

**Table S2. Strains and plasmids used in this study**

Strain #	Strain name	Relevant genotype or features	Source/reference
<b><i>C. difficile</i> strains – 630<math>\Delta</math><i>erm</i></b>			
756	630 $\Delta$ <i>erm</i> $\Delta$ <i>pyrE</i>	<i>erm</i> -sensitive deriviate of 630 with a deletion in <i>pyrE</i>	[1]
846	630 $\Delta$ <i>erm</i> -p	<i>erm</i> -sensitive deriviate of 630 with <i>pyrE</i> restored	[2]
849	630 $\Delta$ <i>erm</i> $\Delta$ <i>spo0A</i> -p	630 $\Delta$ <i>erm</i> $\Delta$ <i>spo0A</i> with <i>pyrE</i> restored	[2]
925	630 $\Delta$ <i>erm</i> $\Delta$ <i>sleC</i> -p	630 $\Delta$ <i>erm</i> $\Delta$ <i>sleC</i> with <i>pyrE</i> restored	[2]
950	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> $\Delta$ <i>pyrE</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>pyrE</i> with <i>gerS</i> deleted ( <i>CD630_34640</i> )	This study
1075	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> -p	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> with <i>pyrE</i> restored	This study
1084	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> / <i>gerS</i> *	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> with <i>gerS</i> in the <i>pyrE</i> locus	[3]
1611	630 $\Delta$ <i>erm</i> $\Delta$ <i>pdaA</i> $\Delta$ <i>pyrE</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>pyrE</i> with <i>pdaA</i> deleted ( <i>CD630_14300</i> )	This study
1617	630 $\Delta$ <i>erm</i> $\Delta$ <i>cwlD</i> $\Delta$ <i>pyrE</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>pyrE</i> with <i>cwlD</i> deleted ( <i>CD630_01060</i> )	This study
1677	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> / <i>gerS</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>gerS</i> with <i>gerS</i> - <i>alr2</i> in the <i>pyrE</i> locus	This study
1686	630 $\Delta$ <i>erm</i> $\Delta$ <i>cwlD</i> -p	630 $\Delta$ <i>erm</i> $\Delta$ <i>cwlD</i> with <i>pyrE</i> restored	This study
1689	630 $\Delta$ <i>erm</i> $\Delta$ <i>pdaA</i> -p	630 $\Delta$ <i>erm</i> $\Delta$ <i>pdaA</i> with <i>pyrE</i> restored	This study
1692	630 $\Delta$ <i>erm</i> $\Delta$ <i>cwlD</i> / <i>cwlD</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>cwlD</i> with <i>cwlD</i> in the <i>pyrE</i> locus	This study
1695	630 $\Delta$ <i>erm</i> $\Delta$ <i>pdaA</i> / <i>pdaA</i>	630 $\Delta$ <i>erm</i> $\Delta$ <i>pdaA</i> with <i>pdaA</i> in the <i>pyrE</i> locus	This study
<b><i>E. coli</i> strains</b>			
41	DH5 $\alpha$	F <sup>-</sup> $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ ( <i>lacZYA</i> - <i>argF</i> ) U169 <i>recA1 endA1 hsdR17</i> (rK <sup>-</sup> , mK <sup>+</sup> ) <i>phoA supE44</i> $\lambda$ - <i>thi-1 gyrA96 relA1</i>	D. Cameron
531	HB101/pRK24	F <sup>-</sup> <i>mcrB mrr hsdS20</i> (rB <sup>-</sup> mB <sup>-</sup> ) <i>recA13 leuB6 ara-13 proA2 lavYI galK2 xyl-6 mtl-1 rpsL20</i> carrying pRK24	C. Ellermeier
892	BL21 (DE3)	F <sup>-</sup> <i>ompT hsdSB</i> (rB <sup>-</sup> mB <sup>-</sup> ) <i>gal dcm</i> (DE3)	C. Ellermeier
1338	pET22b- <i>pdaA</i>	pET22b- <i>pdaA</i> in BL21(DE3)	This study
1375	pET28a- <i>cspBA</i> +TAA	pET28a- <i>cspBA</i> including its stop codon in DH5 $\alpha$	This study
1539	pMTL-YN3	pMTL-YN3 in DH5 $\alpha$	[1]
1604	pET29a- $\Delta$ 22- <i>gerS</i>	pET29a- <i>gerS</i> (deletion of N-terminal 26 aa) in DH5 $\alpha$	[3]
1617	pET22b-empty vector + pET29a- $\Delta$ 22- <i>gerS</i> +TAA	Co-expression of pET22b empty vector with pET29a- <i>gerS</i> +TAA (untagged deletion of N-terminal 26 aa) in BL21(DE3)	This study
1660	pMTL-YN3- $\Delta$ <i>sleC</i>	pMTL-YN3- $\Delta$ <i>sleC</i> in HB101/pRK24	[2]
1662	pMTL-YN1C	pMTL-YN1C in HB101/pRK24	[1]
1747	pMTL-YN1C- <i>gerS</i> *	pMTL-YN1C- <i>gerS</i> in HB101/pRK24	This study
1933	pMTL-YN1C- <i>gerS</i>	pMTL-YN1C- <i>gerS</i> - <i>alr2</i> in HB101/pRK24	This study
1941	pMTL-YN3- $\Delta$ <i>cwlD</i>	pMTL-YN3- $\Delta$ <i>cwlD</i> in HB101/pRK24	This study
1944	pMTL-YN3- $\Delta$ <i>pdaA</i>	pMTL-YN3- $\Delta$ <i>pdaA</i> in HB101/pRK24	This study
1969	pMTL-YN1C- <i>cwlD</i>	pMTL-YN1C- <i>cwlD</i> in HB101/pRK24	This study
1970	pMTL-YN1C- <i>pdaA</i>	pMTL-YN1C- <i>pdaA</i> in HB101/pRK24	This study
2045	pET22b- $\Delta$ 25- <i>cwlD</i> -His <sub>6</sub>	pET22b- <i>cwlD</i> (deletion of N-terminal 25 aa with His <sub>6</sub> tag) in BL21(DE3)	This study
2072	pET22b- $\Delta$ 25- <i>cwlD</i> -His <sub>6</sub> + pET29a-empty vector	Co-expression of pET22b- <i>cwlD</i> (deletion of N-terminal 25 aa with His <sub>6</sub> tag) with pET29a-empty vector in BL21(DE3)	This study

