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Research Article 研究报告

Analysis of the SKWP effectors to bacterial fitness in host plant by a novel competition assay

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Abstract: [Objective] By testing the internal bacterial growth of SKWP mutants generated in *Ralstonia solanacearum* strain OE1-1, we evaluated the contribution of these SKWP effectors to bacterial fitness towards host plants. **[Methods]** The deletion mutant and complementation mutant of *R. solanacearum* OE1-1 were constructed. A competition assay in mixed infection was adopted to evaluate the contribution of the SKWP to bacterial fitness in tobacco (*Nicotiana tabacum* cv. Bright Yellow) and eggplant (*Solanum melongena* cv. Senryo-nigou). **[Results]** Eggplant was the appropriate host for competitive index (CI) assay of *R. solanacearum* strain OE1-1. All 6 SKWP mutants affected bacterial fitness in eggplant to some extent while SKWP4 appeared to be most important. **[Conclusion]** The SKWP effectors were important for bacterial proliferation in eggplant tissues according to the CI analysis, which paving a new way for further identification of the function on whole SKWP family.

Keywords: *Ralstonia solanacearum* OE1-1, SKWP, CI assay, bacterial proliferation

Ralstonia solanacearum has been established as a model bacterium for plant pathology thanks to pioneering molecular and genomic studies[1–2]. This pathogen endangers the food safety in tropical and subtropical agriculture, especially in China, Bolivia, Bangladesh and Uganda. As for many bacterial pathogens, the main virulence determinant in *R. solanacearum* is the type III secretion system (T3SS) which injects a number of effector proteins into plant cell causing lethal wilting disease in hosts or a hypersensitive response (HR) in resistant plants[3–4]. It has been reported that type III secretion effectors (T3Es) are involved in virulence development in host plants but the mechanism remains unclear.

Generally pathogenicity assays based on quantification of wilting symptoms failed to detect a significant contribution of *R. solanacearum* T3Es in this process, thus revealing the collective effect of T3E in pathogenesis. So far there are three methods mainly for studying the contribution of the each single T3E to bacterial fitness in planta, (i) measure the bacterial population inside a given host plant; (ii) measure the growth of *P. syringae* heterologously expressing *R. solanacearum* T3E in *Arabidopsis*[5–8];

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(iii) competitive index assay (CI assay) between co-infection wild type strain and mutant strains[9]. CI was defined as the mutant/wild-type ratio within the output sample divided by the corresponding ratio in the inoculum. In such *in vivo* competitive assay, the wild-type and mutant strains are co-inoculated in the same plant, thus reducing plant-to-plant variation and enhancing sensitivity in the detection of differential fitness between the two strains[9]. Macho et al. has successfully evaluated 12 T3E genes of strain GMI1000 to their contributions to bacterial fitness in host plants by using CI assay[10–11]. CI assay has been proved to be a sensitive and reliable assay that allows quantifying a differential fitness of *R. solanacearum* strains in planta.

In this study, we evaluated the contribution of SKWP effectors of the Japanese *R. solanacearum* strain OE1-1 to bacterial fitness in host plant by using CI assay. Strain OE1-1 (race1, biovor3) causes lethal wilting disease on tobacco and eggplant, encodes more than 70 T3Es and shares high homologous with those effectors in model strain GMI1000. SKWP effectors (6 members) contain a novel amino acid repeat domain, designated as SKWP repeats from its conserved motif. The studies on SKWP effectors of *R. solanacearum* in virulence activity in host plants has been well developed while their bacterial fitness in host plant is scarce. Therefore, we provided the evidence of SKWP's contribution to bacterial fitness by using CI assay. Analysis of the contribution of SKWP effectors will prove invaluable for understanding *R. solanacearum* invasion strategies and seeking new ways to prevent disease.

1 Materials and Methods

1.1 Bacterial strains, plasmids and growth conditions

The bacterial strains and plasmids used for this study are described in Table 1. *R. solanacearum* strain OE1-1 (race1, biovar3) and its derivative mutants were streak on BG medium at 28 °C. *Escherichia coli* DH12S (Invitrogen Corp., Carlsbad, CA, USA) and S17-1 were grown on Luria-Bertani (LB) medium and incubate at 37 °C overnight.

