

Supplementary Figure S1. PCR screening of the *dotC* disrupted transformants

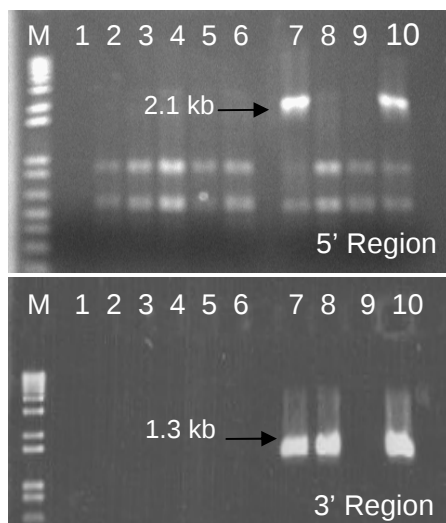
a. Schematic map of PCR screening of *dotC* gene replacement transformants



Primer positions for screening the transformants are indicated by arrows.

5' and 3' elements refer to the *dotC* gene

b. PCR screening results



Top panel: PCR results for 5' region using primers 5'*dotD* RACE and 5'*hphout*. Transformants FJT15 and FJT16 gave 2.1 kb PCR products, indicating correct gene replacement at the 5' end of *dotC*.

Top panel: PCR results for 5' region using primers 3'*hphout* and 100down*dotC*Rep3. Transformants FJT15 and FJT16, as well as FJT19 gave 1.3 kb PCR products, indicating correct gene replacement at the 3' end of *dotC*.

Lane M: 1 kb⁺ ladder

Lane 1: negative control (no DNA);

Lane 2: NZE10 wild type genomic DNA as template

Lanes 3-10: Genomic DNA template from transformants:

Lane 3: FJT 43

Lane 4: FJT 42

Lane 5: FJT 17

Lane 6: FJT 18

Lane 7: FJT 16

Lane 8: FJT 19

Lane 9: FJT 41

Lane 10: FJT15

Only FJT15 and FJT16 were confirmed as *dotC* gene replacement mutants.

Supplementary Table S1:
***dotC* knockout, complementation and *gfp*-fusion primers**

Primer name	Lab. code	Sequence (5'to 3')	Purpose
dotC3'fwd	395	<u>GGGGACAGCTTTCTTGTACAAAGTGG</u> GCACTCCAGACCAAGATCAAGCAGA	Prep dotC KO construct pR260 Incl <u>attB2</u>
dotC3'rev	396	<u>GGGGACACCTTTGTATAATAAAGTTG</u> GTCTGCATCGTGCGGTTGTACCTGT	Prep dotC KO construct pR260 Incl <u>attB3</u>
dotC5'fwd	397	<u>GGGGACAACCTTTGTATAGAAAAGTTGATC</u> TTACGATGCGACTCGATGTGTG	Prep dotC KO construct pR260 Incl <u>attB4</u>
dotC5'rev	398	<u>GGGGACTGCTTTTTTGTACAACTTG</u> CGGATCCTTCTCGGACAAGTTGTCG	Prep dotC KO construct pR260 Incl <u>attB1</u>
100downdot C-Rep3	385	TTGTGGCGAATCAGGATCCA	Screen dotC KO
3'hphout	36	TCCTTGAACCTCTCAAGCCTACAG	Screen dotC KO & complementation
5'hphout	35	GAATCTCCGGTGTGGAAGA	Screen dotC KO
5'dotD RACE	608	TAGACGGCGAGGTCGCGAGAGAT	Screen dotC KO
dotCzf1	400	ATCTTACGATGCGACTCGATGTGT	Prep. dotC-gfp fusion
dotCzf2	401	TCCTCGCCCTTGCTCACCATGGACTTTTG GGCCTTCTCCA	Prep. dotC-gfp fusion
dotCzf3	402	TGGAGAAGGCCCAAAAGTCCATGGTGAG CAAGGGCGAGGA	Prep. dotC-gfp fusion
pPN81 2978rev	425	TCTCAACTCCGGAGCTGA	Prep. dotC-gfp fusion
DCSE1	386	GAGAGACCTTGCAAGATC	Screen dotC-gfp fusion and complementation
egfp rev	693	AGAAGATGGTGCGCTCCT	Screen dotC-gfp fusion
PdotA-fus	219	GAACAGCCCCGGGAGATTTGG	Prep. dotA-gfp fusion
egfp-dota-c	218	CTCGCCCTTGCTCACCATTCGGAAAGCA CCACCGTC	Prep. dotA-gfp fusion
dota-egfp-c	217	GACGGTGGTGCTTTCCGAATGGTGAGCA AGGGCGAG	Prep. dotA-gfp fusion
TtrpC-fus	220	ATACCCGGGTACTTGTACAGC	Prep. dotA-gfp fusion
pUC/M13 fwd	29	CGCCAGGGTTTTCCAGTCACGAC	Prep. dotC complementation
MF4151P3	99	GGACCAGAGGAACATACTTGG	Screen dotC complementation
MF4152P2	103	CTATCATTGTGCTTCGTAACG	Screen dotC complementation
MF4152P4	105	AGACCAGCAGGCAGATGACAG	Screen dotC complementation
MF4151P1	97	ACTTTCAGATGTCCATGGCAGC	Screen dotC complementation

