

Prediction of antibiotic resistance genes and resistance mechanisms of *Pseudomonas syringae* pv. *actinidiae* strain L211 based on whole-genome sequencing

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Abstract

The objective of this paper was to analyze of antibiotic resistance genes and mechanisms of antibiotic resistance in the causal agent of kiwifruit bacterial canker in western China. *Pseudomonas syringae* pv. *actinidiae* (Psa) strain L211, previously isolated from canker-infected kiwifruit (*Actinidia chinensis*) and identified in the laboratory, was used as the study material for whole-genome sequencing and annotation of gene functions. The antibiotic resistance genes and resistance mechanisms were predicted by comparison with the CARD database, and verified by antibiotic sensitivity tests. Our results showed that in total, 482 resistance genes were predicted in the strain L211 genome involved in resistance to 28 antibiotics. The predicted genes were classified in six categories of resistance mechanisms, namely antibiotic efflux, antibiotic inactivation, antibiotic target alteration, antibiotic target protection, reduced antibiotic penetration, and mixed resistance mechanisms. Resistance was mainly characterized as active efflux, β -lactamase degradation, mutation of target genes, plasmid mediated, and reduced biofilm permeability. Antibiotic sensitivity tests revealed that strain L211 was resistant to carbenicillin, cefadroxil, clindamycin, isoniazid, sulfadiazine, bacitracin, norfloxacin, dapson, and chloramphenicol, and sensitive to oxytetracycline, streptomycin, triclosan, and rifampin. Psa may be a broad-spectrum antibiotic-resistant bacterium, with antibiotic efflux as its main resistance mechanism. Multiple resistance mechanisms were predicted to act in concert, resulting in increased antibiotic resistance and resistance to multiple antibiotics. Frequent use of agricultural antibiotics is interpreted to have led to proliferation in resistance genes that target two antibiotics. To avoid further increase in the resistance spectrum of Psa, the use of chemical antibiotics in agriculture should be strictly controlled.

Introduction

Kiwifruit (*Actinidia chinensis*) is a perennial vine belonging to the Actinidiaceae and is among the most economically successful fruit crops domesticated during the twentieth century. Its fruit is rich in dietary fiber, vitamins, mineral elements, amino acids, and bioactive compounds, and the flesh has an especially high content of vitamin C (90–420 mg per 100 g fresh weight). Accordingly, kiwifruit is sometimes termed the “king of fruits”. The fruit can be eaten fresh or processed into jam or juice, and has important health benefits. It has a broad market prospect, the global kiwifruit market is predicted to reach USD\$2.1 billion by 2030^{1–3}.

The kiwifruit industry is a cornerstone of the drive for poverty reduction and upliftment of farmers in western China. However, the frequent incidence of kiwifruit canker in this region in recent years has devastated kiwifruit orchards in many areas, resulting in severe economic losses and seriously hampering the development of the kiwifruit industry⁴. Kiwifruit canker is a bacterial disease caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), which is a widespread, rapidly spreading, highly pathogenic bacterium that is difficult to control and can rapidly cause extensive vine mortality⁵. Antibiotics and copper-based preparations can effectively control the incidence of kiwifruit canker, but their long-term use has led to a gradual increase in prevalence of Psa resistance. Nakajima⁶ reported that Psa strains

collected in 1984–1987 carried only the copper resistance genes *copA* and *copB*, but the continued use of high-copper preparations has resulted in the evolution of two new copper-resistance factors, *copR* and *copS*. Colombi⁷ tested pathogenic *Psa* isolates collected in 2015–2016 and observed that 25% of the strains were resistant to copper preparations. Han⁸ identified the streptomycin resistance genes *strA–strB* from *Psa* strains that cause kiwifruit canker. Thus, the frequent use of agricultural antibiotics can lead to an increase in the number of resistance genes in *Psa*.

To date, most genetic analyses of *Psa* have focused on pathogenicity and few studies have investigated antibiotic resistance genes in detail. On this basis, in the present study, the *Psa* strain L211 isolated in a previous study was selected as representative material to explore the antibiotic resistance genes and antibiotic resistance mechanisms of a pathogenic bacterium by performing whole-genome sequencing. The results provide insight into the antibiotic resistance characteristics of the causal agent of kiwifruit canker in western China and provide a theoretical basis for effective control of kiwifruit canker using antibiotics.

Results

Genomic element analysis and gene prediction. The Prokka and RepeatModeler software were used for gene element prediction and repetitive sequence prediction in the genome assembly, respectively. A total of 5741 genes were predicted, with a GC content of 58.53% and a total length of 5,412,072 bp. The longest gene was 17,859 bp, the average gene length was 942.71 bp, the N50 length was 1191 bp, and 522 repetitive sequences were predicted (Table 1, Fig. 1). In addition, the genome assembly was predicted to contain 59 tRNA and four rRNA genes.

Table 1
Genomic element prediction in the genome assembly for *Pseudomonas syringae* pv. *actinidiae* strain L211

Class	GC(%)	Protein Coding Genes	Average length(bp)	Total coding gene(bp)	tRNA	rRNA	N50	RepeatSeq
Number	58.53	5741	942.71	5412072	59	4	1191	522

COG functional classification. Based on functional annotations in the COG database, the predicted genes of strain L211 were classified into 21 categories according to their predicted functions (Fig. 2). Among the primary functional categories, the highest number of genes were classified as metabolism-related proteins. Of the secondary categories, relatively high numbers of genes encoded proteins involved in categories E (Amino acid transport and metabolism), G (Carbohydrate transport and metabolism), K (Transcription), P (Inorganic ion transport and metabolism), and T (Signal transduction mechanisms).

