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Research Article 研究报告

Involvement of GALA effectors in *Ralstonia solanacearum* disease development towards two host plants

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Abstract: [Objective] By testing the virulence of single and multiple GALA mutants generated in *Ralstonia solanacearum* strain OE1-1, we evaluated the pathogenicity of these effectors in different host plants. **[Methods]** The deletion mutant of *R. solanacearum* OE1-1 was constructed through double crossover. Deletion of the target gene was confirmed by colony PCR. Virulence assay and bacterial multiplication were measured by root-cutting and leaf infiltration respectively. Tobacco (*Nicotiana tabacum* cv. Bright Yellow) and tomato (*Lycopersicon esculentum* cv. Moneymaker) were adopted as host plants to inoculate. **[Results]** Deletion mutants of each effector gene barely affected the bacterial virulence in tobacco. A mutant RK7022 with the deletion of all seven *gala* genes showed 2-days delayed virulence on tobacco compared with the wild type. The bacterial multiplication capacity of deletion mutants correlated with the phenotypes on host plants. Pathogenicity test on tomato among GALA mutants and wild type OE1-1 showed no difference. **[Conclusion]** GALA effectors contributed collectively to pathogenicity of OE1-1 on tobacco but not on tomato. Some effectors might play more important roles in *R. solanacearum* pathogenicity.

Keywords: *Ralstonia solanacearum* OE1-1, GALAs, pathogenicity, bacterial multiplication, tobacco, tomato

Many Gram-negative pathogenic bacteria infect plant and animals through the type III secretion system (T3SS) to cause symptoms on their respective hosts[1–2]. The T3SS is a syringe needle-like structure consisting of inner and outer membrane rings and a protruding filament called a pilus which works as a conduit to deliver an array of bacterial proteins into host cells[3–5]. Those bacterial proteins, named type III effectors (T3Es) are injected into the cytosol of eukaryotic cells to modulate host defense signaling pathways and to promote disease. However, some effectors are recognized by a cognate resistance protein, thereby triggering host defenses, resulting in a rapid and intense host defense response known as the hypersensitive response (HR)[6]. Previous researches focused on

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the role of the T3Es which could modify various plant cellular function to promote the fitness of the pathogen[7–8], however limited success was achieved.

Ralstonia solanacearum is a Gram-negative β-proteobacterium pathogenic to plants and responsible for the development of bacterial wilting disease on more than 200 plant species from 50 botanical families, including economical crops such as eggplant, tomato, tobacco and banana[9]. It has been ranked the top two most important bacterial plant pathogen, following the first one *Pseudomonas syringae*. This pathogen endangers the food safety in tropical and subtropical agriculture, especially in China, Bolivia, Bangladesh and Uganda. It has been reported that T3Es are involved in controlling the host invasion stages but the mechanism remains unclear[10].

It is a tough work to study the physiological and molecular functions of the T3Es[11–13]. So far, only a few number of T3E of *R. solanacearum* strain GMI1000 have been biochemically characterized, such as GALA family (seven effectors), AWR family (five effectors). GALAs possess an F-box domain and Leu-rich repeat (LRR) which interact with *Arabidopsis* ASK proteins, mimicing plant ubiquitin E3 ubiquitin ligases[14–17]. Single *gala* mutants derived from GMI1000 barely affected their pathogenicity, whereas the septuple mutant shows less pathogenic on *Arabidopsis* and delayed virulence on tomato.