Table 1. *R. solanacearum* stains and plasmids used in this study

Designation	Relevant characteristics	Reference or source
E. coli		
DH12S	*araD*139 Δ(*ara, leu*)7697 Δ*lacX*74 *galU ga*lK *mcr*A Δ(*mrr-hsd*RMS*mcr*BC) *rps*L *deo*R Ø80d*lacZ*ΔM15 *nupG recA*1/F9*proAB*1 *lacIq* ZΔM15	Invitrogen
S17-1	*thi pro hsdR*$^{-}$ *hsdM*$^{+}$ *recA pro hsdR* RP4-2-Tc::Mu-Km::Tn*7*	[24]
Ralstonia solanacearum		
OE1-1	wild type (race 1 biovar 3)	[25]
RK7197	OE1-1derivative, Gm^{r}	This study
RK7029	OE1-1 Δ*rsc3401 (skwp1)*	This study
RK7058	OE1-1 Δ*rsp1374 (skwp2)*	This study
RK7028	OE1-1 Δ*rsp0930 (skwp3)*	This study
RK7033	OE1-1 Δ*rsc1839 (skwp4)*	This study
RK7050	OE1-1 Δ*rsp0296 (skwp5)*	This study
RK7032	OE1-1 Δ*rsc2130 (skwp6)*	This study
Plasmids		
pBlueScript II ks(+)	Phagemid sequencing vector, Ap^{r}	Stratagene, La Jolla CA, U.S.A.
pTNS2	T7 transposase expression vector	
pUC18mini-Tn7T-Gm	Gm^{r} on mini-Tn7T; for gene insertion in Gm^{r} bacteria	[26]
pK18*mobsacB*	Allelic exchange vector, Km^{r} oriT sacB	[27]

Escherichia coli DH12S was used for plasmid construction and S17-1 was used in conjugation experiments. Antibiotics were used at the following concentrations: ampicillin (Ap), 100 mg/L; kanamycin (Km), 50 μg/mL; gentamicin (Gm), 50 mg/L; polymyxin B (PB), 50 mg/L; 5-bromo-4-chloro-3-indolyl-beta-D-galactopy-ranoside (X-gal), 40 mg/L, isopropyl-beta-D-thiogalactopyranosiade (IPTG), 100 μmol/L.

1.2 Construction of single deletion mutant and complementation mutant

Two 500-bp fragments were PCR amplified by PrimeStar HS DNA Polymerase (TaKaRa Bio, Otsu, Japan) using two pairs of primers (Table 2): OEC(P)xxxxA51 and OEC(P)xxxxB51 primers for upstream fragment, and OEC(P)xxxxA31 and OEC(P)xxxxB31 for downstream fragment. Then the fragments were cloned on pre-digested vector pBluescriptII KS(+)/*Eco*R V to generate pBC(P)xxxx-5 and pBC(P)xxxx-3 plasmids. The *Eco*R I-*Bam*H I fragment of pBC(P)xxxx-5 and the *Bam*H I-*Hin*d III fragment of pBC(P)xxxx-3 were ligated to pre-digested vector pK18mobsacB/*Eco*R I-*Hin*d III to generate pKC(P)xxxx. The sequences of the plasmids were determined with Bigdye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) with primer either M13-47 (5′-TGTAAAACG ACGGCCAGT-3′) or RV-M (5′-CAGGAAACAGCT ATGACC-3′). pKC(P)xxxx was transferred from *E. coli* S17-1 into *R. solanacearum* strain. Deletion mutant strain was generated through two consecutive homologous recombination events (Figure 1). The plasmid pUC18-mini-Tn7T-Gm was electroporated with a transposase-containing helper plasmid pTNS2 into OE1-1 derivative. The transformant was selected on BG agar medium supplemented with Gm and PB. The insertion was verified using colony PCR with primer glms and Tn7R (Figure 2). The strain of OE1-1 derivative was named as RK7197 which carried resistance to gentamicin but did not affect virulence.