Resistance gene annotation. Through comparative analysis with the CARD database, 482 resistance genes were annotated in strain L211. These genes were associated with six resistance mechanisms, namely, antibiotic efflux, antibiotic inactivation, antibiotic target alteration, antibiotic target protection,

reduced permeability to antibiotic, and mixed resistance mechanisms, and were associated with resistance to 28 antibiotics (Fig. 3). The highest number of annotated resistance genes (203) was associated with the antibiotic inactivation mechanism, followed by 146 for the antibiotic efflux mechanism (Table 2). The 20 resistance genes that showed the highest homology and matches comprised seven genes associated with the efflux pump system (mexF homology was highest at 85.72%), five genes associated with fluoroquinolone resistance (parE homology was highest at 89.49%), five genes associated with fenifase antibiotic resistance (EF-Tu homology was highest at 78.34%), and the remaining three genes were associated with polymyxin resistance (arnA homology was 78.43%), macrolide antibiotic resistance (PhoP homology was 83.48%), and fosfomycin resistance (GlpT homology was 71.91%), respectively.

A: cephamycin; B: cephalosporin; C: fluoroquinolone antibiotics; D: penam; E: macrolide antibiotics; F: monobactam; G: carbapenem; H: phenicol antibiotics; I: tetracycline antibiotics; J: aminoglycoside antibiotics; K: diaminopyrimidine antibiotics; L: aminocoumarin antibiotics; M: penem; N: peptide antibiotics; O: triclosan; P: rifamycin antibiotics; Q: glycyclcyclines; R: sulfonamide antibiotics; S: disinfecting agents and intercalating dyes; T: acridine dye; U: elfamycin antibiotics; V: lincosamide antibiotics; W: fosfomycin; X: glycopeptide antibiotics; Y: bicyclomycin; Z: isoniazid; A1: sulfone antibiotics; A2: fusidic acid.

Table 2

Predicted antibiotic resistance genes in the genome assembly for *Pseudomonas syringae* pv. *actinidiae* strain L211

Resistance mechanisms	Gene number	Resistant gene name
antibiotic efflux	146	abeS acrA acrB acrD F adeB adeG adel K amrB arlR baeR baeS bcr-1 ceoB cmeB cpxR CRP emrA emrB emrE emrY golS kdpE macB macC mdtB G mexA F mexK mexL mexN mexQ mexS mexY mtrA mtrD mtrE nfxB OpmH oprA oprJ oprM oprN qacH rosA smeA F smeR smeS TriA C
antibiotic inactivation	203	ACC-1 5 ACT-5 ACT-7 ACT-9 ACT-10 ACT-12 ACT-14 25 ACT-27 ACT-28 ACT-30 32 ACT-35 37 AQU-1 CepS CFE-1 CMY-1 2 CMY-4 34 CMY-37 39 CMY-41 44 CMY-47 48 CMY-50 51 CMY-53 62 CMY-65 72 CMY-74 79 CMY-81 85 CMY-90 CMY-93 94 CMY-98 105 CMY-110 119 DHA-1 3 DHA-5 7 DHA-10 DHA-12 22 FOX-1 5 FOX-7 10 LAT-1 LRA-13 MIR-1 6 MIR-8 17 MOX-1 9 OCH-1 8 PDC-1 10 SRT-1 SRT-2
antibiotic target alteration	43	alaS arnA bacA cysB EF-Tu folP GlpT gyrA gyrB gyrA/parC katG LpxA LpxC murA parC parE PmrF rpsL vanA vanB vanD vanRF vanRI
antibiotic target protection	75	mfd QnrB1 38 QnrB40 50 QnrB54 62 QnrB64 74 QnrD1 QnrD2 QnrVC4 QnrVC5 QnrVC7
reduced permeability to antibiotic	3	oprD
antibiotic target alteration/antibiotic efflux	11	PhoP PmrA
antibiotic target alteration/antibiotic target substitution	1	rpoB

Annotation of genes associated with antibiotic efflux mechanisms. Antibiotic efflux is the expression of resistance by reducing the intracellular antibiotic concentrations through specific or universal efflux pumps that expel antibiotics from the cell. The efflux pump is present in multiple forms in most bacteria. There are five main types of extrusion pumps classified according to their structure and energy supply, namely, the ATP-binding cassette (ABC), small multidrug resistance (SMR), large facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), and resistance–nodulation–cell division (RND)⁹. Strain L211 was predicted to have 146 resistance genes associated with antibiotic efflux mechanisms, which belonged to four efflux pump families involving 34 efflux pumps (Table 3), comprising 25 RND family efflux pumps, five MFS family efflux pumps, three SMR family efflux pumps, and one ABC family efflux pump. Of these genes, MexAB–OprM, adeIJK, MexCD–OprJ, MexEF–OprN, smeABC, and smeDEF of the RND family were associated with multidrug efflux pumps that confer resistance to multiple antibiotics (Table 3). Resistance to diaminopyrimidine antibiotics, tetracycline

antibiotics, phenicol antibiotics, lincosamide antibiotics, triclosan, bicyclomycin, glycylicycline, fusidic acid, acridine dye, and disinfecting agents/intercalating dyes was solely responsible for the antibiotic efflux mechanisms, with an abundance of associated resistant genes of 100%. In addition, the number of genes responsible for resistance to penam (60%), penem (67.65%), carbapenem (43.28%), aminocoumarin antibiotics (88.89%), aminoglycoside antibiotics (97.62%), fluoroquinolone antibiotics (46.99%), Fosfomycin (40%), peptide antibiotics (37.93%), sulfonamide antibiotics (90.91%), macrolide antibiotics (92.65%), and rifamycin antibiotics (94.12%) comprised a high percentage of the resistance genes.