In present study, we isolated a *R. solanacearum* strain OE1-1 from Japan. Previous genomic analysis showed that GALA effectors from strain OE1-1 and GMI1000 shared high homologous and identity (97%–99%). Strain OE1-1 (race 1, biovor 3) causes lethal wilting disease on tobacco and tomato whereas GMI1000 results in wilting disease on *Arabidopsis* and tomato[18]. For GMI1000, none of the seven single *gala* mutants are affected in their pathogenicity, whereas the septuple mutant shows less pathogenic on *Arabidopsis* and delayed virulence on tomato. To our best knowledge, the contribution of GALA effectors to pathogenicity of *R. solanacearum* strain OE1-1 remains unknown. In this study, we constructed single and multiple deletion mutants of *gala* generated from OE1-1, investigated the role of these effectors by observing the phenotypes of infected host plants and measuring the internal bacterial multiplication. Tobacco and tomato were chosen as the susceptible host plants for OE1-1 instead of *Arabidopsis*. Through comparing and analysis, we found that GALA effectors of OE1-1 were essential for disease on tobacco but not on tomato which was different from GMI1000. Single *gala* mutants of OE1-1 barely affected their virulence on tobacco, but the absence of all seven *gala* genes resulted in delayed virulence on tobacco.

1. Material 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 (race 1, biovar 3) and its derivative mutants were streak on BG medium (1.0% of bacto peptone, 0.1% of yeast extract, 0.1% of casamino acids, 0.5% of glucose, and 1.5% of agar) and incubate at 28 °C for 2 days. B medium (1.0% of bacto peptone, 0.1% of yeast extract, and 0.1% of casamino acids) was used to inoculate with cells of a selected single colony and incubated overnight at 28 °C. *Escherichia coli* DH12S (Invitrogen Corp., Carlsbad, CA, USA) and S17-1 were grown on Luria-Bertani (LB) medium (Miller, 1992; 1.0% tryptone, 0.5% yeast extract, and 0.5% NaCl) and incubate at 37 °C overnight. *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 μg/mL; kanamycin (Km), 50 μg/mL; polymyxin B (PB), 50 μg/mL. And 5-bromo-4-chloro-3-indolyl-beta-D-galactopy-ranoside (X-gal), 40 μg/mL, isopropyl-beta-D-thiogalactopyranosiade (IPTG), 100 μmol/L.

Table 1. Strains and plasmids used in this study

Strains	Description	References
R. solanacearum		
OE1-1	Wild type (race 1 biovar 3)	[19]
RK7007	OE1-1 Δ*gala3*	This study
RK7008	OE1-1 Δ*gala2*	This study
RK7009	OE1-1 Δ*gala6*,*7*	This study
RK7017	OE1-1 Δ*gala4*,*5*,*6*,*7*	This study
RK7018	OE1-1 Δ*gala3*,*4*,*5*,*6*,*7*	This study
RK7019	OE1-1 Δ*gala2*,*3*,*4*,*5*,*6*,*7*	This study
RK7020	OE1-1 Δ*gala1*	This study
RK7022	OE1-1 Δ*gala1*,*2*,*3*,*4*,*5*,*6*,*7*	This study
RK7024	OE1-1 Δ*gala7*	This study
RK7025	OE1-1 Δ*gala4*	This study
RK7026	OE1-1 Δ*gala5*	This study
RK7036	OE1-1 Δ*gala6*	This study
E. coli DH12S	*araD139 Δ*(*ara*, *leu*)*7697 ΔlacX74 galU galK mcrA Δ*(*mrr-hsdRMSmcrBC*) *rpsL deoR Ø80dlacZΔM15 nupG recA1/F9proAB1 lacIq ZΔM15*	Invitrogen
S17-1	*recA pro hsd*R RP4-2-Tc::Mu-Km::Tn*7*	[20]
Plasmids		
pBluescript II KS+	Ampicillin resistance	Stratagene
pK18mobsacB	Kanamycin resistance, *oriT sacB*	[21]
pKP0914-1	Deletion of rsp0914 (*gala1*)	This study
pKP0672-1	Deletion of rsp0672 (*gala2*)	This study
pKP0028-1	Deletion of rsp0028 (*gala3*)	This study
pKC1800-1	Deletion of rsc1800 (*gala4*)	This study
pKC1801-1	Deletion of rsc1801 (*gala5*)	This study
pKC1356-1	Deletion of rsc1356 (*gala6*)	This study
pKC1357-1	Deletion of rsc1357 (*gala7*)	This study

