TABLE S1 Bacterial strains, plasmids, and primers used in this study.

	Description	Reference
Streptomyces strains		
S. coelicolor M600	SCP1$^-$ SCP2$^-$	Kieser et al. 2000
S. coelicolor H2004	Δ*ddlA* (SCO5560)::*apr*, SCP1$^-$ SCP2$^-$	Novotna et al. 2012
S. coelicolor H2009	H2004 + pGN8	This study
S. coelicolor H2012	H2004 + pGN17	This study
S. coelicolor H2027	H2004 + pIJ10257	This study
S. coelicolor H2077	M600 + pIJ10257	Novotna et al. 2012
S. coelicolor H360	H2009 + pMK2	This study
S. coelicolor H361	H2012 + pMK2	This study
S. coelicolor J2175	Δ*vanRS*::*apr*, SCP1$^-$ SCP2$^-$	Hutchings et al. 2006
S. coelicolor J3130	Δ*femX*::*apr*, SCP1$^-$ SCP2$^-$	Hong et al. 2005
E. coli strains		
ET12567 (pUZ8002)	ET12567 containing helper plasmid pUZ8002	Paget et al. 1999
BW25113 (pIJ790)	BW25113 containing helper plasmid pIJ790	Gust et al. 2003
Plasmids		
pIJ773	pBluescript KS(+) containing the apramycin resistance gene and *oriT* of Plasmid RP4, flanked by FRT sites (Ampicillin^R)(Apramycin^R)	Gust et al. 2003
pIJ790	Modified λRED recombination plasmid pKD20 (Chloramphenicol^R)	Gust et al. 2003
pIJ6902	integrative (ϕC31 *attP-int*) and conjugative (*oriT* RK2), *tipAp* expression vector (Apramycin^R)(Thiostrepton^R)	Huang et al. 2005
pIJ10257	integrative (ϕBT1 *attP-int*) and conjugative (*oriT* RK2), *ermEp* expression vector (Hygromycin^R)	Hong et al. 2005
pSET152	ϕC31 *attP-int* derived integration vector for the conjugal transfer of DNA from *E. coli* to *Streptomyces* spp. (Apramycin^R)	Bierman et al. 1992
pUZ8002	Non-trasmissible oriT-mobilising plasmid (Kanamycin^R)	Keiser et al. 2000
pHJH5	*ddlA* gene cloned into pGEM T-easy vector (Ampicillin^R)	This study
pGN8	*ddlA* gene cloned into pIJ10257 under *ermEp* control (Hygromycin^R)	This study
pGN15	*vanA* gene cloned into pGEM T-easy vector (Ampicillin^R)	This study
pGN17	*vanA* gene cloned into pIJ10257 under *ermEp* control (Hygromycin^R)	This study
pHJH4	derivative of pSET152 containing *vanJp-neo* (Apramycin^R)	This study
pMK1	derivative of pSET152 containing *tsr* (Apramycin^R)(Thiostrepton^R)	This study
pMK2	derivative of pSET152 containig *tsr* and *vanJp-neo* (Apramycin^R)(Thiostrepton^R)	This study
Primers		
ddlA KO F	TCTCGAGGCACCGCGGGCGGGTACTCTCAACGCGATATGATTCCGGGGATCCGTCGACC	
ddlA KO R	GGGAGTCGCCTTCTCTGTGGTCACGACACGAAAGCGTCATGTAGGCTGGAGCTGCTTC	
ddlA KO Test F	TGAAGGAACTGATGTCGCGCA	
ddlA KO Test R	TTCCCGGACCAGACAGGAAAC	
ndeI-ddlA F	CCGGCATATGATGAGCACCGAGAACCTCCCCCAGA	
pacI-ddlA R	CCGGTTAATTAATCAGCGCAGTCCGGTGGGCCGCC	
ndeI-vanA F1	CCGGCATATGATGGCTGAGTCGGACAAGTTGGCCA	
bln-vanA R	CCGGCCTAGGTCATCGGAGCTTCCCCGTCAACGCC	
qvanH F	ACGAGAATCACGGTTTACGG	
qvanH R	CTTGTGGTCAATGCTGATGC	
qvanK F	CCGCAGTTCAAGTACGAGGT	
qvanK R	GGACGTAGAGGTCGTGGAAC	
vanH S1 FOR	TTCGACCTCTATATGAAGCGACGT	
vanH S1 REV	TGAGAGTCGCCTCGACGCCGAAA	
G1680	CACCGACCGGCAGGTGCGCG	
G1681	TCGAGCACCTCGCCCATGTC	

