**SGE1 is involved in conidiation and pathogenicity of
Fusarium oxysporum f. sp. cubense**

Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2017-0638.R2
Manuscript Type:	Article
Date Submitted by the Author:	27-Dec-2017
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Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	<i>Fusarium oxysporum</i> f. sp. cubense, pathogenicity, colonization, proteomics analysis

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Fusarium oxysporum f. sp. *cubense*

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18

19 **Abstract**

20 The ascomycete fungus *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc TR4) causes
21 vascular wilt diseases in banana (*Musa* spp.). In the present study, the roles of SGE1
22 in regulating growth, conidiation, and pathogenicity of Foc TR4 were investigated.
23 Deletion of *SGE1* did not influence vegetative growth, but impaired the conidiation of
24 Foc TR4. Besides, the *SGE1* deletion mutant basically lost pathogenicity on banana
25 plantlets. Observation under the microscope indicated that the penetration and
26 colonization processes were severely impaired in the *SGE1* deletion mutant.
27 Proteomics analysis suggested that SGE1 regulated the production of a series of
28 proteins of Foc TR4. Taking together, our results suggest that SGE1 plays important
29 roles in regulating conidiation and pathogenicity in fungal pathogen Foc TR4.

30

31 **Keywords:** *Fusarium oxysporum* f. sp. *cubense*; pathogenicity; colonization;
32 proteomics analysis

33

34

Introduction

The fungus *Fusarium oxysporum* is found in soils throughout the world (Beckman, 1987). The pathogenic strains of *F. oxysporum* were grouped into formae speciales depending on their host range (Nelson, 1981). *Fusarium oxysporum* f.sp. *cubense* (Foc) is the causal agent of wilt disease in banana (*Musa* spp.), which caused serious economic losses worldwide. Foc consist of 4 physiological races, among which the *Fusarium oxysporum* f.sp. *cubense* race 4 (also named *Fusarium oxysporum* f. sp. *cubense* tropical race 4, Foc TR4; taxid:1229665) could infect almost all the banana cultivars including ‘Dwarf Cavendish’ (*Musa* sp. AAA group) (Guo et al., 2014). Foc TR4 could persist in soil for a long time as a saprophyte and there are few effective means for managing the pathogen. Foc TR4 could switch from a saprophyte into a pathogen after successfully colonizing on roots of banana plantlets.

To overcome the plant immune systems, pathogenic fungi have evolved complex ways to ensure its infection and colonization of the host, including secretion of cell wall-degrading enzymes and effectors (Chisholm et al. 2006), production of toxic secondary metabolites (Stergiopoulos et al. 2013), modulation of host cell pH value (Miyara et al. 2010) and so on. A transcriptional regulator SGE1 (SIX Gene Expression 1) was found to be participated in regulating the growth and pathogenicity processes in several plant pathogens. *SGE1* was first identified in the pathogen *Fusarium oxysporum* f. sp. *lycopercisi* (Fol) (Michielse et al. 2009a), and shows homology to the transcriptional regulators of the human pathogen *Candida albicans* *WOR1* and *Histoplasma capsulatum* *RYPI* (Huang et al. 2006; Nguyen and Sil 2008). Further research revealed that SGE1 serve as a controller of parasitic growth and is required for full virulence in Fol (Michielse et al., 2009b). Similar phenotypes were found in the *SGE1* deletion mutant of other plant pathogen *Botrytis cinerea*

(Michielse et al. 2011), *Fusarium graminearum* (Jonkers et al. 2012), *Verticillium dahliae* (Santhanam and Thomma 2013), *Fusarium verticillioides* (Brown et al., 2014), and *Zymoseptoria tritici* (Mirzadi et al. 2014). Furthermore, SGE1 also regulate secondary metabolite biosynthetic especially synthesis of toxin in *Fusarium graminearum* (Jonkers et al. 2012) and *Fusarium verticillioides* (Brown et al. 2014). Besides, the protein kinase A phosphorylation (Pka) sites are required for the normal function of SGE1 proteins (Michielse et al. 2009b; Yu et al., 2015). RT-PCR analysis showed that SGE1 is required for the expression of effector genes in Foc (Michielse et al. 2009a); furthermore, the RNAseq analysis revealed that constitutive expression of the *SGE1* gene induced the expression of a similar set of plant-responsive genes, including most effector genes (van der Does et al. 2016). However, whether SGE1 regulated the production of other functional proteins as a transcriptional regulator remains unclear.

