Supplementary Data

Detection of Rhodococcus fascians, the causative agent of lily fasciation in South Korea

Joon Moh Park et al.

| No | Location | Lily cultivar |
|----|------------------------------|---------------------------|
| 1 | X :128.172848, Y :37.3113234 | Oriental hybrids |
| 2 | X :128.185448, Y :37.3120449 | Asiatic hybrids |
| 3 | X :128.167835, Y :37.3066157 | Oriental hybrids |
| 4 | X :128.267412, Y :37.2866898 | Asiatic hybrids |
| 5 | X :128.229448, Y :37.5283488 | Oriental hybrid 'Siberia' |

Table S1. Sampling place of lilies located in Gangwon province of South Korea.

| Primer name | Primer sequence (5'-3') | Target gene | Reference | | |
|-------------|-------------------------|-------------|--------------------|--|--|
| 27F | AGAGTTTGATCMTGGCTCAG | | Lane et al. 1991 | | |
| 1492R | GGTTACCTTGTTACGACTT | 105 fKINA | Turner et al. 1999 | | |
| vicA44-F | TCCTATTCGATTTCGTCGAGAAG | | | | |
| vicA737-R | GGGTCGATCTGGATCTCGAA | VICA | This study | | |
| fasD-F | ATTGTTGTTGCCGACCGTATC | fasD | This study | | |
| fasD-R | AAGGACGCCGTGCTCGACATAC | jusD | This study | | |



Figure S1. Multiple sequence alignment of the *vicA* genes of *Rhodococcus* species. The alignment was performed using ClustalW. The conserved region for *R. fascians* strains are represented by yellow rectangles. The red arrows indicate *vicA* primers developed in this study for diagnosis of *R. fascians*.

