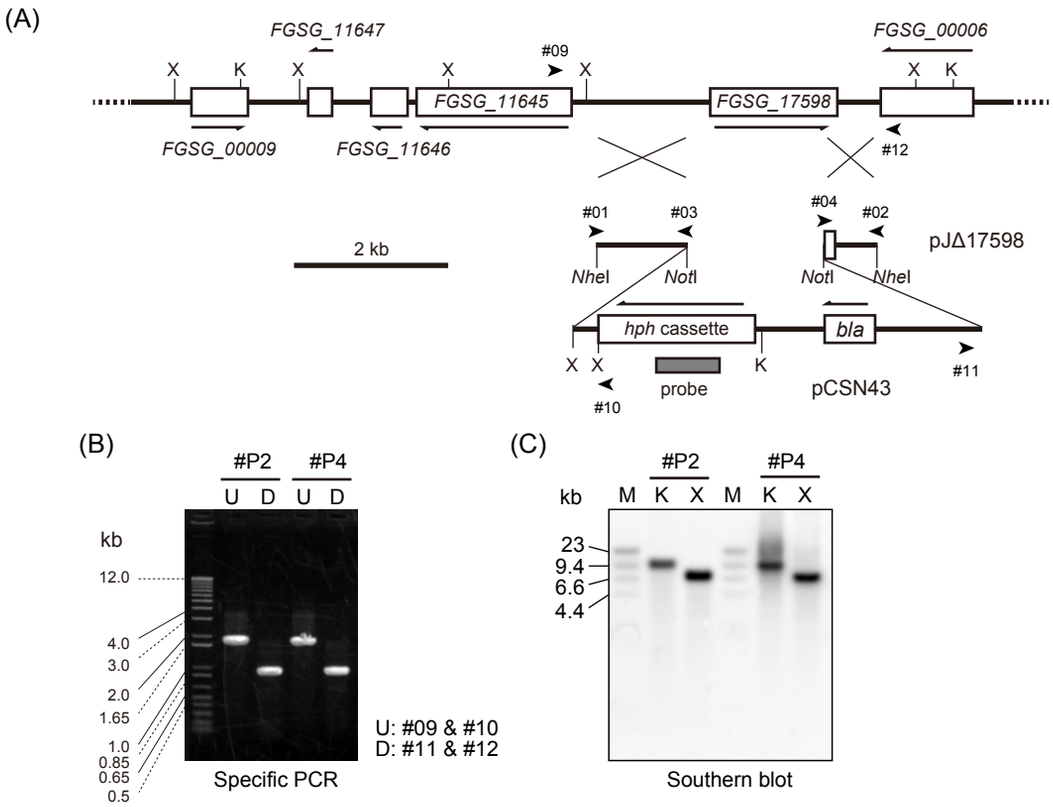
Fig. S1

Disruption of *FGSG_17598*



Disruption of *FGSG_10397*

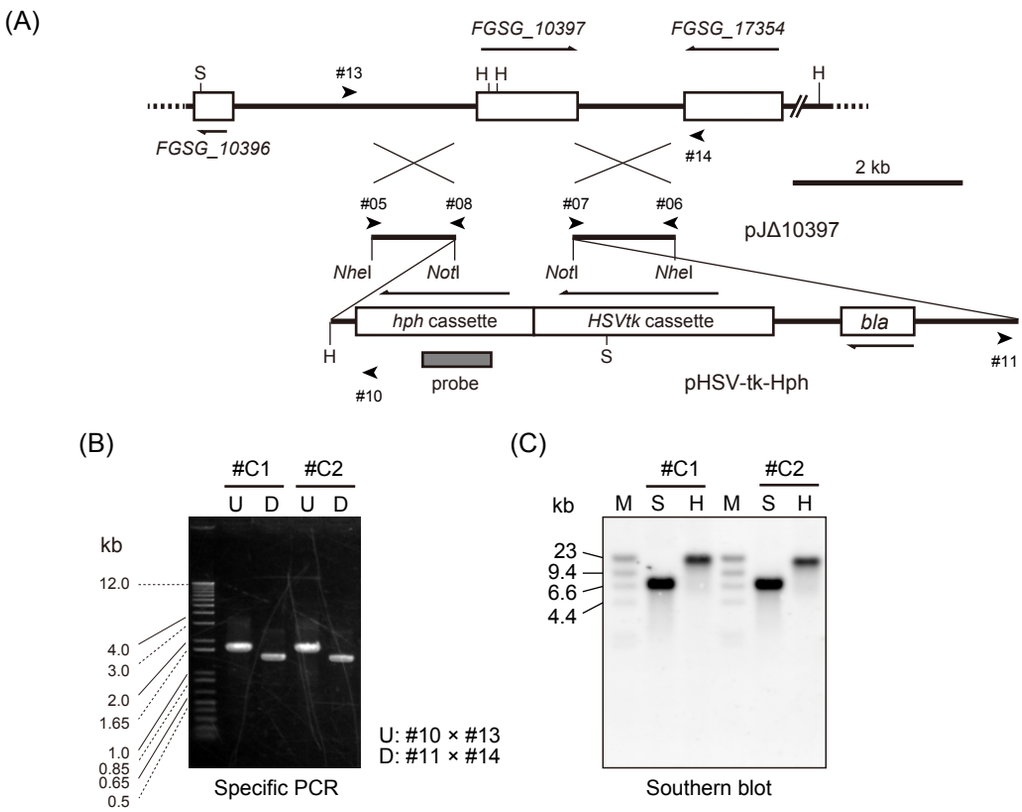


Fig. S1. Disruption of FGSG_17598 and FGSG_10397 genes. (A) Structures of the vectors used for disruption of FGSG_17598 and FGSG_10397 genes. The gene disruption vectors were constructed by replacing the coding region of FGSG_17598 and FGSG_10397 with pCSN43 or its derivative, pHSV-tk-Hph, respectively, that contain the *hph* cassette. The homologous regions upstream and downstream of the deleted region were cloned by inverse PCR (IPCR) as follows: (1) DNA fragments containing the region to be deleted were amplified by long PCR with inward primers #01 and #02 (for FGSG_17598) or #05 and #06 (for FGSG_10397) that have a *Nhe*I recognition site; (2) the amplified products were self-ligated after digestion with *Nhe*I; (3) DNA fragments flanking the region to be deleted were amplified by IPCR with the outward primers #03 and #04 (for FGSG_17598) or #07 and #08 (for FGSG_10397) that have a *Not*I recognition site; and (4) the IPCR products were cloned into the *Not*I site downstream of the *trpC* terminator in pCSN43 