In the present study, the roles of SGE1 in regulating the growth, conidiation and pathogenicity of Foc TR4 were investigated; and the potential downstream targets of SGE1 were explored using the proteomics assay.

Materials and methods

Fungal strains and culture conditions

Fusarium oxysporum f.sp. *cubense* race 4 (Foc4) was isolated from diseased rhizomes of banana (*Musa* spp.) cultivars ‘Brazil’ (AAA group) in Hainan of China (Guo et al. 2014). All the Foc TR4 strains including wild type (WT) and the mutants were maintained on the Malt extract agar medium (Oxoid, Basingstoke, England) at 28 °C.

Vector constructions

Vector pBS-NEO carrying the Neomycin phosphotransferase gene (NPTII)

86 conferred resistance to Geneticin (G418) was used to construct the replacement vector.
87 The gene knocking-out strategy was described in Fig. S1A: the flanking region of the
88 *SGE1* nucleotide were amplified, excised with EcoRI/BamHI and NotI/SacI
89 respectively, and ligated into vector pBS-NEO to construct the replacement vector.
90 The replacement vector was linearized with SacI before transformation. Vector V21
91 carrying the Hygromycin B phosphotransferase gene (HPT) conferred resistance to
92 Hygromycin B was used to construct the complementation vector. A 2.4 kb fragment
93 containing the *SGE1* nucleotide together with a 1.4 kb upstream nucleotide was
94 amplified, excised with BamHI/XbaI, and ligated into vector Vector V21 to construct
95 the complementation vector. The replacement vector was linearized with XbaI before
96 transformation.

97 **Transformation of Foc TR4, PCR diagnosis, and single conidia purification**

98 Conidia of Foc TR4 were cultured in 200 mL liquid complete medium with the
99 initial concentration of 10^6 conidia mL^{-1} . Then the mycelium were collected and
100 washed two times with 1 M sorbitol, transferred to the lysing buffer (1 M sorbitol
101 containing 10 mg mL^{-1} lysing enzyme) and incubated for 3 h at 28 °C, 100 rpm to
102 digest the cell wall. The cell debris were removed by filtering with nylon membrane.
103 After that, the protoplasts were collected by centrifugation with 2000 rpm at 4 °C,
104 washed two times and resuspended with STC buffer (1 M sorbitol, 50 mM Tris-Cl, 10
105 mM CaCl_2 , pH 7.4) to the final concentration of 10^8 mL^{-1} CFU. For the protoplast
106 transformation, 200 μL protoplasts were mixed with 100 μL linearized vector and
107 incubated on ice for 20 min. Then the mixture was added with 1 ml 40% PEG
108 (dissolved in STC buffer) and reversed gently. After that, 5 mL liquid regeneration
109 medium (1g L^{-1} yeast extract, 1g L^{-1} casein, 6 M sucrose) was added into the
110 protoplast and cultured at 28 °C, 100 rpm for 4 h. For the mutant selection, the

111 regenerated protoplasts were mixed with the regeneration medium with 1% agar at
112 about 50 °C and spread on the petri dish. After solidification of the regeneration
113 medium, another same volume of medium containing 200 $\mu\text{g mL}^{-1}$ G418
114 (Sigma-Aldrich, St Louis, MO, USA) was spread on the upper level. After culture for
115 2-3 days, the G418-resistant strains which grow into the upper medium were isolated
116 and analyzed by PCR with the primer pairs showed in Table. S2. Single conidial
117 isolates of the correct transformants were obtained by spreading 100 μL of conidial
118 suspension (10^4 conidia mL^{-1}) on Malt extract agar medium. The Single conidial
119 isolated strain were confirmed by PCR. The correct mutant was then named as ΔSGEI .
120 For generation of the complementation mutants, the deletion mutant ΔSGEI was used
121 as the recipient strain to conduct the transformation, and 300 $\mu\text{g mL}^{-1}$ Hygromycin B
122 (Roche, Basel, Switzerland) was used to select the transformants.

