaF-RS IPO_00609     | NP                                   | Cytoplasm                |
| rfbA-RS IPO_02316     | NP                                   | Cytoplasm                |
| rpoN2-RS IPO_03239    | NP                                   | Cytoplasm                |
| RS IPO_01504          | NP                                   | Cytoplasm                |
| thrC-RS IPO_01494     | NP                                   | Cytoplasm                |
| cheD1-RS IPO_03688    | NP                                   | Cytoplasmic Membrane     |
| dctA1-RS IPO_00355    | NP                                   | Cytoplasmic Membrane     |
Table 3: Results of Virulence

| Protein ID(s)          | Pathogenic (P) / Non-Pathogenic (NP) | Subcellular Localization* |
|------------------------|--------------------------------------|---------------------------|
| dctA2-RSIPO_02972      | NP                                   | Cytoplasmic Membrane      |
| epsB-RSIPO_04042       | NP                                   | Cytoplasmic Membrane      |
| ftsW-RSIPO_02594       | NP                                   | Cytoplasmic Membrane      |
| narH-RSIPO_03620       | NP                                   | Cytoplasmic Membrane      |
| pmrK-RSIPO_01498       | NP                                   | Cytoplasmic Membrane      |
| RSIPO_04402            | NP                                   | Unknown                   |

* Consensus Results from PSORTb and CELLO2GO Tools

Figure 1: Workflow of Antibacterial Targets Identification in *Ralstonia solanacearum* IPO1609.

Figure 2: Localization Prediction Results of Unique Essential Proteins in *Ralstonia solanacearum* IPO1609.