Supplementary Table S2: Real-time PCR primers

Gene/ purpose	Primer name	Lab. code	Sequence (5' to 3')	Amplicon Size cDNA(bp)	Amplicon Size gDNA(bp)
general ribosomal primer (qRT- PCR)	NS7	666	GAGGCAATAACAGGTCTGTGAT GC	377	377
	NS8	667	TCCGCAGGTTACCTACGGA		
<i>vbsA</i> (qRT- PCR)	rt VBS fw I	674	CCGAGCCACAAGAGGG	430	n/a*
	rt VBS rev I	675	CGGGTGAATGGGCTGA		
<i>dotA</i> (qRT- PCR)	rt dotA fw 1	691	CTGGTGATGAATTCGACCG	462	n/a
	rt dotA rev 1	692	AAGCACCACCGTCAATAC		
<i>pksA</i> (qRT- PCR)	rt pksA rev 1	695	CGAACAGAACTACCGACC	409	n/a
	rt pksA fw 1	696	CATTATGTCGTCCGAGCAC		
<i>tubA</i> (qRT- PCR)	rt TUB fw I	709	CCGGCGTGTACAATGG	392	n/a
	rt TUB rev I	710	CATGCGGTCTGGAAC		
<i>dotC</i> (qRT- PCR)	rt DotC fw1	711	GCTTCTTCATCATCGGCG	518	518
	rt DotC rev I	712	TGGTCCGTTGCCGATAC		
<i>tubA</i> (gDNA copy no.)	rt TUB fw II	728	CGGTATGGGTACGCTCT	-	321
	TUB 4	227	TTGCGGAGATCACTGTTGAGC TG		
<i>dotC</i> (gDNA copy no.)	MF4151p3	99	GGACCAGAGGAACATACTTGG	-	438
	MF4152p4	105	AGACCAGCAGGCAGATGACAG		

*n/a One of the primers in each case was designed to flank intron sequence so no amplification could occur with genomic DNA template

Supplementary Table S3:
Expression of dothistromin genes measured by real-time PCR

	Wild type		<i>dotC</i> disruption		complemented
gene	NZE10	NZE7	FJT15	FJT16	FJT93
<i>dotA</i>	1	1.28 ± 0.47	0.26 ± 0.06	0.23 ± 0.05	3.96 ± 0.98
<i>pksA</i>	1	0.53 ± 0.09	0.21 ± 0.03	0.20 ± 0.07	1.28 ± 0.48
<i>vbsA</i>	1	0.50 ± 0.06	0.40 ± 0.16	0.16 ± 0.03	1.90 ± 0.34
<i>tubA</i>	1	0.94 ± 0.03	0.89 ± 0.03	1.15 ± 0.06	0.88 ± 0.07
<i>dotC</i>	1	1.15 ± 0.44	0.00 ± 0.00	0.01 ± 0.01	11.95 ± 0.84

Expression of *dotC*, dothistromin genes *dotA*, *pksA* and *vbsA*, and beta tubulin (*tubA*, constitutive control) in wild type, *dotC* disruption and complemented strains. These are the values corresponding to the chart in Figure 2. The 18S ribosomal RNA was used as a reference gene for standardization in real-time PCR. Values are normalised expression ratios relative to the NZE10 wild type (from which *dotC* disruptant strains were derived), shown as mean ± standard error (n = 6). Significant differences from NZE10 (P<0.05) are shown by grey shaded boxes. Significant differences from NZE7 (P<0.05) follow the same pattern except that FJT15 *dotA*, *vbsA* and *tubA* are not significantly different from NZE7 values.