123 For generation of the GFP-tagged strains, the plasmid pCT74 containing a sGFP
124 expressing cassette and a Hygromycin B resistant cassette was used to transform the
125 Foc TR4. The protoplasts of Foc TR4 were transformed as mentioned above with
126 linearized pCT74, and the transformants were selected using 300 $\mu\text{g mL}^{-1}$
127 Hygromycin B. Then the Hygromycin B-resistant strains were confirmed with PCR
128 analysis and microscope observation. After that, the strains were inoculated into the
129 banana plantlets to analyze the pathogenicity. The strains that showed full
130 pathogenicity compared with the Foc TR4 were selected for the further work.

131 **Growth and conidiation assay**

132 For the growth rate assays, all the Foc TR4 strains were grown for 3 day on Malt
133 extra medium, then the mycelium with 1 mm in diameter together with agar were
134 removed from the edge of the colony and transformed to the new complete medium or
135 the minimal medium. All strains were cultured for 5 days, and the diameters of the

colonies were recorded and the growth rate was calculated. For the conidia quantitation, conidia of WT and the mutant strains were collected and inoculated into 200 mL liquid CM medium and adjusted to the concentration of 10^3 mL^{-1} . Then Foc TR4 strains were cultured at 28°C, 150 rpm for 3-4 days, and the conidia number were counted under microscope.

Pathogenicity assay

Banana plantlets (*Musa acuminata* L. AAA group, 'Brazilian') were obtained from the Tissue Culture Center of Chinese Academy of Tropical Agricultural Sciences. The plants (1 plant/pot) were raised in a glass greenhouse. The plants at the five-leaf stage with a healthy root system were used for the pathogen treatment. The Foc TR4 strains were cultured in the liquid complete medium for 3 days, then the conidia were collected, washed two times and resuspended with ddH₂O to a final concentration of $10^6 \text{ conidia mL}^{-1}$. For the pathogenicity assay, 200 mL of the conidia suspension were irrigated into the root of one banana plantlet, and each treatment with different strain contained at least 20 banana plantlets. After one week, the inoculation was repeated once. Disease symptoms were recorded at 3, 4 and 5 week post-inoculation. The disease index was defined as follows: the index of the plantlet showed no disease symptoms was defined as 0; the index of the plantlet in which less than 25% of leaves showed disease symptoms were defined as 1; that more than 25% but less than 50% was defined as 2; that more than 50% but less than 75% was defined as 3; that more than 75% was defined as 4. The experiments were repeated twice.

Microscopic assay of colonization of banana root

For the microscopic assay, the banana tissue culture seedling and the GFP expressing Foc TR4 strains were used for inoculation and the subsequent analysis. The conidia suspension was prepared as mentioned above. The intact roots of the

161 banana seedlings were inoculated with 20 ml conidia suspension of 10^6 conidia mL^{-1}
162 and incubated at room temperature in a petridish. After 12 h or 24 h, the young roots
163 of the seedlings were rinsed with water, cut and placed on a glass slide. Images of the
164 banana roots were captured and analyzed with the Leica DM5000 microscope. A total
165 of 5 seedlings were used for each treatment and experiment were repeated twice.

166 **Proteomic analysis**

167 For the intracellular proteome assay, protein extraction, two-dimensional (2D) gel
168 electrophoresis, image analysis and in-gel digestion were carried out according to An
169 et al. (2016) with some modification. Briefly, cellular proteins were extracted from
170 hyphae cultured on cellophane paper plated on the Malt extract agar medium for 3 day.
171 Then aliquots of 700 μg of protein were used for 2D gel electrophoresis using the
172 Ettan IPGphor III and the Ettan DALTsix (GE Healthcare, USA) systems. Protein
173 spots of which abundance changed by > 2 -fold were excised for protein identification
174 using a MALDI-TOF/ TOF mass spectrometer (Bruker Daltonics, Billerica, MA,
175 USA). MS data were searched against the Matrix Science public website and the
176 NCBI genome database of *Fusarium oxysporum* f.sp. *cubense* race 4
177 (GCA_000350365).