Table 3

Predicted genes associated with the efflux pump mechanism of antibiotic resistance in the genome assembly for *Pseudomonas syringae* pv. *actinidia* strain L211

Type	efflux pumps number	efflux pumps name
RND	25	AcrAB-TolC acrD AcrEF-TolC adeABC adeFGH adeIJK AmrAB-OprM CeoAB-OpcM CmeABC MdsABC MdtABC-TolC MdtEF-TolC MexAB-OprM MexCD-OprJ MexEF-OprN MexJK-OprM MexJK-OpmH MexMN-OprM MexPQ-OpmE MexXY-OprM MexXY-OprA MtrCDE smeABC smeDEF TriABC-OpmH
MFS	5	bcr-1 EmrAB-TolC emrY mdtG RosAB
SMR	3	abeS emrE qac
ABC	1	MacAB-TolC

Annotation of genes associated with antibiotic inactivation mechanisms. Antibiotic inactivation refers to the bacterial inactivation of antibiotics through degradation or substitution of the active group, resulting in resistance. In total, 203 resistance genes were associated with β -lactamase, an enzyme that hydrolyzes the β -lactam ring of β -lactam antibiotics, which inhibits their binding to target proteins and thereby generates resistance. The antibiotic inactivation-associated resistance genes of strain L211 were mainly directed against six β -lactam antibiotics, namely, cephalexin, cephalosporin, penam, penem, carbapenem, and monobactam. Among these genes, the proportions of antibiotic resistance genes that targeted cephamycin, cephalosporin, monocyclic lactam antibiotics, and penicillin were 81.12%, 61.63%, 72.06%, and 52.24%, respectively, which were much higher than that observed for other resistance mechanisms.

Annotation of genes associated with antibiotic target alteration mechanisms. The mechanism of antibiotic target alteration refers to the development of resistance by bacteria through mutations or enzymatic modifications that alter the structure of antibiotic-acting target genes to reduce the affinity of the antibiotic for the cellular target site. In total, 43 resistance genes associated with antibiotic target alteration mechanisms were annotated and were responsible for resistance to 10 antibiotics. Among these genes, resistance to elfamycin antibiotics, glycopeptide antibiotics, isoniazid, and sulfone antibiotics was solely responsible for antibiotic target alteration mechanisms. The percentage of

associated genes was 100%. In addition, an isoniazid resistance gene (*katG*), a sulfonamide and sulfone antibiotic resistance gene (*folP*), and a streptomycin resistance gene (*rpsL*) were identified.

Annotation of genes associated with antibiotic target protection mechanisms. The target protection mechanism involves the physical association of the target protection protein (TPP) with the target. Unlike target alteration mechanisms (mutations and chemical modifications), target protection does not involve a permanent change in the nature of the target; instead, sustained or repeated interactions between the TPP and the target are required to confer resistance. Target protection can be classified into three different types, namely, TPPs that directly remove the antibiotic from the target to achieve resistance, TPPs that induce structural changes within the target to separate the antibiotic from the target to confer resistance, and TPPs that induce structural changes within the target to restore the original function without removing the antibiotic that has bound to the target¹⁰. Seventy-five resistance genes associated with antibiotic target protection mechanisms were annotated in strain L211, mainly comprising the *mfd* and *Qnr* series of genes responsible for fluoroquinolone antibiotic resistance. The percentage of relevant resistance genes was 45.18%, second only to those associated with antibiotic efflux mechanisms.

Annotation of genes associated with the reduced permeability to antibiotic mechanism.

Reduced antibiotic permeation is a class of resistance mechanisms specific to Gram-negative bacteria. The presence of a lipopolysaccharide layer in the outer membrane of Gram-negative bacteria forms a permeability barrier, which affects the entry of antibiotics into the bacterium owing to reduced permeability of the outer membrane, resulting in antibiotic resistance. Three copies of resistance genes associated with antibiotic target protection mechanisms (*OprD*) were identified in strain L211, which were responsible for resistance to cepharmycin, cephalosporin, penam, penem, carbapenem, and monobactam.

Annotation of genes associated with mixed resistance mechanisms. The analysis revealed two mixed resistance mechanisms for strain L211, namely, the antibiotic target alteration/antibiotic efflux mechanism and the antibiotic target alteration/antibiotic target substitution mechanism. The former mechanism included peptide antibiotic and macrolide antibiotic resistance genes, and the latter included rifamycin antibiotic resistance genes.

Antibiotic sensitivity. The Kirby Bauer disk-diffusion method was used to verify the antibiotic resistance of strain L211. The MIC of 13 antibiotics against strain L211 were assessed separately. Strain L211 was sensitive to oxytetracycline hydrochloride, streptomycin sulfate, triclosan, and rifampicin with MIC values of 1 µL/ml, 4 µL/ml, 32 µL/ml, and 16 µL/ml, respectively (Table 4).

Table 4
Antibiotic sensitivity of *Pseudomonas syringae* pv. *actinidiae* strain L211

Antibiotic Type	Antibiotic name	MIC(μ L/ml)
tetracycline antibiotic	Oxytetracycline hydrochloride	≥ 1
aminoglycoside antibiotics	Streptomycin sulfate	≥ 4
triclosan	Triclosan	≥ 32
rifamycin antibiotic	Rifampicin	≥ 16
penam	Carbenicillin	none
cephalosporin	Cephalexin hydrate	none
lincosamide antibiotic	Clindamycin hydrochloride	none
isoniazid	Isoniazid	none
sulfonamide antibiotic	Sulfadiazine	none
peptide antibiotic	Bacitracin zinc	none
Chloramphenicol	Chloramphenicol	none
fluoroquinolone antibiotic	Norfloxacin	none
sulfone antibiotic	Diaminodiphenyl sulfone	none

Discussion

In this study, the molecular mechanism of antibiotic resistance in Psa was examined in detail by whole-genome sequencing. A total of 482 resistance genes were annotated in the genome assembly, involving resistance to 28 antibiotics, which were classified into six resistance mechanisms, namely, antibiotic efflux mechanisms, antibiotic inactivation mechanisms, antibiotic target alteration mechanisms, antibiotic target protection mechanisms, reduced antibiotic penetration mechanisms, and mixed resistance mechanisms. Resistance was characterized by active efflux, β -lactamase degradation, target gene mutation, plasmid-mediated, and reduced biofilm permeability, which can be categorized as intrinsic, acquired, and adaptive resistance.