1.3 Plant tests

R. solanacearum cells were incubated overnight in B medium and resuspended in 10 mmol/L $MgSO_4$ at an optimal density at 600 nm (OD_{600}) of 0.1 (1.4×10^8 CFU/mL). For root-cutting inoculation, bacterial suspension was poured onto the root wounded plants to achieve a final concentration of 10^7 CFU/mL. Plants were cultivated in a temperature-controlled culture room at 25 °C under 10000 lux (16 h light/8 h dark). Virulence assays were tested on four-week old eggplant (*Solanum melongena* cv. Senryo-nigou) and tobacco (*Nicotiana tabacum* cv. Bright Yellow) respectively. Eggplant plants were cultivated in

Table 2. Primers used in this study

OEC3401A51	TCCGCTTCCGATACGGTGAGTGGTC
OEC3401B51	ggatccGGTTCCTGCACGCTTTGCATTCG
OEC3401A31	ggatccACGCGGCCGCGCGGCGGCAGG
OEC3401B31	ATCATCCTGCGCAGCTTCCTGCGGC
OEP1374A51	ACGGCGGCAAACTGCTGCGC
OEP1374B51	ggatccTTAGGGTTCTGCCGATGTTTTTATG
OEP1374A32	ggatccCTGCTTCAGACGATGCAG
OEP1374B32	ACGGTGGCGGCATAGGTC
OEP0930A51	TGATCCCGGGTGAATCCATTCGCGGAC
OEP0930B51	ggatccCCCCGATCCGCTCCCCTGCGTG
OEP0930A31	ggatccTCGTACTTTCCGTGCAAAATC
OEP0930B31	CAGCGCATCGCCGCGCCGATG
OEC1839A51	CGTARGAGGCCCGCGCCG
OEC1839B51	ggatccGCGGCGAGGGAGCGACCTG
OEC1839A31	ggatccCCCAGGCGCGGCAACGGGC
OEC1839B31	GTCATCGAGGTCGTCGCG
OEP0296A51	TTCGCCGGTGGCCGCCTT
OEP0296B51	ggatccAGGTTGTATTCACAATAGTTC
OEP0296A31	ggatccTGACCCGGTACGCCGGCA
OEP0296B31	CACCAACTACGGCGGGCAG
OEC2130A51	GCGGCGATCATTGCGTCG
OEC2130B51	ggatccTTCCAAAGCGTTGCGTGAGCG
OEC2130A31	ggatccGCGCGTGCGGCCCCACG
OEC2130B31	GTATCGATGAGCGCGGCAAG
glms	GCGCTCAAGCTCAAGGAGATC
Tn7R	CACAGCATAACTGGACTGATTTC

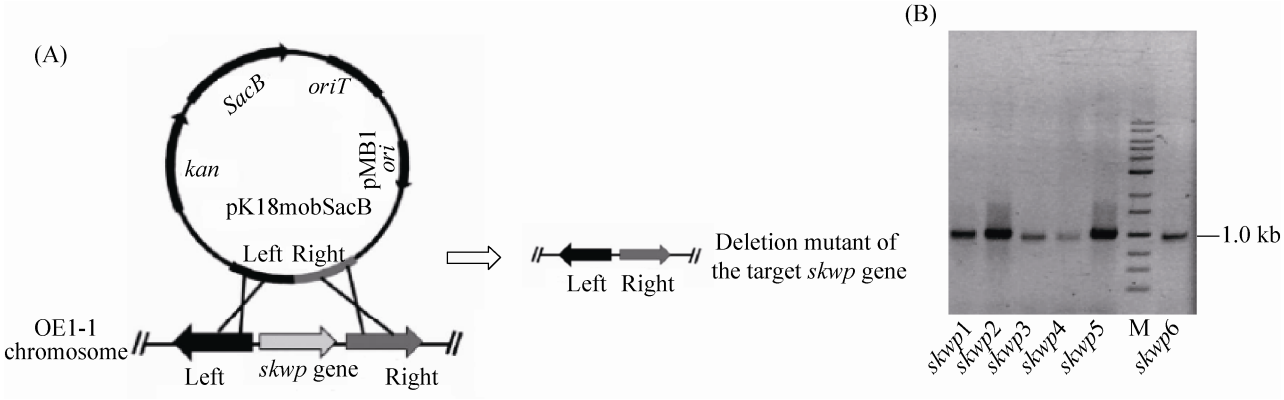

Figure 1. Construction of single SKWP deletion mutants. A: schematic diagram of single *skwp* deletion using pK18mobSacB based on homologous recombination; B: colony PCR results of single *skwp* deletions. M: 10 kb DNA ladder.