1.2 Construction of single deletion 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. The amplified PCR fragments were run on 0.8% agarose gel electrophoresis and purified by E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, Doraville, GA, USA), then cloned on pre-digested vector pBluescript II 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. Plasmid DNA was purified using GenElute Plasmid Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). 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′-TGTAAAACGACGGCC AGT-3′) or RV-M (5′-CAGGAAACAGCTATGAC C-3′) and analyzed with Applied Biosystems 3130 genetic analyzer (Applied Biosystems).

pKC(P)xxxx was transferred from *E. coli* S17-1 into *R. solanacearum* strain. Deletion mutant strains were generated through two consecutive homologous recombination events (Figure 1). A donor *E. coli* S17-1 containing pKC(P)xxxx plasmid (1 mL) and a recipient *R. solanacearum* OE1-1 strain

Table 2. Primers used in this study

Primers	Sequences (5′→3′)	References
OEC1356A51	CCGAGCCGAAGCTGATCGGCCACG	This study
OEC1356B51	ggatccCGCCGTCTCCGTTACCTATCCATG	This study
OEC1356A31	ggatccATGTGACGTGTGCTTGAGGTGC	This study
OEC1356B31	ATATAGGGATGGGAAGCGCTG	This study
OEC1357A51	CCAAGGTGCTGGAGGCCAATAC	This study
OEC1357B52	ggatccCAGCTCCACTGCATGACCATG	This study
OEC1357A31	ggatccGGCGGGGCGGCACTGTTTGCCTTG	This study
OEC1357B31	CTCTCTCCTTGTGTGATCGATCCATC	This study
OEC1800A51	TCACGGCGGCGGATGTCGAGCGCG	This study
OEC1800B51	ggatccCGGCGGTGCCACCCACCGCGCCCG	This study
OEC1800A31	ggatccGGCACCGCCATCGGGCCCG	This study
OEC1800B31	CCGGATAGTTGCCCGCGCGC	This study
OEC1801A51	GACATCGGCAACAACGGCATC	This study
OEC1801B51	ggatccGCGCCACGCCCAGTCTGCTC	This study
OEC1801A31	ggatccACATGCCGGTGAGTTTGCCGGCG	This study
OEC1801B31	AGACCCACCTGCTGTGGGTGCCG	This study
OEP0028A51	GACCTGGATGCTCGTGCTGCGCG	This study
OEP0028B51	ggatccTTCCGTCCGTGGCTCCGGCAAACG	This study
OEP0028A31	ggatccGGGGTGCCAGGGCATCCTCGCAAC	This study
OEP0028B31	CGACAAATTCCTGATCGCCTGATC	This study
OEP0914A51	CACTACGGAAACGAGGTCGCATTCAC	This study
OEP0914B51	ggatccGGCAGTGCGATCGTCCTTTCTG	This study
OEP0914A31	ggatccGATCGCCCCGCGCCGGGCAGGACG	This study
OEP0914B31	GCCTTCGCAATCCGGGCCGTGGGCGCG	This study
OEP0672A51	TAATCAAAAGTGACTCCGAAGTGC	This study
OEP0672B51	ggatccGGAAGCCAGCCCGCCCAGGGGTG	This study
OEP0672A31	ggatccCGCTGCGGGACCGTTCTGCAACG	This study
OEP0672B31	GTGGTGGAGCACGATGCCGTGTTCG	This study