Intrinsic resistance, also termed natural resistance, is a resistance mechanism possessed by the wild type of the bacterium¹¹. A three-copy outer membrane pore protein gene, OprD, was identified in strain L211. Mutation of this gene can confer resistance to β -lactam antibiotics in *Pseudomonasaeruginosa*¹². An additional natural resistance mechanism predicted in strain L211 comprises the antibiotic efflux mechanisms, whereby a large number of multidrug efflux pumps operate in the efflux system, such as MexAB–OprM, adeIJK, and MexCD–OprJ. Pamp¹³ observed that the multidrug efflux pump MexAB–OprM in *P. aeruginosa* was associated with resistance to fluoroquinolone antibiotics, chloramphenicol,

erythromycin, azithromycin, and β -lactam antibiotics. Damier¹⁴ reported that *adeJ* is a multidrug efflux pump for *Acinetobacter baumannii*, which contributes to resistance to β -lactam antibiotics, chloramphenicol, tetracycline, erythromycin, lincomycin, and fluoroquinolone antibiotics. Jeannot¹⁵ reported that the MexCD–OprJ efflux pump in *P. aeruginosa* is associated with mutations in the *NfxB* factor and can efflux fluoroquinolone antibiotics, chloramphenicol, and macrolide antibiotics. These multidrug efflux pumps belong to the RND family of efflux pumps and are composed of endosomal transporter proteins (MexB, *adeJ*, and MexD), periplasmic fusion proteins (MexA, *adeI*, and MexC), and outer membrane channel proteins (OprM, *adeK*, and OprJ), which need to be assembled properly to function. The endosomal transporter protein is responsible for antibiotic recognition, the periplasmic fusion protein is responsible for connecting the endosomal transporter protein to the outer membrane channel protein, and the outer membrane channel protein is responsible for opening the exocytosis channel¹⁶. It is hypothesized that antibiotic efflux is the main resistance mechanism of Psa, which can efflux multiple antibiotics from the cytoplasm through the efflux pump, leading to the development of multiple antibiotic resistance. Therefore, by developing inhibitors that interfere with the assembly of the Psa efflux pump and affect its efflux function, kiwifruit canker could be effectively controlled.

Acquired resistance is a resistance type produced by genetic mutation or acquisition of exogenous resistance genes in bacteria¹⁷. The main mechanisms of acquired resistance predicted in strain L211 were antibiotic inactivation and target mutation. Five families of β -lactamases (CMY, DHA, FOX, ACT, and MIR) were mainly annotated in the mechanism of antibiotic inactivation, which can be induced by β -lactam antibiotics to produce and subsequently hydrolyze the β -lactam ring of antibiotics to inactivate them and inhibit the binding of antibiotics to target proteins, resulting in antibiotic resistance¹⁸. The proportions of resistance genes associated with acquired resistance to cepharmycin, monocyclic lactam antibiotics, cephalosporins, and carbapenem antibiotics were 81.12%, 72.06%, 61.63%, and 52.24% for strain L211, respectively. It is hypothesized that Psa can develop resistance to β -lactam antibiotics through both efflux pump and antibiotic inactivation mechanisms. A streptomycin resistance gene (*rpsL*), an isoniazid resistant gene (*katG*), and a sulfonamide and sulfone resistance gene (*folP*) were predicted in the target mutation mechanism. Marie¹⁹ identified *rpsL* as the gene encoding the ribosomal S12 protein of *Mycobacterium tuberculosis*, which acts to stabilize 16S rRNA to form a highly conserved pseudoknot structure and disrupt the interaction between 16S rRNA and streptomycin to produce resistance to streptomycin. Thwe²⁰ observed that *katG* is responsible for encoding catalase in *Mycobacterium tuberculosis*, that catalase is required for isoniazid to be effective when it enters a pathogenic bacterium, and that *katG* mutations contribute to isoniazid resistance. Buwembo²¹ and Noboru²² reported that *folP* is responsible for encoding dihydropteroate synthase, a target enzyme for sulfonamide and sulfone antibiotics, and that mutations in *folP* can provide pathogenic bacteria with resistance to both antibiotics. These results suggest that there may be a synergistic effect of multiple resistance mechanisms in Psa, which also contains many mutation-prone antibiotic target sites that may increase resistance and multiple drug resistance, which may be the main reason why kiwifruit canker is difficult to control with antibiotics. Thus, the use of chemical antibiotics should be strictly controlled.

Adaptive drug resistance is an environment-dependent resistance mechanism with reversible phenotypic characteristics²³. Adaptive resistance in strain L211 was predicted to be mainly a target protection mechanism. The Qnr family of genes was associated with the antibiotic target protection mechanism of strain L211. These genes can bind to gyrase and topoisomerase IV to block the target sites of fluoroquinolone antibiotics, thereby resulting in resistance²⁴. The percentage of fluoroquinolone antibiotic resistance genes associated with the antibiotic target protection mechanism in strain L211 amounted to 45.18%, which was comparable to the percentage of fluoroquinolone antibiotic resistance genes associated with the antibiotic efflux mechanism (46.99%). It is hypothesized that *Psa* develops resistance to fluoroquinolone antibiotics mainly through the synergistic effect of the antibiotic efflux and antibiotic target protection mechanisms.