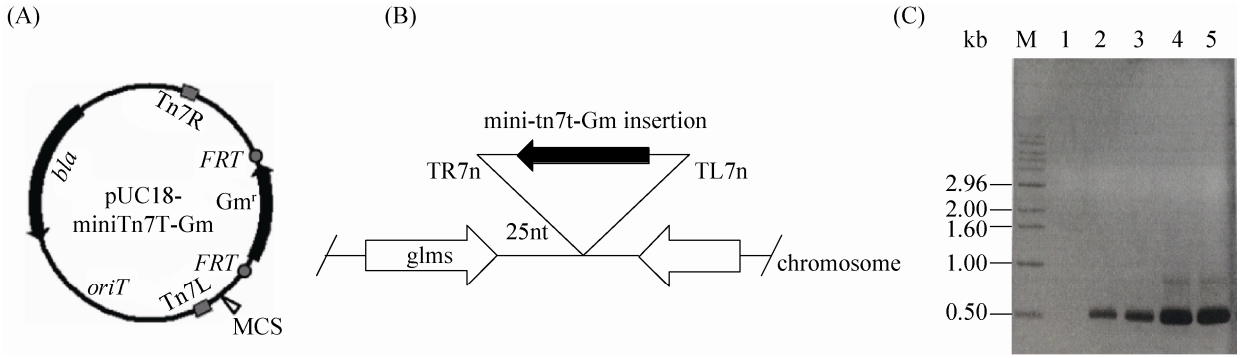

Figure 2. Construction of RK7197. A: structure of pUC18-mini-Tn7T-Gm; B: schematic diagram of site-specific insertion into the chromosome of *R. solanacearum* OE1-1; C: Colony PCR of RK7197. M: 1 kb DNA Ladder; lane 1–5: RK7197.

rock-wool (Nittobo) and tobacco plants were cultivated in pots containing a mixture of vermiculite/peat moss (3:1). Each bacterial inoculation was tested on at least 4 plants and was carried out multi-replicates. Disease symptoms were scored daily for 16 days. Plants were rated according to a scale ranging: 0=no wilting; 1=1% to 25% wilting; 2=26% to 50% wilting; 3=51% to 75% wilting; and 4=76% to 100% wilting or dead.

1.4 Bacterial growth in infiltrated leaf tissue

Internal bacterial populations were determined at selected time intervals after inoculation. Four-week old eggplant (*Solanum melongena* cv. Senryo-nigou) was chosen as host for leaf-infiltration. Bacterial cell suspensions (at 10^6 CFU/mL) in 10 mmol/L $MgSO_4$ were infiltrated into plant leaves. Plants were grown at 25 °C (16 h light/8 h dark). Leaves were sampled every day. Leaf disks (an area of 0.38 cm^2) were cut from infiltrated area by a sterile borer, transferred by a sterile forceps into a sterile tube containing 500 μL of sterile 10 mmol/L $MgSO_4$, and crushed at 3000 r/min for 60 s with a 5 mm-diameter zirconia bead using Micro smash MS-100 (TOMY SEIKO). Standard 10-fold dilution plating onto BG medium supplemented with PB was carried out. Colonies were counted after 2-day incubation at 28 °C, and the bacterial populations were calculated as CFU/cm^2 of leaf area.

1.5 CI assays by leaf-infiltration

Growth attenuation was identified by CI assay. Mutant and WT suspension (10^6 CFU/mL) were mixed at a ratio of 1:1 and then were injected on leaf by a blunt syringe[12]. Leaf tissue was smashed as mentioned above. Serial dilution of the smashed sample was spread onto BG medium with and without the corresponding antibiotic. Both of RK7197 (OE1-1 derivative, Gm^r) and mutant could grow on medium with PB but only RK7197 survived on medium with PB and Gm (Figure 3). The CI was defined as the mutant/wild-type ratio within the output sample divided by the corresponding ratio in the inoculum. CI was calculated as following equation (Eq. 1).

$$CI = \frac{\text{mutanta CFU}}{\text{RK7197 CFU}}(\text{output}) / \frac{\text{mutanta CFU}}{\text{RK7197 CFU}}(\text{input}) \quad \text{(Eq. 1)}$$

2 Results

2.1 Characteristic of SKWP effectors in *Ralstonia solanacearum* strain OE1-1

Plant pathogen *R. solanacearum* OE1-1 encodes a large repertoire (up to 70 candidates) of putative T3Es. These effectors are characterized with various internal repeats which are presumably involved in protein-protein interaction[13]. These effectors are shown in a list of GALA (seven members), AWR (five members), PopP (three members), SKWP (six members) and HLK (three members). SKWP family members contain a novel amino acid repeat domain from its conserved motif which is a type of α-helices structure known to mediate protein-protein interactions. SKWP4 contains 18 tandem SKWP repeats of a 42 aa motif and other members contain 12-15 SKWP repeats[14]. Genomic analysis of the *skwp* genes in strain OE1-1 showed some interesting features: three genes *skwp4*, *skwp1*, *skwp6* (*RSc1839*, *RSc3401*, *RSc2130*) locate on the chromosome whereas *skwp5*, *skwp3*, *skwp2* (*RSp0296*, *RSp0930*, *RSp1374*) live on the megaplasmid (Table 3).