2073	pET22b- $\Delta$ 25- <i>cwID</i> -His <sub>6</sub> + pET29a- $\Delta$ 22- <i>gerS</i> +TAA	Co-expression of pET22b- <i>cwID</i> (deletion of N-terminal 25 aa with His <sub>6</sub> tag) with pET29a- <i>gerS</i> (untagged deletion of N-terminal 26 aa) in BL21(DE3)	This study
2074	pET22b- $\Delta$ 25- <i>cwID</i> -His <sub>6</sub> + pET28a- <i>cspBA</i> +TAA	Co-expression of pET22b- <i>cwID</i> (deletion of N-terminal 25 aa with His <sub>6</sub> tag) with pET28a- <i>cspBA</i> (untagged) in BL21(DE3)	This study

**Plasmids**

pET22b  
pET28a  
pET29a  
pK424

**Relevant features**

*amp*  
*kan*  
*kan*  
Tra<sup>+</sup> Mob<sup>+</sup>; *bla*, *tet*

Novagen  
Novagen  
Novagen  
C. Ellermeier

## References

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2. Donnelly ML, Li W, Li YQ, Hinkel L, Setlow P, Shen A. A *Clostridium difficile*-Specific, Gel-Forming Protein Required for Optimal Spore Germination. mBio. 2017;8(1). Epub 2017/01/17. doi: 10.1128/mBio.02085-16. PubMed PMID: 28096487; PubMed Central PMCID: PMC5241399.
3. Fimlaid KA, Jensen O, Donnelly ML, Francis MB, Sorg JA, Shen A. Identification of a Novel Lipoprotein Regulator of *Clostridium difficile* Spore Germination. PLoS Pathog. 2015;11(10):e1005239. doi: 10.1371/journal.ppat.1005239. PubMed PMID: 26496694; PubMed Central PMCID: PMC4619724.