Antibiotic sensitivity tests verified that strain L211 was resistant to carbenicillin, cephalexin hydrate, clindamycin hydrochloride, isoniazid, sulfadiazine, bacitracin zinc, norfloxacin, diaminodiphenyl sulfone, and chloramphenicol. These results further demonstrated that *Psa* is a broad-spectrum antibiotic-resistant bacterium and resistant to the two antibiotic compounds that are commonly used to control kiwifruit canker in China^{25,26}. The number of resistance genes for tetracycline antibiotics and aminoglycoside antibiotics predicted in the present study were 64 and 42, respectively, an increase of 16 and 18 genes, respectively, compared with the results of Deng²⁷, who studied *Psa* resistance genes in 2020. It is hypothesized that new resistance genes evolved in strain L211 and that the increase in number of bacterial resistance genes is strongly associated with the frequent use of antibiotics. Thus, the use of tetracycline and aminoglycoside antibiotics should be strictly controlled in kiwifruit orchards in western China to avoid the spread of resistance in *Psa* and to protect the effectiveness of existing antibiotics.

Methods

Source of materials. The *Psa* pathogenic strain L211 was isolated previously in our laboratory from a canker-infected branch of kiwifruit 'Hongyang' collected from an orchard in western China. Streptomycin sulfate, oxytetracycline hydrochloride, cephalexin hydrate, triclosan, carbenicillin, rifampicin, clindamycin hydrochloride, isoniazid, sulfadiazine, bacitracin zinc, chloramphenicol, norfloxacin and diaminodiphenyl sulfone were purchased from the Sangon Biotech (Shanghai) Co.Ltd. (Shanghai, China).

Genomic DNA extraction from *Psa* strain L211. Bacterial genomic DNA was extracted using a magnetic bead method. Briefly, *Psa* strain L211 was cultured in fermentation broth, the cells were collected and lysed, and then digested under the joint action of lysozyme and protease. Binding solution and magnetic beads were added to the lysate for absorption of DNA and RNA. The magnetic beads were then washed and the DNA/RNA recovered by elution. The isolated DNA was used for the construction of a genomic library after qualifying concentrations.

Gene library construction. The DNA fragments were randomly disrupted to obtain 200–400 bp DNA fragments. The DNA fragment ends were repaired and an adenine base was added at the 3' end, after which the junction ligand was prepared to connect the junction to the DNA. The ligand product was

amplified and recovered, and finally the PCR product was purified using Hieff NGS™ DNA Selection Beads. The final library was obtained. Sequencing was performed by the Sangon Biotech (Shanghai) Co.Ltd.

Genome assembly. An Illumina platform was used to sequence the genomic libraries that passed the test. The quality of the raw sequence data was assessed with FastQC, and then the sequence data were filtered and trimmed using Trimmomatic software to remove low-quality reads and adapter sequences, and trim low-quality bases. SPAdes was used to splice the cleaned sequence data into contigs. GapFiller was used to close gaps within the contigs obtained by splicing, and finally sequence correction was performed using PilonSeS-G to correct editing errors and insertions of small fragments during splicing.

Gene prediction and annotation. Gene element prediction was performed on the genome assembly results using Prokka. The amino acid sequences of the predicted genes were compared with the Clusters of Orthologous Groups of proteins (COG; <https://www.ncbi.nlm.nih.gov/COG/>) database and the Comprehensive Antibiotic Resistance Database (CARD; <https://card.mcmaster.ca>) to obtain gene function annotations and information on antibiotic resistance genes.

Antibiotic sensitivity test. Each antibiotic was prepared as 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µL/ml solutions, into which 6-mm-diameter absorptive paper disks were immersed. The L211 cell suspension was diluted with sterile saline to 0.5 Maxwell turbidity and evenly coated on the surface of LB solid medium plates. After standing for 10 min, the liquid on the medium surface of each plate was absorbed, and sterilized forceps were used to place the antibiotic-impregnated paper disks of each concentration on the plate. Each concentration of each antibiotic was tested with three replicates. After 48 h of incubation at 20°C, the presence of a zone of bacterial inhibition around the paper disk was observed to determine the minimum inhibitory concentration (MIC) for each antibiotic.

Declarations

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Author contributions

Y.R. contributed to the conception and design and writing of the manuscript. W.L.L.S. contributed to the data analysis, data interpretation and drafting the manuscript. Y.S. contributed to the data interpretation and drafting the manuscript. S.L.F. contributed to the data acquisition and involved in data analysis. P.Q.Z. contributed to the data interpretation and involved in data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Data availability. The genome wide dataset (SRR25900477) analyzed in the current study is available in the NCBI database (<https://www.ncbi.nlm.nih.gov/sra/>)