| | (1) | 1 | ,10 | | 20 | ,30 | | 40 | | 50 | 60 | | ,70 | | 86 |
|--------------|-------------------------|--------------|--------------------|-------------------------|--|---------------------------|-------------------------|--------------------|------------------------------|--|--------------------|---------------------------|------------------------|---------------------------|----------------------------|
| A25f D188 | (1) (1) | ATGA ATGA | AGGAATO | CAACCAT CAACCAT | GGCACAGA GGCACAGA | CGCAAGO | TAGGC AAGGT | TCGATC(TTGATC) | GACG <mark>G</mark> GGGTG | CAATGGGA CGATGGGA | ACCTG ACCCG | GCGTAT) GCGTAT) | ACGCAATC(ACGCAATC(| STCGGTG STCGGTG | CCAC CCAC |
| A2 EF | (87) | 87 | 3 mm c c 3 7 | 100 | 11 |) | 120 | mm.c.a.cm | 130 | 140 | mmcmm | _150 | 160 | 202 C M C M | 172 |
| D188 | (87) | CGGA | ATTGGAA | AAAAGCG | CCGAAGCO | AGCAAGI | TGGCA | TTGAGT | CACTO | GGCTCCGZ | ATTGTT(| GTTGCC | GACCGTAT(| CCAGIGI | TACT |
| A25f | (173) (173) | 173 CCGA | 180 | GTCACC | 190 AGTGGTCG | 200 | GACGC | 210 GAAAGT | 22 ggaag | 20 <mark>GGCTCA</mark> C <mark>C</mark> | 230 | FTGGCT | 240 CGACAACC | ggacc <mark>g</mark> t | 258 ACAT |
| D188 | (173) | CCGA | TCTCCTG | GTCACC | AGTGGTCG | AGCGTTC | GACGC | GAAAGT | GGAAG | GGCTCAAC | CGCGT | TTGGCT(| CGACAACC | GGACCAT | ACAT |
| A25f | (259) (259) (259) | 259 CAGO | GCAACTI | 270 PCGATCC | 280 GGACGAGO | CCTTTGZ | | 300 TGATAA | AGGTA | 310 CTGACCTC | GTACG | 320 | 330 | GCGGTGG | 344 |
| D 100 | (233) | CAGE | OCAACII | COALCO | GGACGAGC | | 100000 | I GAIAA | AAGIA | CIGACUIC | GIACO. | LIGATO | JUBBUBAA | 3000100 | IAAI |
| A25f D188 | (345) (345) (345) | GGAG GGAG | GGCGGTI GGCGGTI | JOL CGATAT CGATAT | CGCTCATI | 370 CTTCGAT CTTCGAT | J8U PTCGCG PTCGCG | CAAACG. CAAACG. | 390 ATATC ATATC | 4 CAACCTAC CAACCTAC | CGTTC CGTTC | 410 CCAGCT(CCAGCT) | GTCGTAAA GTCGTGAA | IZU FGTCATG FGTTATG | 430 CCTA CCTA |
| | (131) | 431 | 44 | 0 | 450 | 460 | | 470 | | 480 | 490 | | 500 | | 516 |
| A25f D188 | (431) (431) (431) | TTCC | TGATAGO TGATAGO | GCAACAC GCAACAC | TACTTTG | CCAGCA | STGCGC STGCGC | AAGGGC AAGGGC | ACGAC ACGAC | AAATGTT AAATGTTZ | | AGATTC: AGATTC: | AACGGGCA AACGGGCA | GGAATTT GGAATTT | G <mark>CTC</mark> ACTC |
| | (517) | 517 | | 530 | 54 | 0 | 550 | | 560 | 570 | | 580 | 590 | | 602 |
| A25f D188 | (517) (517) | ACC6 ACC6 | AACTGGC AACTGGC | CGGAAGC CGGAAGC | GTGGGCT GTGGG <mark>T</mark> T GTGGG | TAGGCGI TAGGCGI | ATCAGC ATCAGC | ACAACT ACAACT | TCGTA TCATA | GCCTCGGI GCCTCGGI | TTGCGG(TTGCGG(| GTCTGG. GTCTGG. | ACTGTGTA ACTGTGTA | CTCGACT CTCGACT | GGTG GGTG |
| 625f | (603) | 603 | 610 | D.C.C.C.D.A.A | 620 | 630 | 200630 | 640 | 65 CTTC 2 C | 50 | 660 | CACCAA | 670 | አ ምርግ አ አ ምርጋ | 688 |
| D188 | (603) | CGCC | ACGCATI | ICCGTAA | CACCAGA | GAGTTG | GCCAAC | CGAGAC | CTCAC | CACGGAGO | GTGCTC | GACGAA | CTGGCGGC | ATCAATG | GGTG |
| A25f | (689) (689) | 689 GCCG | GTATGTO | ,700 CGAGCAC | 710 GGCGTCCT | TCAGCAG | 20 GGAAAT | 730, ATTTT | AAGAA | 740 | reccc | 750 AGGTGT | GACCGCCA | 768 GATGA | |
| D188 | (689) | GCCG | GTATGTO | CGAGCAC | GGCGTCCI | TCAGCA | GAAAT | ATTTT | AAGAA | CCTTCGGI | reccce | AGGTGT | GACCGCCA | GATGA | |

Figure S2. Design of PCR primers for *fasD* gene of *R. fascians*. Most *fasD* genes from pathogenic *R. fascians* can be classified into two types, A25f and D188. The pairwise alignment of two types of genes include from translational initiation codon to stop codon was performed to determine the conserved region. The red arrows indicate *fasD* primers developed in this study.



Figure S3. Representative PCR-based detection of *fasD*, *vicA*, and 16S rRNA genes of bacterial genomic DNA isolates. Total genomic DNA from bacterial isolates was used as template in PCR analysis. A previous isolate of *Rhodococcus* sp. (*R.* sp. KB6) was used as a negative control *for R. fascians*-specific primers for *fasD* and *vicA*. The 16S rRNA was used as an internal control. The amplicon size is 573 bp for *fasD*, 694 bp for *vicA*, and 1517 bp for 16S rRNA, respectively. *R. fascians* (Loewe Biochemica GmbH) was used for positive control of PCR.



Figure S4. Phylogeney analysis of *R. fascians* YWS isolates from symptomatic lily plants. Phylogenetic trees were constructed based on nucleotide sequences of (A) 16S rRNA, (B) *vicA*, and (C) *fasD* using the Neighbor-Joining method by MEGA software. The reference sequences with Genbank accession numbers from *R. fascians* D188 and A25f, and *Rhodococcus* sp. KB6 were gathered from NCBI nr and wgs database. Accession numbers are followings: 16S rRNA gene for D188 (JMET01000045), A25f (CP049744), KB6 (LNAK01000053); *vicA* for D188 (CP015235), A25f (CP049744), KB6 (LNAK01000134); *fasD* for D188 (CP015236), A25f (CP049745).