2.2 A competition assay of *R. solanacearum* in tobacco and eggplant leaves

Macho et al. has developed a sensitive and reliable CI assay that allows quantifying the *R. solanacearum*

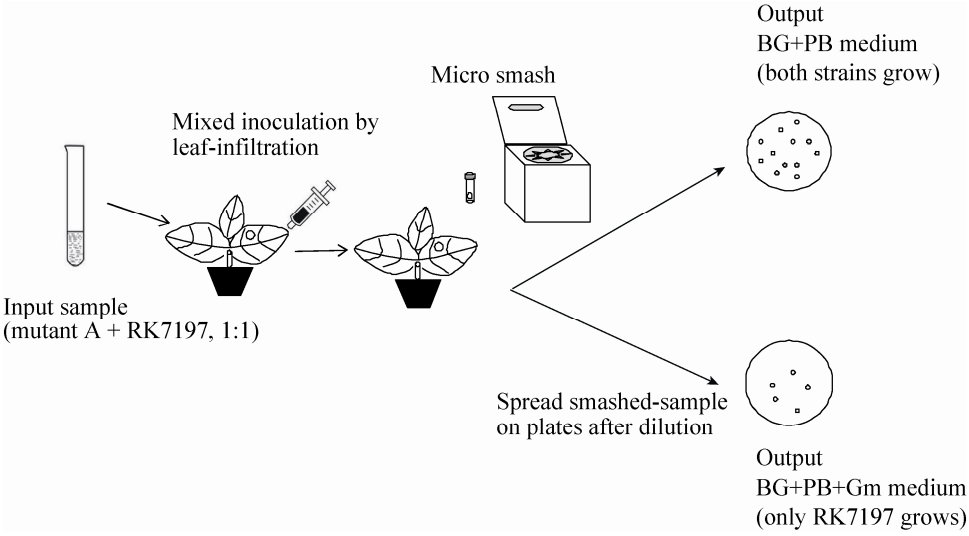

Figure 3. The flow diagram of CI assay on host plant by leaf-infiltration. Mutant and RK7197 (OE1-1 derivative, Gm^r) are separated on BG medium supplemented with different antibiotics gentamicin (Gm) and polymyxin B (PB).

Table 3. Characteristics of SKWP effectors

Gene in OE1-1	SKWP	Size/aa	Features	Distribution in Rs species[a]
RSc3401	SKWP1	2574	Heat/armadillo-related repeats	Variable
RSp1374	SKWP2	2467		Conserved
RSp0930	SKWP3	2208		Conserved
RSc1839	SKWP4	2574		Conserved
RSp0296	SKWP5	2298		Variable
RSc2130	SKWP6	817		Variable

[a]Distribution is considered "conserved" when the gene is found in >90% of a set of 45 *R. solanacearum* strains through comparative genomic hybridization, otherwise it is considered "variable".

bacterial fitness in planta by leaf-infiltration. Although stem injection and soil-drenching infection are main infection modes for pathogenicity assay with *R. solanacearum*, there is a complementation effect appears between wild type strain and mutant strain in mixed infection during the inoculation procedures, which indicated those two modes were not appropriate for CI assay. We infiltrated tobacco and eggplant leaves with wild type OE1-1 and its derivative strain RK7197. 500 μL bacterial suspension of RK7197 strain (10^6 CFU/mL) and 500 μL bacterial suspension of wild type strain OE1-1 (10^6 CFU/mL) were performed for single and mixed inoculation. CI was calculated as above Eq. 1. CI=1 would reflect that the mutant strain is able to grow as efficiently as the wild type, and CI<1 would indicate that growth of the mutant strain is attenuated.