178 For the extracellular protein extraction, conidia of the WT and the mutant strain
179 were cultured in 200 mL modified Czapeck liquid medium in which glucose was
180 replaced with apple pectin (Sigma-Aldrich, St Louis, MO, USA) at the initial
181 concentration of 10^3 conidia mL^{-1} . After culture for 3 days, the supernatant was
182 filtrated and centrifuged to remove impurities. To fully precipitate the proteins,
183 sodium deoxycholate (Sigma-Aldrich, St Louis, MO, USA) was added into the
184 supernatant to the final concentration of 0.03% (wt/vol), followed by incubation on
185 ice for 30 min; subsequently, the trichloric acetic acid (Sigma-Aldrich, St Louis, MO,

USA) was added into the mixture to the final concentration of 10% (wt/vol), followed by incubation on ice for another 30 min. After that, the proteins were collected by centrifugation at $16,000 \times g$ at 4°C for 30 min and were washed three times with cold acetone. For proteome assay, the dry proteins were resolved, reduced, alkylated, and digested using the filter-aided sample preparation (FASP) method. Then the tryptic peptides were labeled with the iTRAQ Reagents Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Samples of WT and the mutant were labeled with iTRAQ tags 113 and 121, respectively. After the pre-separation with Strong Cation Exchange Chromatography (SCX), the combined iTRAQ-labeled peptides were submitted for LC-MS/MS analysis. Protein identification and quantification for the iTRAQ experiments were performed using ProteinPilot™ 4.5 software (AB SCIEX Redwood City, CA, USA). The mass spectra data were used to search the Foc TR4 genome database (GCA_000350365).

Results

Generation of the $\Delta SGE1$ and the complementation mutants

The *SGE1* coding gene (FOC4_g10009186) in Foc TR4 consists of 993 bp without introns and codes a predicted protein composed of 330 amino acids. To explore its functions in Foc TR4, the nucleotide sequence of the gene was deleted using a replacement strategy; the neomycin phosphotransferase 2 (*NPTII*) gene conferring resistance to G418 was used as the selective marker. After the protoplast transformation, the G418 resistant colonies were analyzed by PCR to verify the integration locus, primer pairs 5/6 and 7/8 with one primer being located outside and one inside the construct were used (shown in Fig. S1A). As shown in Fig. S1B, at least three mutants showed the both diagnostic fragments of 5' and 3' flanking region, indicating successful integration of the transforming DNA and the loss of the target

gene sequence, and the transformants were named as $\Delta SGE1$. Then the transformants were purified by single conidia isolation. The Single conidial isolated strain were confirmed by PCR with the primer pair primer pair 9/10. In our preliminary experiment, three mutants showed similar phenotype both in growth rate and pathogenicity, so only one strain of the mutant was chosen for detailed studies. Complemented mutant strains showed Hygromycin B resistance were diagnosed by PCR with the primer pairs 11/12. The correct transformants were also purified by single conidia isolation. For generation of the GFP-tagged *SGE1* deletion mutant, the Foc TR4 expressing GFP was used as the recipient strain, and the transformation procedure was same as mentioned above.

***SGE1* is not required for vegetative growth but needed for conidiation**

For the vegetative growth assays, both complete medium and minimal medium were used to explore potential metabolic defects of $\Delta SGE1$. The results in Fig. 1A showed that there are no obvious differences in growth rate between the mutant and WT. When cultured in liquid complete medium, $\Delta SGE1$ produced about 4-fold less conidia (including both microconidia and macroconidia) compared with WT (Fig. 1B).

***SGE1* is required for pathogenicity**

Banana plantlets at five-leaf stage were used to analyze the pathogenicity of the mutant strains. As shown in Fig. 2, after 5 weeks, the banana plants treated with the WT strain showed serious disease symptoms, and the rhizome together with pseudostem were significantly blackened. While $\Delta SGE1$ were non-pathogenic on banana plants in the root dip bioassay, with a slight blackening of the rhizome. Banana plants treated with the complemented mutant strain showed similar disease symptoms as compared to the WT strain. These results indicated that *SGE1* is required

236 for pathogenicity of Foc TR4.