and pHSV-tk-Hph. The directions of the inserted *Not*I fragments were determined by PCR with primers #11 and #12 (for FGSG_17598) or #10 and #13 (for FGSG_10397). (B) PCR amplification of the border regions of the FGSG_17598 gene disruptants (strains #P2 and #P4) and FGSG_10397 gene disruptants (strains #C1 and #C2). Targeted integrations of vectors were demonstrated by successful amplification of amplicons with primers #09 and #10 (1,804 bp; upstream of FGSG_17598) and primers #11 and #12 (951 bp; downstream of FGSG_17598), and primers #10 and #13 (1,721 bp; upstream of FGSG_10397) and primers #11 and #14 (1,402 bp; downstream of FGSG_10397). The left lane was loaded with 1 kb Plus DNA ladders (Life Technologies, Carlsbad, CA, USA). (C) Southern blot analysis of the disruption mutants. The DNA of the disruption mutant was digested with *Kpn*I or *Xba*I (FGSG_17598), and *Sma*I or *Hin*dIII (FGSG_10397). A DIG-labeled probe (indicated as a thick line) was prepared using a PCR DIG Probe Synthesis kit with the primers listed in Table S1. DNA Molecular Marker II, DIG-labeled (Roche Diagnostics GmbH; Mannheim, Germany) was loaded on the left lane labeled “M”.