References

1. Richardson, D. P., Ansell, J., Drummond, L. N. The nutritional and health attributes of kiwifruit: a review. *J. European journal of nutrition* 57, 2659–2676 (2018).
2. Gao, X., Huang, Q., Zhao, Z. *et al.* Studies on the infection, colonization, and movement of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit tissues using a GFPuv-labeled strain. *J. PLoS One* 11, e0151169 (2016).
3. Butler, M. I., Stockwell, P. A., Black, M. A. *et al.* *Pseudomonas syringae* pv. *actinidiae* from recent outbreaks of kiwifruit bacterial canker belong to different clones that originated in China. *J. PLoS One* 8, e57464 (2013).
4. Lin, L. W., Luo, S. N., Xiao, F. L. *et al.* Incidence and control measures of kiwifruit canker disease in Xiangxi autonomous prefecture. *J. Hunan Agricultural Sciences* 05, 42–44 (2015).
5. Vanneste, J. L., Yu, J., Cornish, D. A. *et al.* Identification, Virulence, and Distribution of Two Biovars of *Pseudomonas syringae* pv. *actinidiae* in New Zealand. *J. Plant disease* 97, 708–719 (2013).
6. Masami, N., Masao, G., Tadaaki, H. Similarity between Copper Resistance Genes from *Pseudomonas syringae* pv. *actinidiae* and *P. syringae* pv. *Tomato*. *J. Journal of General Plant Pathology* 68, 68–74 (2002) .
7. Colombi, E., Straub, C., Künzel, S. *et al.* Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* through acquisition of integrative conjugative elements and plasmids. *J. Environmental Microbiology*. <https://doi.org/10.1111/1462-2920.13662> (2017).
8. Han, H. S., Koh, Y. J., Hur, J. S. *et al.* Occurrence of the strA-strB Streptomycin Resistance Genes in *Pseudomonas* Species Isolated from Kiwifruit Plants. *J. The Journal of Microbiology* 42, 365–368 (2004).
9. Reygaert, W. C. An overview of the antimicrobial resistance mechanisms of bacteria. *J. AIMS Microbiol* 4, 482–501 (2018).
10. Wilson, D. N., Hauryliuk, V., Atkinson, G. C. *et al.* Target protection as a key antibiotic resistance mechanism. *J. Nat Rev Microbiol* 18, 637–648 (2020).
11. Kong, K. T., Alian, A., Lisa, S. *et al.* *Pseudomonas aeruginosa* β -lactamase induction requires two permeases, AmpG and AmpP. *J. BMC Microbiol* 10, 328 (2010)
12. Jean, P. P. D., Dimitris, M., Alain, V. *et al.* Analysis of the *Pseudomonas aeruginosa* oprD gene from clinical and environmental isolates. *J. Environmental Microbiology* 4, 872–882 (2002).

13. Pamp, S. J., Gjermansen, M., Johansen, H. K. *et al.* Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. *J. Mol Microbiol* 68, 223–240 (2008).
14. Damier, P. L., Magnet, S., Bremont, S. *et al.* AdelJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in *Acinetobacter baumannii*. *J. Antimicrob Agents Chemother* 52, 557–562 (2008).
15. Jeannot, K., Elsen, S., Köhler, T. *et al.* Resistance and virulence of *Pseudomonas aeruginosa* clinical strains overproducing the MexCD-OprJ efflux pump. *J. Antimicrobial agents and chemotherapy* 52, 2455–2462 (2008).
16. Blair, J. M., Piddock, L. J. Structure, function and inhibition of RND efflux pumps in Gram-negative bacteria: an update. *J. Curr Opin Microbiol* 12, 512–519 (2009).
17. Dunham, S. A., McPherson, C. J., Miller, A. A. The relative contribution of efflux and target gene mutations to fluoroquinolone resistance in recent clinical isolates of *Pseudomonas aeruginosa*. *J. European Journal of Clinical Microbiology and Infectious Diseases* 29, 279–288 (2010).
18. Bush, K., Jacoby, G. A. Updated functional classification of β -lactamases. *J. Antimicrobial agents and chemotherapy* 54, 969–976 (2010).
19. Marie, B., Paul, H., Serej, L. *et al.* Drug resistance-conferring mutations in *Mycobacterium tuberculosis* from Madang, Papua New Guinea. *J. BMC microbiology* 12, 191–195 (2012).
20. Thwe, E. P., Namwat, W., Pinlaor, P. *et al.* Novel Mutations Detected from Drug Resistant *Mycobacterium Tuberculosis* Isolated from North East of Thailand. *J. World J Microbiol Biotechnol* 37, 1–9(2021).
21. Buwembo, W., Aery, S., Rwenyonyi, C. M. *et al.* Point mutations in the folP gene partly explain sulfonamide resistance of *Streptococcus mutans*. *J. International Journal of Microbiology*. <http://dx.doi.org/10.1155/2013/367021> (2013).
22. Nakata, N., Kai, M., Makino, M. *et al.* Mutation analysis of the *Mycobacterium leprae* folP1 gene and dapsone resistance. *J. Antimicrobial agents and chemotherapy* 55, 762–766 (2011).
23. Sommer, L. M., Johansen, H. K., Molin, S. Antibiotic resistance in *Pseudomonas aeruginosa* and adaptation to complex dynamic environments. *J. Microb Genom* 6, 177–192 (2020).
24. Jacoby, G. A., Walsh, K. E., Mills, D. M. *et al.* qnrB, another plasmid-mediated gene for quinolone resistance. *J. Antimicrob Agents Chemother* 50, 1178–1182 (2006).
25. Wang, Q., Zhang, C., Long, Y. H. *et al.* Bioactivity and Control Efficacy of the Novel Antibiotic Tetramycin against Various Kiwifruit Diseases. *J. Antibiotics* 10, 01–11 (2021).
26. Yang, X., Yi, X. K., Chen, Y. *et al.* Identification of *Pseudomonas syringae* pv. *actinidiae* strains causing bacterial canker of kiwifruit in the Anhui Province of China, and determination of their streptomycin sensitivities. *J. Genetics and Molecular Research* 14, 8201–8210 (2015).
27. Deng, B. H., Liu, D. L., Chen, Q. J. *et al.* Sequencing and analysis of the whole genome scanning of 1 strain of *Pseudomonas syringae* pv. *Actinidiae* from biovar 3. *J. Jiangsu Agricultural Sciences* 48, 67–74 (2020).