237 ***SGE1* is essential for colonization of banana root**

238 To determine whether the colonizing ability were impaired in $\Delta SGE1$, the conidia
239 of WT strain or $\Delta SGE1$ mutant expressing GFP were used to infect banana seedlings.
240 After incubation for 12 h, mycelium of WT successfully colonized on the banana
241 roots, showing strong green fluorescence; while only a few of hyphae of $\Delta SGE1$
242 colonize on the banana roots. After incubation for 24 h, mycelium with strong green
243 fluorescence were observed in the banana root infected with WT strain; in contrast,
244 the root infected with $\Delta SGE1$ showed only background fluorescence, with few hyphae
245 growing in the banana roots. These results suggested that the colonizing process of
246 $\Delta SGE1$ is interfered.

247 **Proteomic identification of potential downstream targets of SGE1**

248 To identify the potential downstream targets of SGE1, the cellular proteome
249 profiles between WT and $\Delta SGE1$ were examined using 2D gel electrophoresis. The
250 experiment was performed with at least three biological replicas, and the gel images
251 were analyzed using Image Master 2D software. A total of 1055 spots were detected
252 on each 2D gel after ignoring very faint spots and spots with undefined shapes and
253 areas. The quantitative image analysis revealed 13 protein spots exhibited significant
254 changes in abundance ($P < 0.05$) in $\Delta SGE1$ compared with the WT strain. (Fig. 4).
255 After tandem mass spectrometry and Mascot search analysis, the 13 protein spots
256 representing 11 genes were successfully identified (Fig. 5).

257 To obtain sufficient extracellular proteins, the Foc TR4 strains were cultured in
258 the modified Czapeck liquid medium using apple pectin as the carbon resource, and
259 proteins were extracted from the supernatant. The iTRAQ-based quantitative
260 proteomic approach was used to investigate the secretory proteome profiles of $\Delta SGE1$

261 and WT strain. Using the Foc TR4 genomic database, a total of 1091 proteins were
262 identified; and the abundance of 119 proteins were changed more than 1.5 fold in
263 $\Delta SGE1$ compared with WT strain (Table S1), with 65 down-regulated and 54
264 up-regulated. According to their biological functions, the 119 proteins were classified
265 into 17 categories, among which the functional classes “cell wall degradation”,
266 “metabolic process”, and “unkown” contained the largest number of proteins of which
267 the abundance changed in $\Delta SGE1$.

268 Discussion

269 The ascomycete fungus *Fusarium oxysporum* can live in different environments
270 and exhibit different lifestyles. As for the banana pathogen Foc TR4, it can live as
271 saprophyte in soil on diverse substrates, or live as parasitic pathogen by colonizing in
272 the xylem of banana plants (Beckman, 1987). SGE1 was found to be required for
273 colonization of the xylem system and disease development in several plant pathogens.
274 In the present study, the functions of SGE1 of Foc TR4 were investigated. The $\Delta SGE1$
275 mutant showed the same growth rate as the WT when cultured on complete medium
276 or minimal medium (Fig. 1A), indicating that SGE1 is not required for the normal
277 vegetative growth of Foc TR4. In the present study, the Foc TR4 WT strain produced
278 a mass of microconidia and a few of macroconidia when cultured in the liquid
279 complete medium; and $\Delta SGE1$ produced about 4-fold less conidia compared with WT
280 (Fig. 1B). Same phenotypes were observed in other fungi, including *F. graminearum*,
281 *Fol*, *V. dahliae* (Jonkers et al. 2012; Michielse et al. 2009b, 2011; Santhanam and
282 Thomma 2013) except *F. verticillioides* (Brown et al., 2014).

