

Table S1. Strains and plasmids used in this study

<i>Clostridioides difficile</i> strains			
Lab Notation	Strain Name	Description	Reference
RT273	<i>C. difficile</i> R20291	Ribotype 027 strain	(1)
RT1693	<i>recV flg</i> OFF	R20291 <i>recV::ermB (flgB UTR^{OFF})</i>	(2, 3)
RT1702	<i>recV flg</i> ON	R20291 <i>recV::ermB (flgB UTR^{ON})</i>	(2)
RT1566	<i>sigD</i>	R20291 <i>sigD::ermB</i>	(2)
RT1658	<i>spo0A</i>	R20291 <i>spo0A::ermB</i>	(4)
RT2555	<i>flgBΔUTR</i>	R20291 <i>flgBΔUTR</i>	This work
RT2603	<i>flg-3sub</i> OFF	R20291 <i>flg-3sub</i> OFF	This work
RT2604	<i>flg-ΔRIR</i> OFF	R20291 <i>flg-ΔRIR</i> OFF	This work
RT2609	<i>flg-Δ3</i> OFF	R20291 <i>flg-Δ3</i> OFF	This work
RT2610	<i>flg-3sub</i> ON	R20291 <i>flg-3sub</i> ON	This work
RT2611	<i>flg-ΔRIR</i> ON	R20291 <i>flg-ΔRIR</i> ON	This work
RT2612	<i>flg-Δ3</i> ON	R20291 <i>flg-Δ3</i> ON	This work
RT2196	WT vec	R20291 with pRT1611 (vector control)	(5)
RT1562	WT pRecV	R20291 with pRT1611:: <i>recV</i>	This work
RT2614	<i>flg-3sub</i> ON vec	R20291 <i>flg-3sub</i> ON with pRT1611	This work
RT2615	<i>flg-3sub</i> ON pRecV	R20291 <i>flg-3sub</i> ON with pRT1611:: <i>recV</i>	This work
RT2616	<i>flg-ΔRIR</i> ON vec	R20291 <i>flg-ΔRIR</i> ON with pRT1611	This work
RT2617	<i>flg-ΔRIR</i> ON pRecV	R20291 <i>flg-ΔRIR</i> ON with pRT1611:: <i>recV</i>	This work
RT2618	<i>flg-Δ3</i> ON vec	R20291 <i>flg-Δ3</i> ON with pRT1611	This work
RT2619	<i>flg-Δ3</i> ON pRecV	R20291 <i>flg-Δ3</i> ON with pRT1611:: <i>recV</i>	This work
<i>Escherichia coli</i> strains			
Lab Notation	Strain Name	Description	Reference
	<i>Escherichia coli</i> DH5α	F- φ80 <i>lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rk -, mk+) phoA supE44 thi-1 gyrA96 relA1 λ- tonA</i>	Invitrogen (6)
RT270	<i>Escherichia coli</i> HB101(pRK24)	<i>E. coli</i> used in conjugations with <i>C. difficile</i> , Amp ^R , Cm ^R	(7)
RT1310	pFig OFF pRecV (Ec)	DH5α co-transformed with pRT1324 and pRT1164	(2)
RT2587	pFig-3sub OFF pRecV (Ec)	DH5α co-transformed with pRT2582 and pRT1164	This work
RT2588	pFig-ΔRIR OFF pRecV (Ec)	DH5α co-transformed with pRT2583 and pRT1164	This work
RT2589	pFig-Δ3 OFF pRecV (Ec)	DH5α co-transformed with pRT2584 and pRT1164	This work
RT2602	pFig ON pRecV (Ec)	DH5α co-transformed with pRT1323 and pRT1164	This work
RT2590	pFig-3sub ON pRecV (Ec)	DH5α co-transformed with pRT2585 and pRT1164	This work
RT2593	pFig-ΔRIR ON pRecV (Ec)	DH5α co-transformed with pRT2592 and pRT1164	This work
RT2591	pFig-Δ3 ON pRecV (Ec)	DH5α co-transformed with pRT2586 and pRT1164	This work

Plasmids			
Lab notation	Plasmid Name	Description	Citation
pRT709	pRPF185	pMTL960-derivative, contains ATc-inducible <i>P_{tet}</i> promoter	(8)
pRT1611	pRPF185 EV	<i>gusA</i> removed from pRPF185	(2)
pRT1529	pRecV	pRT1611:: <i>recV</i>	(2)
pRT1391	pMWO-074	Low copy vector with ATc-inducible <i>P_{tet}</i> promoter	(9)
pRT1164	pRecV (Ec)	pMWO-074:: <i>recV</i>	(2)
pRT2460	pMSR0	<i>E. coli</i> - <i>C. difficile</i> shuttle vector for toxin-mediated allele exchange mutagenesis	(10)
pRT2546	pMSR0 <i>flgB</i> ΔUTR	pMSR0 with construct for <i>flgB</i> UTR deletion	This work
pRT2570	pMSR0 <i>flg</i> -3sub OFF	pMSR0 with construct for <i>flg</i> -3sub OFF	This work
pRT2571	pMSR0 <i>flg</i> -ΔRIR OFF	pMSR0 with construct for <i>flg</i> -ΔRIR OFF	This work
pRT2572	pMSR0 <i>flg</i> -Δ3 OFF	pMSR0 with construct for <i>flg</i> -Δ3 OFF	This work
pRT2573	pMSR0 <i>flg</i> -3sub ON	pMSR0 with construct for <i>flg</i> -3sub ON	This work
pRT2580	pMSR0 <i>flg</i> -ΔRIR ON	pMSR0 with construct for <i>flg</i> -ΔRIR ON	This work
pRT2574	pMSR0 <i>flg</i> -Δ3 ON	pMSR0 with construct for <i>flg</i> -Δ3 ON	This work
pRT264	pMC123	pMC- <i>P_{cpr}</i> with nisin-inducible <i>cpr</i> promoter	(11)
pRT1324	pFlg OFF	pMC123:: <i>flg</i> OFF	(2)
pRT2582	pFlg-3sub OFF	pMC123:: <i>flg</i> -3sub OFF	This work
pRT2583	pFlg-ΔRIR OFF	pMC123:: <i>flg</i> -ΔRIR OFF	This work
pRT2584	pFlg-Δ3 OFF	pMC123:: <i>flg</i> -Δ3 OFF	This work
pRT1323	pFlg ON	pMC123:: <i>flg</i> ON	(2)
pRT2585	pFlg-3sub ON	pMC123:: <i>flg</i> -3sub ON	This work
pRT2592	pFlg-ΔRIR ON	pMC123:: <i>flg</i> -ΔRIR ON	This work
pRT2586	pFlg-Δ3 ON	pMC123:: <i>flg</i> -Δ3 ON	This work

References

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