A control experiment was carried out to test whether mixed inoculation affects the growth of two equally virulent bacteria towards tobacco and eggplant (Figure 4). Bacterial population were

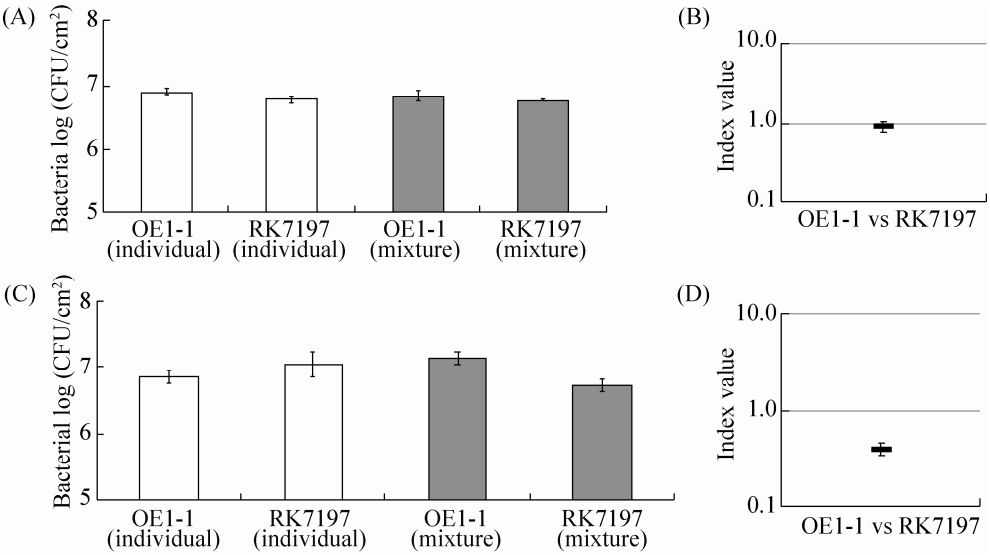

Figure 4. Bacterial growth and competitive index (CI) values of OE1-1 versus RK7197 individually or mixed inoculated in eggplant and tobacco leaves. RK7197 is a derivative of the wild type OE1-1 carrying a gentamicin resistance cassette that does not affect virulence. A: bacterial growth of OE1-1 and RK7197 at 3 days post inoculation (DPI), either individually inoculated or mixed-inoculated in eggplant by leaf infiltration; B: CI mean value generated from mixed inoculation of OE1-1 and RK7197 in eggplant leaf; C: bacterial growth of OE1-1 and RK7197 at 3 days post inoculation (DPI), either individually inoculated or mixed-inoculated in tobacco by leaf infiltration; D: CI mean value generated from mixed inoculation of OE1-1 and RK7197 in tobacco leaf. Each experiment is repeated in triplicate and error bars represent the standard error.

calculated since day post inoculation and differentiated using antibiotic selection. Both WT and RK7197 strains grew to the same level in single and mixed inoculations in eggplant and tobacco (Figure 4-A, C). The CI value in eggplant was close to 1 which indicated that the bacterial multiplication was not affected by limiting thresholds or genetic drift (Figure 4-B). The mutant strain cannot be complemented by the wild-type or interference between co-inoculated strains barely appeared during CI analysis. However, the CI value in tobacco was less than 1 indicated that RK7197 bacterial growth was attenuated (Figure 4-D). These observation correlated to Macho's speculation that bacterial fitness could vary from one plant to another. Therefore, we chose eggplant as host to analyze the contribution of T3Es to bacterial fitness.

2.3 Contribution of SKWP effectors to bacterial fitness in eggplant

Previous research suggests that most T3Es are collectively essential but individually dispensable for the ability to produce symptoms in plants. The contribution of single T3Es is hard to be identified during the pathogenicity process. Solé identified AWR2 as the major contributor of its family to virulence in tomato by a series of complementation experiments[8]. Chen found HLK2 played more important role than other two members by construction of multiple deletion mutant[15]. In this study, all six mutants with single deletion of *skwp* gene were pathogenicity on tobacco and eggplant by root-cutting (Table 4).

Take eggplant for instance, the contribution of each member was hard to clarify during the pathogenicity process. Although there were slight differences of wilting symptoms among six deletion mutants, the function of each SKWP member remains unclear (Figure 5). This observation suggested that plant pathogenicity assay was not sensitive enough to reveal subtle phenotypic differences.