Fig. S2

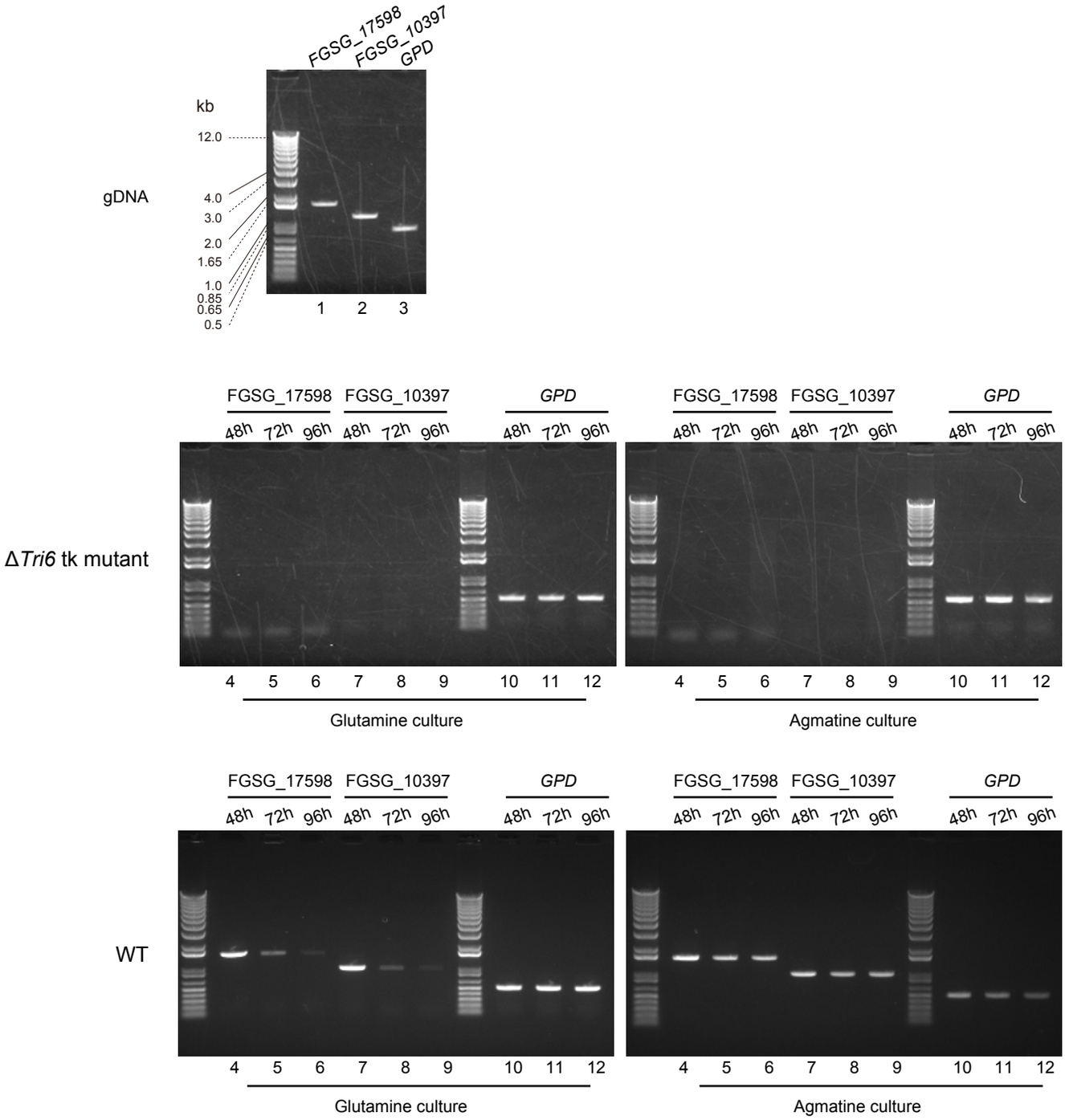

FGSG_17598
FGSG_10397
GPD
kb
12.0
gDNA
4.0
3.0
2.0
1.65
1.0
0.85
0.65
0.5
1 2 3
FGSG_17598 FGSG_10397 GPD
48h 72h 96h 48h 72h 96h 48h 72h 96h
ΔTri6 tk mutant
4 5 6 7 8 9 10 11 12
Glutamine culture
Agmatine culture
WT

Fig. S2. RT-PCR analyses of FGSG_17598 and FGSG_10397 genes using the Δ*Tri6* tk mutant. Primers that allow the amplification of genomic DNA across the intron regions (lanes 1-3; upper panel) were used (Table S1). cDNA was synthesized from 1 μg of total RNA (treated with the RNase-free DNase I) using the Superscript First-Strand Synthesis System (Life Technologies). A 0.1 aliquot was used as a template for the PCR using the primers listed in Table S1. While no amplicons were obtained for FGSG_17598 and FGSG_10397 after 35 cycles (lanes 4-9; middle panel), specific amplicons were consistently obtained for the glyceraldehyde-3-phosphate dehydrogenase gene (*GPD*) after 25 cycles (lanes 10-12). As positive controls, amplicons from the WT cDNA (25 cycles) at each time point are shown (lanes 4-9; lower panel).

Fig. S3

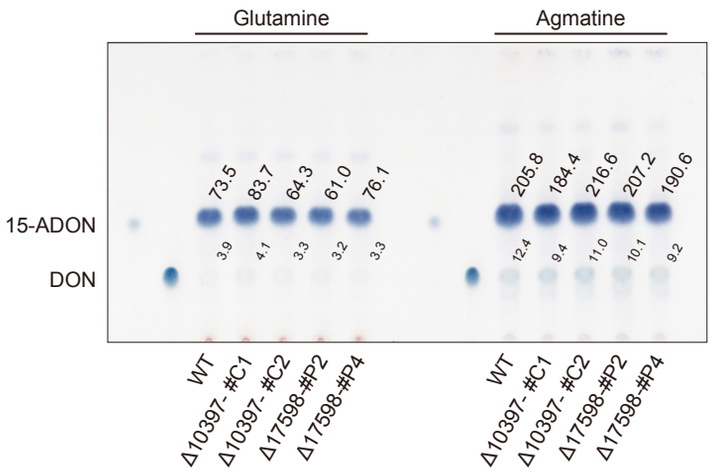

Glutamine
Agmatine
15-ADON
DON
73.5
83.7
64.3
61.0
76.1
205.8
184.4
216.6
207.2
190.6
3.9
4.1
3.3
3.2
3.3
12.4
9.4
11.0
10.1
9.2
WT
Δ10397- #C1
Δ10397- #C2
Δ17598-#P2
Δ17598-#P4
WT
Δ10397- #C1
Δ10397- #C2
Δ17598-#P2
Δ17598-#P4

Fig. S3. TLC analysis for 15-ADON and DON in extracts of cultures of the WT, FGSG_17598 disruption mutants (Δ17598-#P2 and Δ17598-#P4), and FGSG_10397 disruption mutants (Δ10397-#C1 and Δ10397-#C2) grown in 100 μL of synthetic liquid media distributed to a 96-well plate. The concentrations of trichothecenes (indicated as μg/mL over the spots of 15-ADON and DON) in the culture were determined as described in the legend of Table 1.