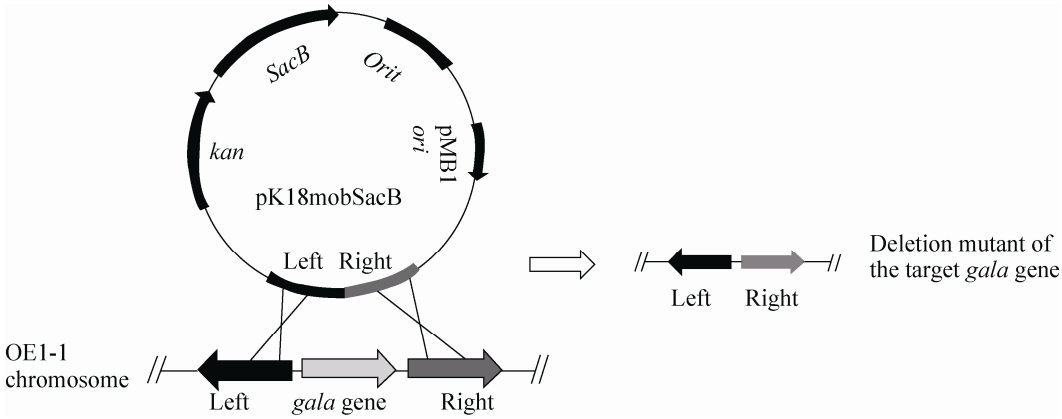

Figure 1. Construction of deletion mutant of target *gala* gene.

(1 mL) were incubated overnight in LB medium with kanamycin and B medium, respectively, and then mixed together. After centrifugation, cells were suspended in 5 mL of 10 mmol/L autoclaved $MgSO_4$, and collected on a 0.45 μm-membrane filter. The membrane was incubated on BG agar medium at 28 °C overnight. Cells grown on the membrane were immersed in 10 mmol/L $MgSO_4$ solution, and spread on BG supplemented with kanamycin and polymyxin B. Cells from several colonies were grown in B media for 5 h and spread on B agarose media supplemented with 10% sucrose. Colonies were replicated on both BG agar with polymyxin B and BG agar with kanamycin and polymyxin B. Clones, which grew only on BG agar with polymyxin B, were subjected to colony PCR (Figure 2-A). Paq5000 polymerase with 10×Paq PCR buffer (Stratagene) and 10×PCR enhancer (Invitrogen Corp.) were used for colony PCR under the condition 85 °C for 5 min, 35 repeats of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 1 min.

1.3 Construction of multiple deletion mutants

A strategy was exactly same for single gene deletion. Instead of OE1-1, the already-constructed deletion mutant was used for conjugation with *E. coli* S17-1 containing pK18mobsacB-based plasmid. They were incubated respectively overnight in LB medium with kanamycin and B medium respectively and mixed together. After centrifugation, cells were suspended in 5 mL of 10 mmol/L autoclaved $MgSO_4$, and collected on a 0.45 μm-membrane filter. The membrane was incubated on BG agar medium at 30 °C overnight. Cells grown on the membrane were immersed in 10 mmol/L $MgSO_4$ solution, and spread on BG supplemented with kanamycin and polymyxin B. Cells from several colonies were grown in B media for 5 h and spread on B agarose media supplemented with 10% sucrose. Colonies were replicated on both BG agar with polymyxin B and BG agar with kanamycin and polymyxin B. Clones, which grew only on BG agar with polymyxin B, were subjected to colony PCR (Figure 2-B).

1.4 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/g. 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 tomato (*Lycopersicon esculentum* cv. Moneymaker) and tobacco (*Nicotiana tabacum* cv. Bright Yellow) respectively. Tomato plants were cultivated in rock-wool (Nittobo) and tobacco plants were cultivated in pots containing a mixture of

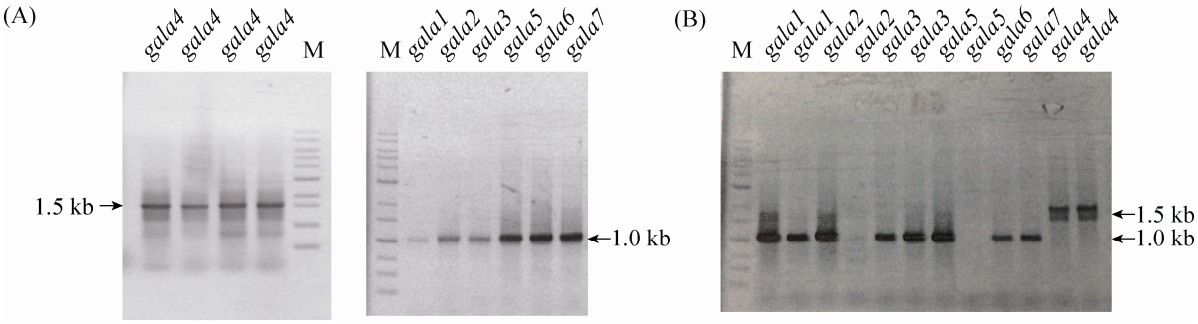

Figure 2. Colony PCR results of mutant. A: colony PCR results of single GALA deletion mutant; B: colony PCR results of RK7022 deletion mutant.