To unravel the role of each SKWP effector in contributing to bacterial multiplication inside host plant, we used competition assay in mixed infection to analysis the single *skwp* gene behavior in eggplant. All deletion mutants were found to multiply as efficient as RK7197 except RK7033 which was the mutant of *skwp4* deficiency. The bacterial population of all five mutants varied from 6-log to 7-log while the bacterial population of RK7033 was below this range (Figure 6-A). Interestingly, we found that RK7033 strain exhibited CI values significantly less than 1 which indicated that its fitness in eggplant tissues was reduced (Figure 6-B). Other five mutants showed the CI value slightly higher than 1 or close to 1. This suggested that SKWP effectors affected the bacterial proliferation on some extent while

Table 4. Pathogenicity tests of single *skwp* deletion mutant on host plants by root-cutting

Strain	Gene						Virulence	
	skwp1	*skwp2*	*skwp3*	*skwp4*	*skwp5*	*skwp6*	Eggplant	Tobacco
OE1-1	+	+	+	+	+	+	Virulence	Virulence
RK7029	–	+	+	+	+	+	Virulence	Virulence
RK7058	+	–	+	+	+	+	Virulence	Virulence
RK7028	+	+	–	+	+	+	Virulence	Virulence
RK7033	+	+	+	–	+	+	Virulence	Virulence
RK7050	+	+	+	+	–	+	Virulence	Virulence
RK7032	+	+	+	+	+	–	Virulence	Virulence

Tobacco (*N. tabacum* cv. Bright Yellow) and eggplant (*Solanum melongena* cv. Senryo-nigou) plants were inoculated with *R. solanacearum* cells to achieve the final concentration at 10^7 CFU/mL by root-cutting. "–" means the effector gene was deleted while "+" means the effector gene existed in the mutant strain.

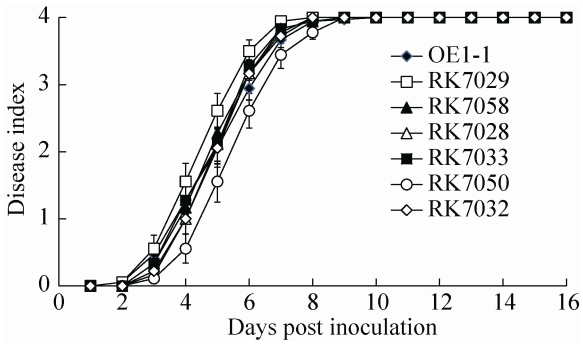

Figure 5. Pathogenicity test of *skwp* single deletion mutants on eggplant (*Solanum melongena* cv. Senryo-nigou). Bacterial suspension was poured onto the root wounded plants to achieve a final concentration of 10^7 CFU/mL. Disease symptoms were scored daily for 16 days. Plants were rated according to a scale ranging of 0 to 4 (0, no wilting; 1, 1%–25% wilting; 2, 26%–50% wilting; 3, 51%–75% wilting; 4, 76%–100% wilting). Each bacterial inoculation was tested on at least 4 plants and was repeated in triplicate. The average and standard error were calculated.

SKWP4 was most significant. This speculation co-related with previous study that SKWP4 of *R. solanacearum* GMI1000 provided a competitive advantage only on eggplant but not tomato and pea.

3 Discussion

The plant pathogen *R. solanacearum* typically enters hosts through root wound and colonizes the water-transporting xylem tissue, spreading up into plant stem. It encodes a large repertoire (up to 70 candidates) of putative T3SS effectors. These effectors are major determinant controlling the virulence of bacterial pathogens on both mammal and plant hosts[16–17]. Generally, these effectors are collectively essential but are individually dispensable for wilting symptoms in plant. GALA effectors jointly contribute to pathogenicity much more on *Arabidopsis* than on tomato[18]. AWR gene family collectively contributes to bacterial virulence on tomato, although AWR2 is the major contributor to virulence. HLK2 (HLK family, three members) played an important role in bacterial fitness in planta. All these symptoms are explained to a functional accumulation among effectors. Since the normal plant pathogenicity assays was not sensitive enough to reveal subtle phenotypic differences[19–20]. Macho developed a CI assay to detect individual contribution of T3E to bacterial fitness in a more sensitive manner than monitoring symptom development in pathogenicity assay.

In present study, the CI assay on *R. solanacearum* OE1-1 bacterial fitness in different host plants was investigated and eggplant was proved to be appropriate for this novel assay.