Figures

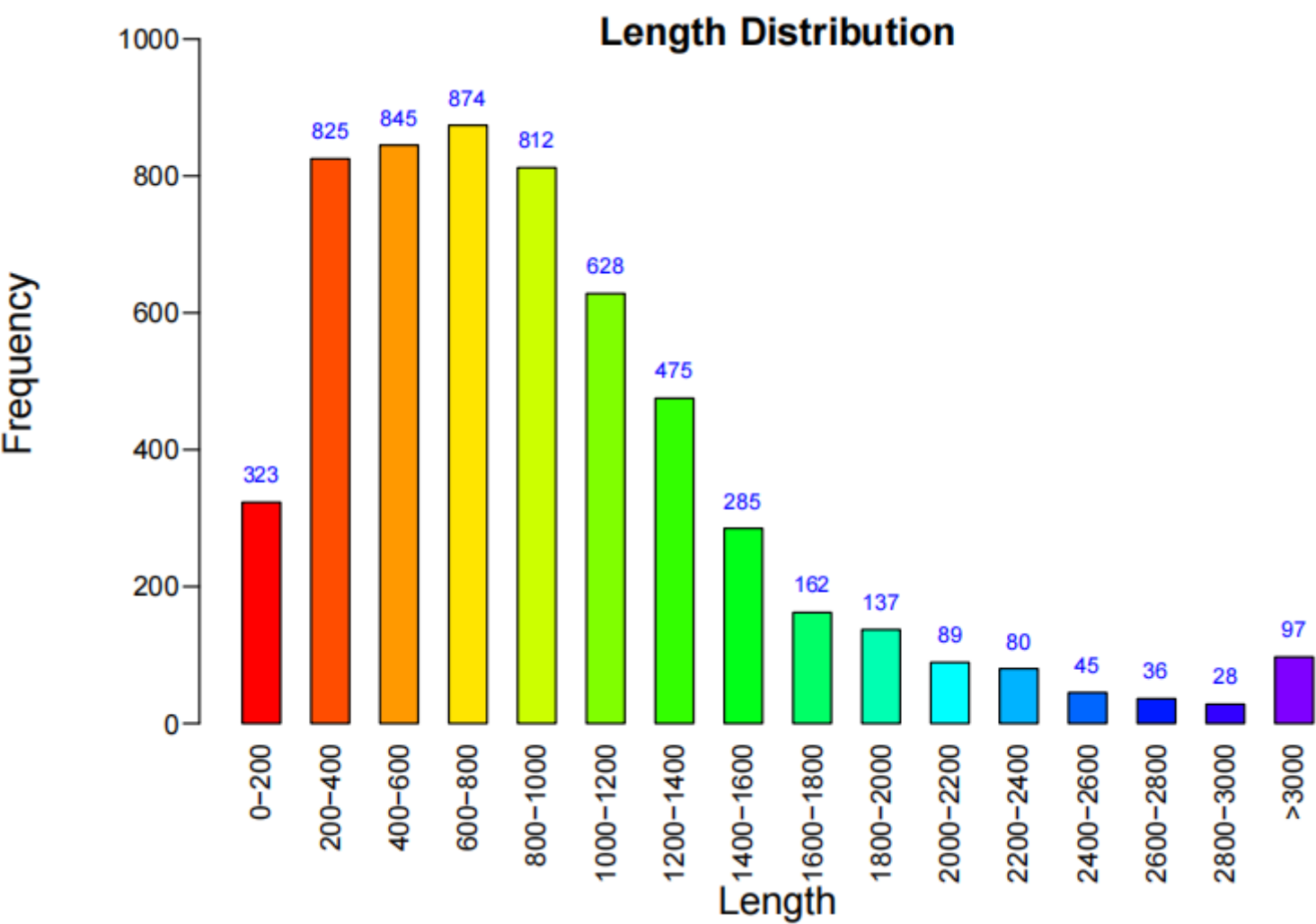


Figure 1

Frequency distribution of predicted gene lengths in the genome assembly for *Pseudomonas syringae* pv. *actinidiae* strain L211