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.

1.5 Bacterial population dynamics in infiltrated leaf tissue

Internal bacterial populations were determined at selected time intervals after inoculation. 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 B agar 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.

2. Results

2.1 Comparison of GALA effectors between strain OE1-1 and GMI1000

In 2006, Angot et al. identified a group of seven genes from *R. solanacearum* GMI1000 that contained a conserved GAxALA sequence in their Leucine-rich repeats (LRR) and named as "GALA" protein[15]. GMI1000 contains 74 effectors of which several effectors are very much conserved in phylotype I Japanese strain OE1-1[22]. Genomic analysis of the *gala* genes in strain OE1-1 showed some interesting features: four of the *gala* gene *gala4*, *gala5*, *gala6* and *gala7* (*RSc1800*, *RSc1801*, *RSc1356*, *RSc1357*) located on the chromosome whereas *gala1*, *gala2* and *gala3* (*RSp0914*, *RSp0672*, *RSp0028*) lived on the megaplasmid. The GALA family effectors are mostly 458-1029aa in size (Table 3). Members of this family contain a Leucine-rich repeat and a F-box domain. Comparison analysis of GALA effector sequences from OE1-1 revealed that they all shared high identity and similarity with GMI1000 (97%–99%).

2.2 GALA effectors are important for disease on tobacco

Deciphering effector function is essential to understand the molecular interaction between pathogens and their hosts[23–25]. It has been reported that multigenic family members contribute to pathogenicity synergistically but dispensable individually. Our experiments verified this consecution in Japanese *R. solanacearum* strain OE1-1. Plants were inoculated with deletion mutants using the root-cutting method described in "Materials and methods". Deletion of single effector gene from *gala* family did not affect the bacterial pathogenicity on tobacco (Figure 3-A). The lack of

Table 3. Comparison of GALA effectors between GMI1000 and OE1-1

Gene in OE1-1	GALA	Size/aa	Features	Distribution in Rs species[a]	Identities to GMI1000/%
RSp0914	GALA1	655	Leucine Rich Repeats	Variable	99
RSp0672	GALA2	1029	F-box proteins	Conserved	98
RSp0028	GALA3	505		Conserved	97
RSc1800	GALA4	458		Conserved	99
RSc1801	GALA5	533		Conserved	99
RSc1356	GALA6	604		Conserved	97
RSc1357	GALA7	646		Variable	99

[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".