283 As a root disease pathogen, Foc TR4 could colonize on root surface of banana
284 plant, then penetrate into root and colonize xylem vessels. After that, the pathogen
285 grows toward rhizome and pseudo stem, and causes death of tissue or the entire plant,

286 leading to the wilt disease of the plant. The effect of the deletion of *SGE1* on
287 pathogenicity of Foc TR4 was examined. As showed in Fig. 2, pathogenicity of
288 Δ *SGE1* was significantly reduced compared with WT, and the complementation of
289 Δ *SGE1* largely restored the virulence. To test whether the colonization process was
290 impaired in Δ *SGE1*, the GFP tagged Foc TR4 strain were used to inoculate the banana
291 tissue culture seedlings. The results showed that only a few hyphae of Δ *SGE1*
292 colonized on the root after incubation for 12 h and 24 h. While a mass of mycelium of
293 WT successfully colonized on the root after 12 h, showing strong green fluorescence,
294 and stronger green fluorescence of mycelium was observed after 24 h (Fig. 3). These
295 results suggested that impair on the colonizing ability may be the main cause of
296 reduction of pathogenicity of Δ *SGE1*. Furthermore, inspection of the pseudostem and
297 rhizome showed that the plantlets inoculated with Δ *SGE1* only turned brown slightly,
298 while that inoculated with WT were already dark and brown (Fig. 2), indicating that
299 colonizing xylem vessels of root was also suppressed in Δ *SGE1*.

300 To explore the underlying mechanism of *SGE1* in regulating the conidiation and
301 pathogenicity of Foc TR4, the intracellular proteome assay was conducted. The results
302 showed that deletion of the *SGE1* gene lead to the change of abundance of 11 proteins
303 (Fig. 4), including 10 down-regulated protein spots representing 9 proteins and 3
304 up-regulated protein spots representing 2 proteins (Fig. 5). Among the 11 proteins, 9
305 proteins are involved in the metabolism and 2 proteins are involved in protein
306 synthesis (Table 1). It has been widely reported that plant fungal pathogens could
307 secret abundance of proteins to the host cell for successful infection, including
308 hydrolases and effectors. So, the extracellular proteome of the Foc TR4 was also
309 investigated in the present study. The iTRAQ method was employed to explore the
310 proteins that changed abundance in Δ *SGE1* compared with WT. Using this technique,

119 proteins that changed abundance over 1.5 fold in $\Delta SGE1$ were identified, and the 119 proteins could be classified into 17 categories according to their biological functions (Fig. 6). Among the 119 proteins, 19 proteins involved in metabolism were down-regulated in $\Delta SGE1$, and 9 proteins involved in plant cell wall degradation were also down-regulated (Table S1). The proteomics assays showed that a lot of proteins involved in metabolism were down-regulated in $\Delta SGE1$, suggesting that suppress of the metabolism may lead to the reduction of conidia production and the colonization of the plant root. 5 proteins involved in the fungal cell wall organization or biogenesis were also down-regulated in $\Delta SGE1$, which may be the cause of reduction of conidiation of the mutant. Besides, degrading plant cell wall is important for fungal pathogen to penetrate into the host cell. The extracellular proteomics assays showed that several hydrolytic enzymes involved in plant cell wall degradation were down-regulated in $\Delta SGE1$ (Table S1), including the Endoglucanase (D22, D48, D52 and D53), beta-1,3 exoglucanase (D24), cellobiose dehydrogenase (D35) and so on (Sreedhar et al., 1999; Götesson et al., 2002).

To summarize, SGE1 regulates the conidiation and pathogenicity of the fungal pathogen Foc TR4.

Acknowledgements

This study was supported by the Natural Science Foundation of Hainan Province (No. 20163046), the National Natural Science Foundation of China (No. 31560044), the National Natural Science Foundation of China (No. 31571967).

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407

408 **Figure captions**

409 **Fig. 1.** Growth and conidiation analysis. (A) Growth rates of WT, the deletion mutant
410 and the complementary mutant strains cultured on complete medium (CM) and
411 minimal medium (MM) for 4 days. (B) Quantitation of conidia of WT, the deletion
412 mutant and the complementary mutant strains. Bars represent standard deviations
413 (SD). Columns with different letters indicate significant difference ($P < 0.05$).