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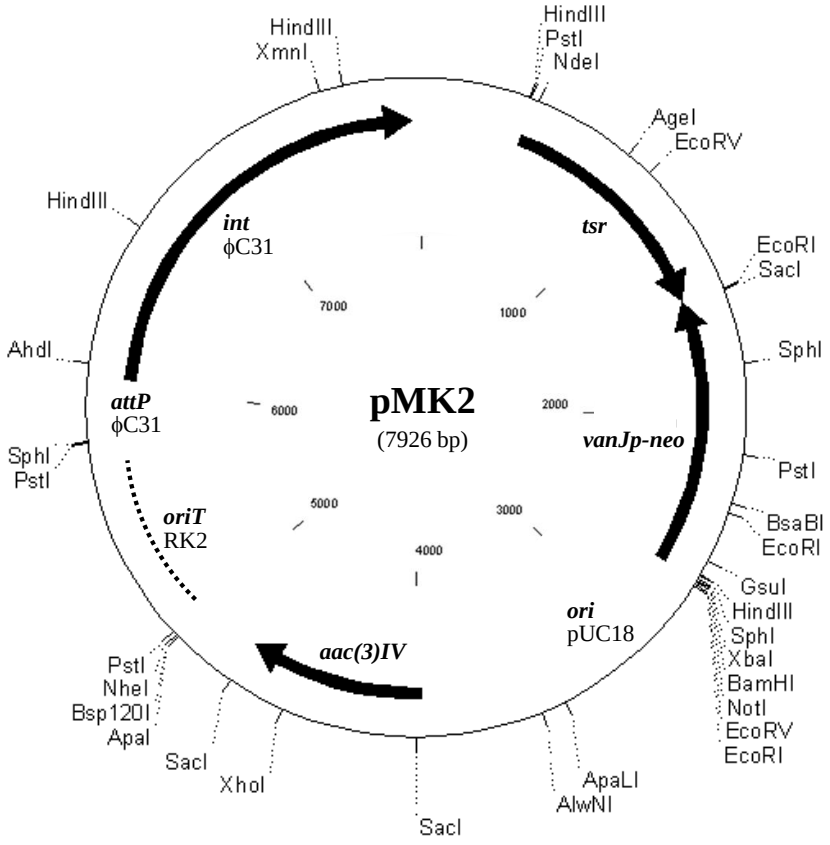

FIG S1 Integrative reporter plasmid pMK2 carrying the *neo*, neomycin/kanamycin resistance gene, expressed under the control of the vancomycin inducible *vanJ* promoter (*vanJp*). *aac(3)IV*, apramycin resistance gene; *tsr*, thiostrepton resistance gene; *oriT* RK2 for conjugation; *attP* and *int* for integration at the ϕC31 phage attachment site.

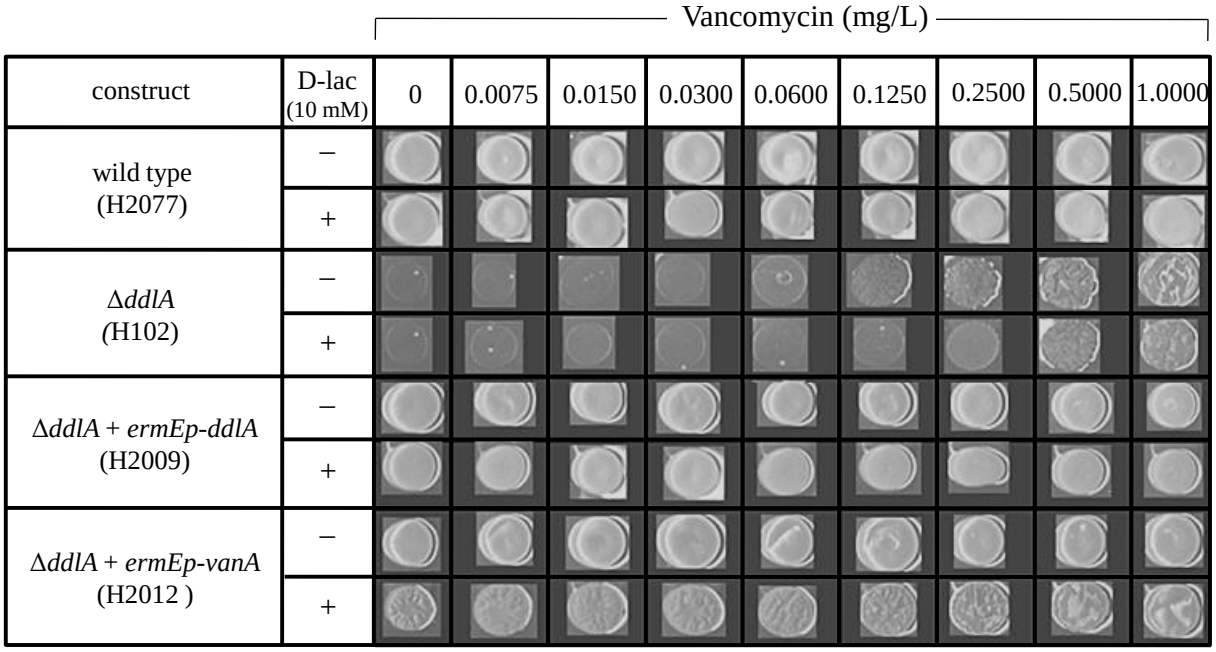

FIG S2 D-Lac supplementation alters the ratio of D-Ala-D-Ala:D-Ala-D-Lac-containing PG precursors in strains expressing the VanA ligase, and increases the concentration of vancomycin required for survival of the vancomycin-dependent Δ*ddlA* mutant strain. Phenotypic analysis of *S. coelicolor* wild type (H2077), Δ*ddlA* (H2027) and Δ*ddlA* complemented by the ligases encoded by *ddlA* (H2009) or *vanA* (H2012) expressed from the constitutive *ermEp*. Approximately 10^5 spores of each strain were spotted onto MMCGT plates containing different concentrations of vancomycin in the presence or absence of 10 mM D-Lac, as indicated. The result was scored after 4 days incubation at 30°C. Both complemented strains H2009 and H2012 grow in the absence of vancomycin and are unaffected by supplementation of the media with 10 mM D-Lac. This contrasts with Δ*ddlA* carrying only the empty pIJ10257 vector (H2027) which requires at least 0.25 mg/ml vancomycin for detectable growth in the presence of the D-Lac supplement, and 0.06 mg/ml in its absence. M600 harbouring pIJ10257 (H2077) is shown as a control.