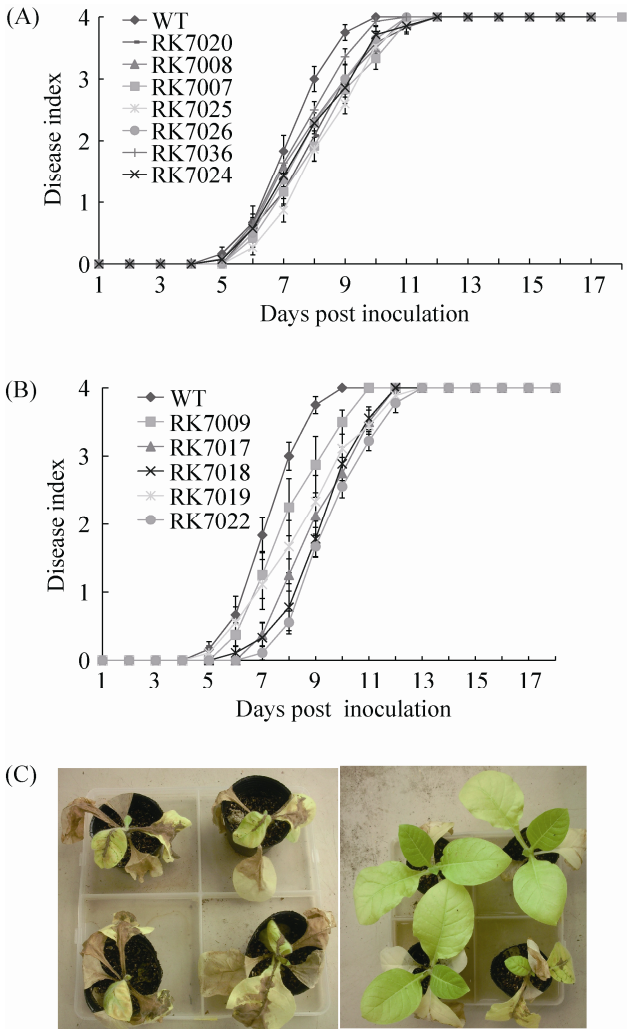

Figure 3. Pathogenicity test of OE1-1 and mutants on tobacco (*Nicotiana tabacum* cv. Bright Yellow). A: pathogenicity test of single deletion mutants on tobacco. B: pathogenicity test of multiple deletion mutants on tobacco. Bacterial suspension was poured onto the root wounded tobacco plants to achieve a final concentration of 10^7 CFU/g. Disease symptoms were scored daily for 18 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% wilted). Each bacterial inoculation was tested on at least 4 plants and was repeated in triplicate. The average and standard error were calculated. C: tobacco plants inoculated with OE1-1 (left) and RK7022 (right). Pictures were taken 9 days post-inoculation.

phenotype of single mutants could be explained by a functional overlap of these proteins. To test this hypothesis, multiple deletion mutants were constructed and tested on tobacco. Plants inoculated with the septuple deletion mutant RK7022 showed 2-days delayed wilting symptom on tobacco, but eventually wilted in 18 days (Figure 3-B, 3-C). Consecutive deletion mutants RK7019 (Δ*gala*2-7), RK7018 (Δ*gala*3-7) and RK7017(Δ*gala*4-7) affected the *R. solanacearum*'s pathogenicity on tobacco variously.

The internal bacterial population was measured by leaf-infiltration, since this methodology was reported to be more sensitive and quantitative than plant disease scoring[17]. As shown in Figure 4-A, no statistical significant difference of bacterial growth was found among single deletion mutants and wild type except RK7025 (Δ*gala4*), RK7026 (Δ*gala*5), RK7036 (Δ*gala*6) and RK2024 (Δ*gala*7) (P<0.05). These results suggested that OE1-1 with the deletion of *gala4,5,6,7* affected the cell growth much more than other *galas*. These data correlated with the phenotypes of the single mutants and supported an important role of *gala4,5,6,7* in *R. solanacearum* OE1-1 virulence.

To better understand the functional role, the bacterial growth of multiple deletion mutants were measured in tobacco leaves (Figure 4-B). The multiplication capacity of the multiple mutants was reduced with the consequent deletion of the *galas*. The bacterial growth of RK7022 was significant reduced compared with the wild type strain. As shown in Figure 5, the initial bacterial growth of RK7022 increased from 5.01-log to 7.11-log after 3 days inoculation while the bacterial growth of wild type varied from 5.03-log to 7.38-log. Wild type strain OE1-1 exhibited 0.64-log preservation of bacterial growth over RK7022 after 2 days inoculation (P<0.01). These results indicated that GALA effectors jointly contributed to the growth of bacterial population.