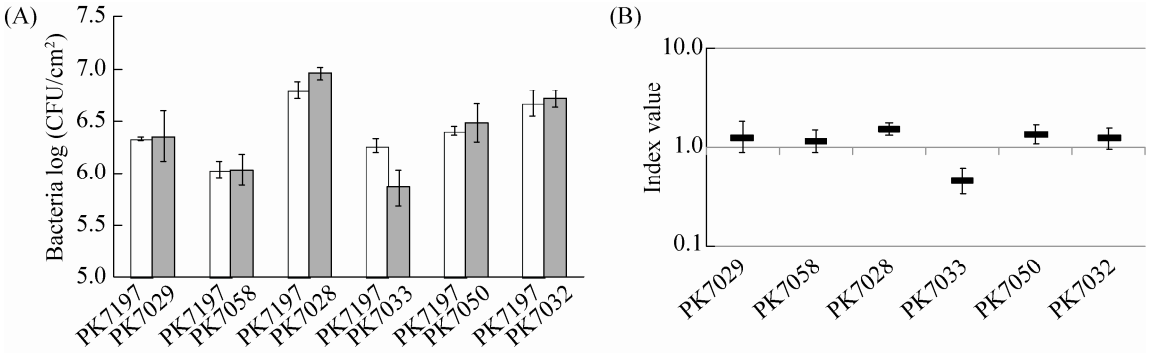

Figure 6. Bacterial growth and CI assay. A: bacterial growth of RK7197 and *skwp* deletion mutant at 3 days post inoculation in a mixed infection in eggplant leaves. B: CI mean value generated from mixed inoculation of RK7197 and single *skwp* deletion mutant in eggplant leaves. Each experiment is repeated in triplicate and error bars represent the standard error.

Furthermore, we identified the contribution of six *skwp* genes to their bacterial proliferation through CI assay. All six SKWP effectors were found to be required for bacterial growth in eggplant tissues but affected the bacterial fitness differently in planta. The mutant with the deletion of *skwp4* exhibited a reduced bacterial fitness in eggplant while others not, which indicated SKWP4 effector play a important role than other members. Macho et al. identified several T3Es among tomato, eggplant and bean, and confirmed that the contribution to fitness of T3Es can differ quite significantly on different host plants[9]. Wild type strain and mutant strains carry kanamycin and gentamicin antibiotics respectively which evaluate the individual contribution among effectors sensitively and efficiently in his method. Our study distinguished the wild type strain and mutant strains by adding gentamicin and polymyxin B antibiotic. The identification was quantitative but not as sensitive as Macho's method. Therefore, the identification of SKWP to bacterial fitness required the mutants more representative characteristic. Further study on consecutive deletion mutant and complemented mutants of SKWP would encourage us characterizing the contribution of these effectors to bacterial fitness and broaden our knowledge of the virulence strategies developed by *R. solanacearum*.

However, we should be clear that the competition experiment in plant leaf tissues is a way to monitor bacterial fitness in specific environment which is not appropriate to be considered as a representative assay to replace traditional pathogenicity assay. Because *R. solanacearum* invasion stage was complex and multistage. The bacteria has to attach to the root, find nutrients, multiply, migrate into plant tissues, and then penetrate the xylem in order to cause wilting disease[21–23]. The current leaf-infiltration injection mode ignores several key infectious processes. Nevertheless, the CI assay provides a new sight to discriminate 6 *skwp* members' contribution on bacterial fitness in specific host plant. This knowledge will be helpful in the future to demonstrate functional involvement or functional independence of the whole SKWP family.

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应用竞争力指数分析 III 型效应子 SKWP 对青枯菌在寄主植物体内增殖能力的影响

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摘要:【目的】研究 III 型效应子 SKWP 对青枯菌 OE1-1 在寄主植物体内增殖能力的影响。【方法】构建青枯菌 RK7197 (野生型突变体，带 Gm 抗性)和 SKWP 单基因缺失突变体(带 PB 抗性)，通过竞争力指数分析 SKWP 各效应子对青枯菌 OE1-1 在叶片组织内增殖能力的影响。【结果】竞争力指数适合在寄主植物茄子上分析各效应子功能，6 个 SKWP 效应子对 OE1-1 细菌增殖能力影响不同，SKWP4 影响最明显。【结论】竞争力指数可提供一个新视野来分析 SKWP 各效应子对青枯菌 OE1-1 在寄主茄子上增殖能力的影响。

关键词：青枯菌 OE1-1，SKWP，竞争力指数分析，细菌增殖

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