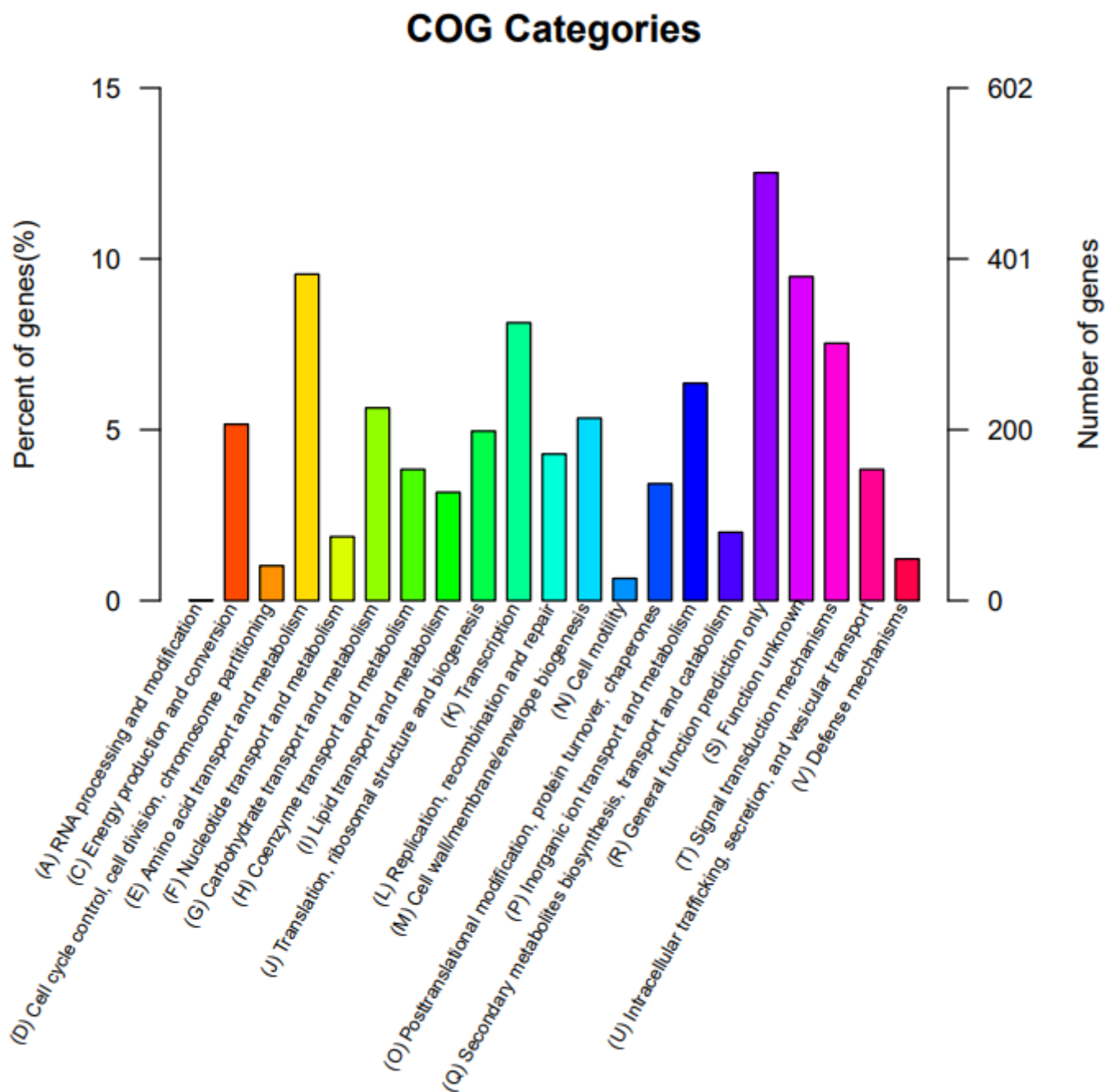


Figure 2

Functional classification of predicted genes in the genome assembly for *Pseudomonas syringae* pv. *actinidia* strain L211 based on annotations in the COG database

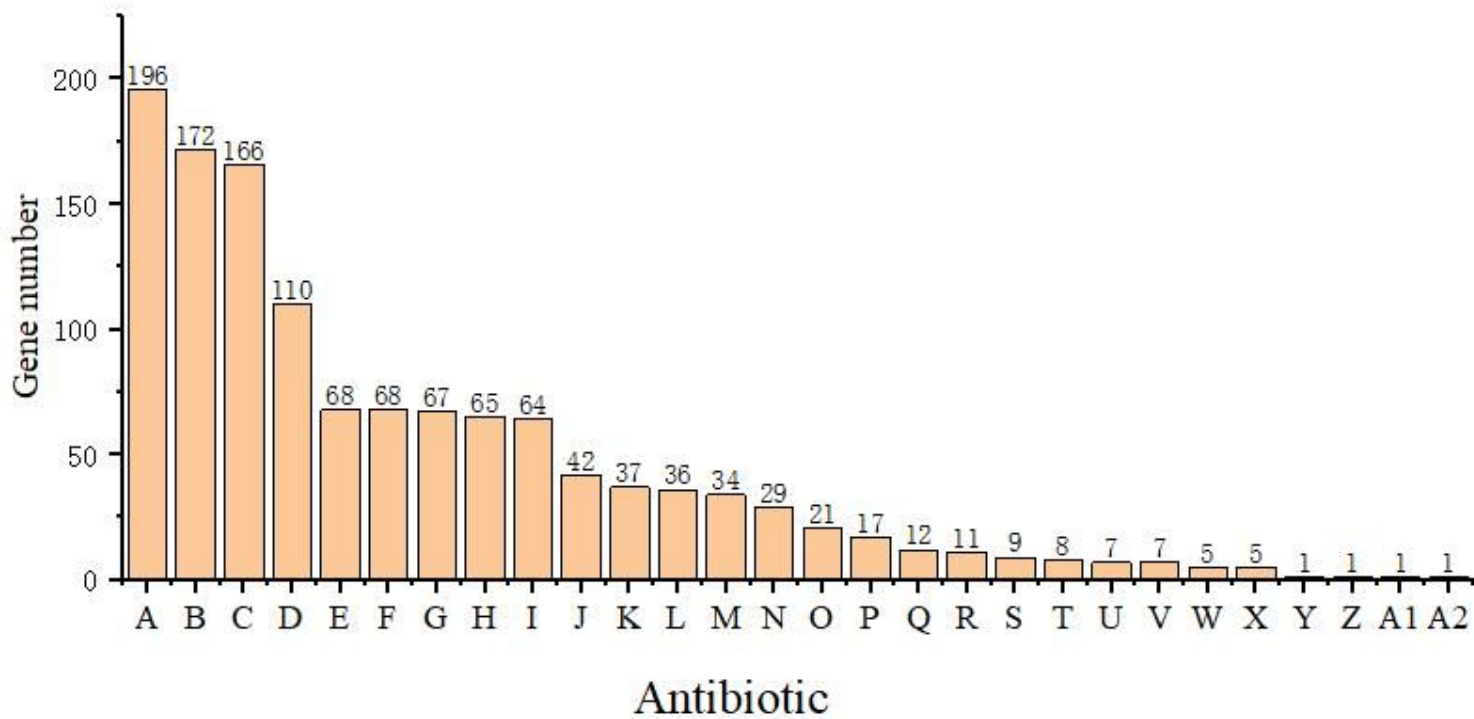


Figure 3

Frequency distribution of predicted resistance genes in the genome assembly for *Pseudomonas syringae* pv. *actinidia* strain L211