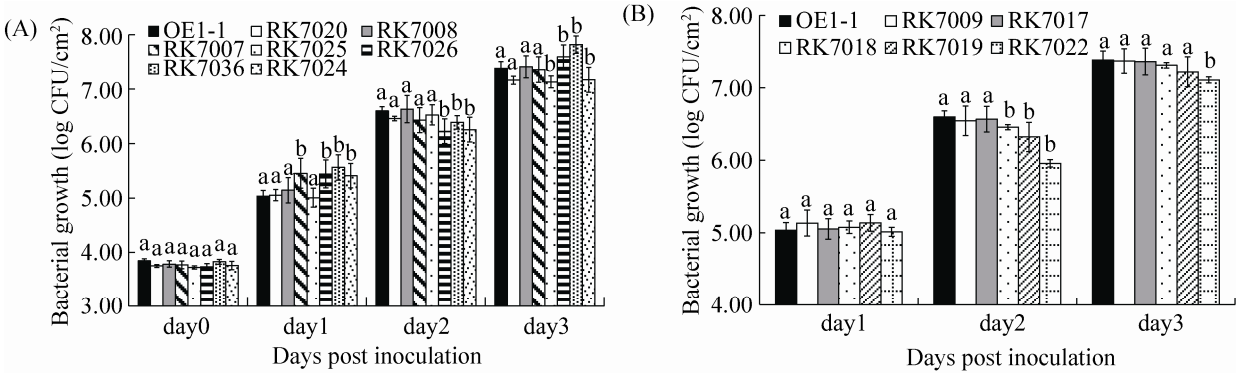

Figure 4. Bacterial population in leaves infiltrated with OE1-1 and mutants. A: bacterial population of single deletion mutants. B: bacterial population of multiple deletion mutants. Bacterial cell suspensions (10^6 CFU/mL) of OE1-1 and mutants were infiltrated into leaves of tobacco (*Nicotiana tabacum* cv. Bright Yellow). Each assay was repeated in three successive trials, and four plants were treated within each trial. The average and standard error were calculated. Statistically significant groups were calculated using *t*- test (P<0.05).

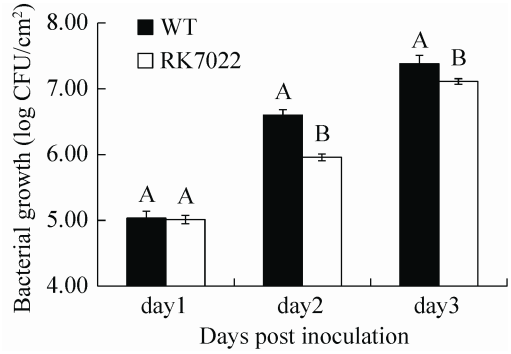

Figure 5. Bacterial population in leaves infiltrated with OE1-1 and RK7022. Bacterial cell suspensions (10^6 CFU/mL) of OE1-1 and RK7070 were infiltrated into leaves of tobacco (*Nicotiana tabacum* cv. Bright Yellow). Each assay was repeated in three successive trials, and four plants were treated within each trial. The average and standard error were calculated. Statistically significant groups were calculated using *t*-test (P<0.01).

2.3 GALA effectors contribute differently towards host plants

We also evaluated the combined contribution of the *gala* gene family in pathogenicity towards different host plants. All the deletion mutants were tested on tomato. Angot et al. (2006) reported that single deletion mutations of *gala* did not affect *R. solanacerum* strain GMI1000 pathogenicity on tomato, but the septuple deletion mutant resulted in a significant reduction of virulence[15]. In the terms of strain OE1-1, tomato inoculated with single deletion mutants and septuple deletion mutant showed similar wilting progression as wild type in 18 days (Figure 6). These results demonstrated that *gala* genes of OE1-1 were essential for bacterial pathogenicity on tobacco but not on tomato.