414 **Fig. 2.** (A) Disease symptoms of banana plantlets inoculated with different pathogen
415 strains after 5 weeks. (B) Disease index of the banana plantlets after the inoculation
416 for 4 and 5 weeks.

417 **Fig. 3.** Microscope analysis of infection behavior. The GFP tagged strains were used
418 to inoculate the banana tissue culture seedlings, and the roots of the seedlings were
419 cut and observed under the Leica DM5000 microscope.

420 **Fig. 4.** Two-dimensional patterns of proteins of WT and $\Delta SGE1$. Arrows indicate
421 protein spots which were down-regulated (marked with 'D') and up-regulated
422 (marked with 'U') in abundance more than 2-fold between WT and $\Delta SGE1$. The
423 protein spots are numbered corresponding to those in Table 1.

424 **Fig. 5.** Protein spots that changed abundance of WT and $\Delta SGE1$. The number of each
425 protein spot corresponds to its listing in Table 1.

426 **Fig. 6.** Biological functional categories of the extracellular proteins that changed
427 abundance in $\Delta SGE1$ compared with WT.

428

Table 1. Proteins Identified by Quadrupole Time-of-Flight Tandem Mass Spectrometry. **SC**: amino acid sequence coverage for the identified proteins. **WT vs $\Delta SGE1$** : average fold change of relative abundance of specific spot of WT versus $\Delta SGE1$ from three biological repeats. ∞ : corresponding spot appeared in the WT but not in the mutant.

Spot	Protein functions	Accession number	SC (%)	WT vs $\Delta SGE1$
D1	CDP-alcohol phosphatidyltransferase	ENH65121.1	26	2.35
D2	Glutathione S-transferase	EMT69530.1	47	2.46
D3	Glutathione S-transferase	EMT69530.1	38	∞
D4	hydrolases	ENH68281.1	28	2.53
D5	pisatin demethylase	EMT70817.1	25	2.92
D6	Phosphonopyruvate decarboxylase	EXM08516.1	21	5.22
D7	Glyceraldehyde-3-phosphate dehydrogenase	ENH68413.1	33	2.48
D8	Sterol 24-C-methyltransferase	ENH71649.1	27	2.22
D9	40S ribosomal protein S0	EMT73310.1	34	3.73
D10	Isoflavone reductase	EMT68137.1	51	2.41
U1	Succinate-CoA ligase	EXM00755.1	44	0.27
U2	Succinate-CoA ligase	EXM00755.1	15	0.51
U3	translation initiation factor	EXM02563.1	47	0.28

Fig. 1. Growth and conidiation analysis. (A) Growth rates of WT, the deletion mutant and the complementary mutant strains cultured on complete medium (CM) and minimal medium (MM) for 4 days. (B) Quantitation of conidia of WT, the deletion mutant and the complementary mutant strains. Bars represent standard deviations (SD). Columns with different letters indicate significant difference ($P < 0.05$).

27x10mm (300 x 300 DPI)

Fig. 2. (A) Disease symptoms of banana plantlets inoculated with different pathogen strains after 5 weeks. (B) Disease index of the banana plantlets after the inoculation for 4 and 5 weeks.

80x40mm (300 x 300 DPI)

Fig. 3. Microscope analysis of infection behavior. The GFP tagged strains were used to inoculate the banana tissue culture seedlings, and the roots of the seedlings were cut and observed under the Leica DM5000 microscope.

97x59mm (300 x 300 DPI)

Fig. 4. Two-dimensional patterns of proteins of WT and the Δ SGE1 mutant. Arrows indicate protein spots which were down-regulated (marked with 'D') and up-regulated (marked with 'U') in abundance more than 2-fold between WT and the mutant. The protein spots are numbered corresponding to those in Table 1.

124x195mm (300 x 300 DPI)

Fig. 5. Protein spots that changed abundance of WT and Δ SGE1. The number of each protein spot corresponds to its listing in Table 1.

72x55mm (300 x 300 DPI)

Fig. 6. Biological functional categories of the extracellular proteins that changed abundance in Δ SGE1 compared with WT.

140x76mm (300 x 300 DPI)