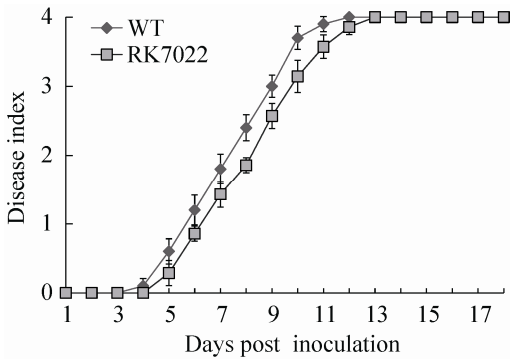

Figure 6. Pathogenicity test of OE1-1 and RK7022 on tomato (*Lycopersicon esculentum* cv. Moneymaker). Bacterial suspension was poured onto the root wounded tomato plants to achieve a final concentration of 10^7 CFU/g. Disease symptoms were scored daily for 18 days. Plants were rated according to a scale ranging of 0 to 4 (0: no wilting; 1: 1%–25% wilting; 2: 26%–50% wilting; 3: 51%–75% wilting; 4: 76%–100% wilted). Each bacterial inoculation was tested on at least 4 plants and was carried out multi-replicates. The average and standard error were calculated.

3. Discussion

In this study, we revealed the involvement of GALA effectors on pathogenicity in the Japanese *R. solanacearum* strain OE1-1. Although strain OE1-1 and GMI1000 both belong to phylotype I, they exhibit different pathogenicity towards host plants. The cumulative disruption of the seven *gala* genes strongly affects virulence of *R. solanacearum* strain GMI1000 on *Arabidopsis* and less on tomato[15]. While in our study, the septuple GALA mutant (RK7022) resulted in delayed virulence (2-day) on tobacco in comparison to wild type strain. No significant difference of wilting symptom was found between OE1-1 and RK7022 when inoculated on tomato. This suggested that GALA effectors from different *R. solanacearum* strains contributed in restriction of host range. Furthermore, we found the multiple mutants derived from OE1-1 showed reduced bacterial growth with the consequent deletion of *galas* in tobacco, but tobacco plants inoculated with mutants eventually died. These results suggested that the effect of GALAs appeared to be additive for full virulence of *R. solanacearum*. One GALA protein might be the virulence determinant, whereas the other members of the family act synergistically, reinforcing this function. Similar results are reported from AWR family and HLK family. The deletion of all *awr* genes severely impairs its capacity to multiply in host plants while AWR2 is the major contributor to virulence[17]. The absence of all three *hlk* genes exhibits reduced virulence on tomato and HLK2 appears to be more important than other two members[22]. These features of the multigenic family suggest that *R. solanacearum* virulence involves a small number of effectors with a key effect and many effectors with a weak, additive contribution.

In *Arabidopsis*, F-box proteins are the essential adaptor proteins linking the protein to be ubiquitinated to the SCF E3-ubiquitin ligase via their interaction. GALAs contain a plant-like F-box domain could alter protein levels through host ubiquitination pathway in *Arabidopsis*[26]. Genome analysis in *Arabidopsis* and rice reveals the presence of F-box proteins with a C-terminal lectin-related domain homologous with Nictaba, a jasmonate-inducible lectin from tobacco that is shown to interact with the core structure of high-mannose and complex N-glycans[27]. Over-expression of *OsDRF1* which containing a highly conserved F-box domain in tobacco results in enhanced disease resistance against tomato mosaic virus (ToMV)[28]. Whether the GALAs of OE1-1 use the similar strategy to promote disease in tobacco need more information to explain. Future complementation studies and a yeast two-hybrid (Y2H) screen would enable us to understand the GALAs function in the interaction between *R. solanacearum* OE1-1 and tobacco.

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III 型效应子 GALA 对青枯菌在两种寄主植物上致病性的影响

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摘要：【目的】研究 III 型效应子 GALAs 对青枯菌 OE1-1 在不同寄主植物致病性上的影响。【方法】构建青枯菌 OE1-1 的多种 GALA 缺失突变体，通过根切和叶片注射等方法研究 GALAs 对青枯菌 OE1-1 致病力和细胞内增殖能力的影响。【结果】GALA 多基因缺失突变体对寄主烟草的致病力减弱，在烟草体内细菌繁殖能力较野生型明显降低，但在寄主番茄上不影响其致病性。【结论】GALA 效应子对青枯菌 OE1-1 在烟草植株致病性上展现协同作用。

关键词：青枯菌 OE1-1，GALAs，致病性，细菌增殖，烟草，番茄

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