

Supporting Information

Figure S1. Domain analysis of *genL* (*Orf6255*)

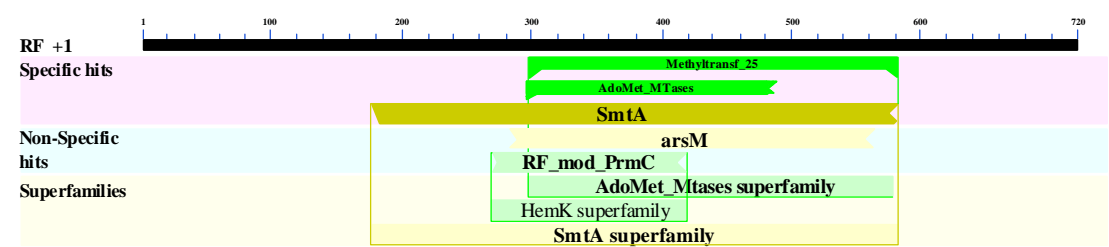
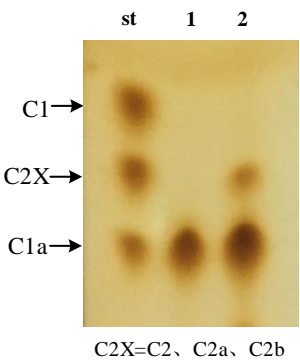


Table S1. List of *GenL* (*Orf6255*) conserved domain hits.

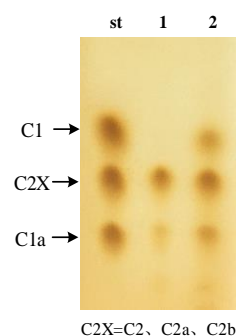
Name	Description	Interval (aa)	E-value
Methyltransf_25	Methyltransferase domain; This family appears to be a methyltransferase domain.	298-579	9.32e-11
AdoMet_MTases	S-adenosylmethionine-dependent methyltransferases (SAM or AdoMet-MTase)	295-486	3.53e-07
SmtA	SAM-dependent methyltransferase, Secondary metabolites biosynthesis, transport and catabolism,	175-579	1.52e-04

Figure S2. Thin layer chromatography analysis of metabolites of GbKL202



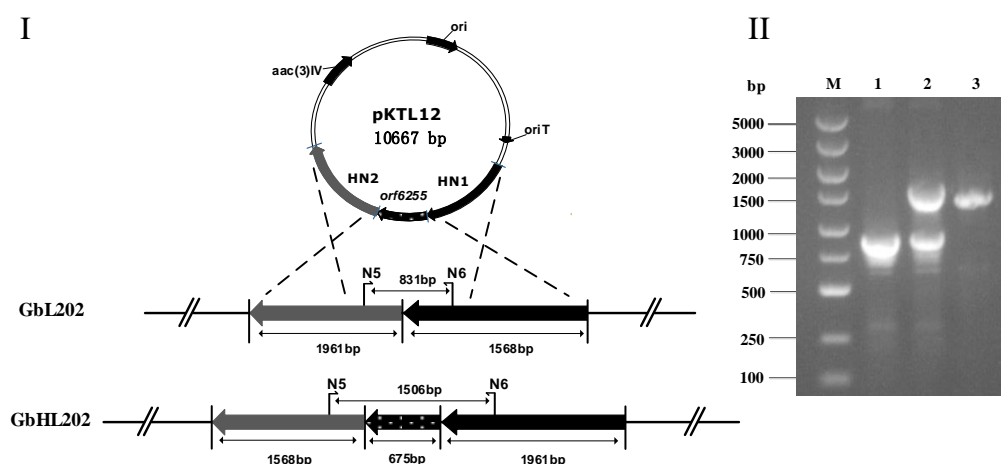
Analysis of metabolites by TLC: Lane st: Standard gentamicin C complex including C1, C1a, C2, C2a and C2b (C2a and is C2 stereoisomer, they have the same Rf); Lane 1: Metabolites of GbKL202 mutant strain producing only C1a; Lane 2: Metabolites of GK1101 producing C2b and C1a.

Figure S3. Thin layer chromatography analysis of metabolites from GbL202



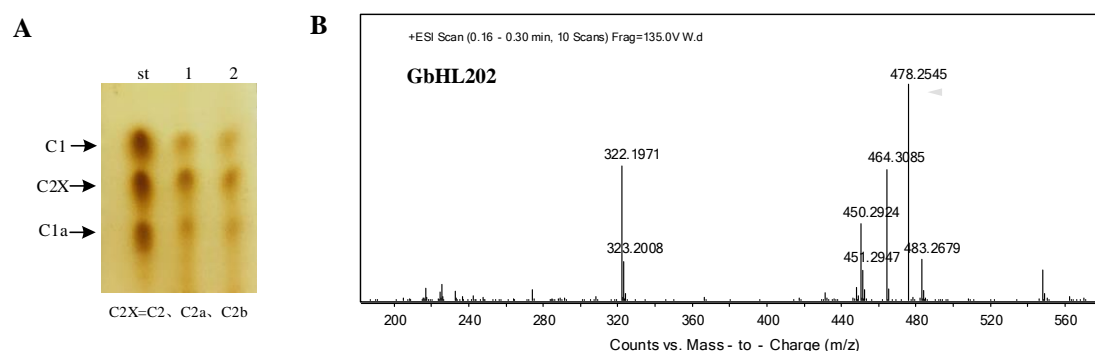
Analysis of metabolites by TLC: Lane st: Standard gentamicin C complex including C1, C1a, C2, C2a and C2b (C2a and is C2 stereoisomer, they have the same Rf); Lane 1: Metabolites of GbL202; Lane 2: Metabolites of wild type strain *M.purpurea* Gb1008 producing C1, C2, C2a, C2b and C1a.

**Figure S4. Construction of GbHL202 (*genL* anaplerosis of GbL202) and Confirmation**



(I) Schematic presentation of the in-frame deletion; (II) DNA gel results. PCR analysis of the genomic DNA fragments from GbL202 and GbHL202 using primers N5 and N6. The gel results show: a 831 bp band (deleted 675 bp of *genL*) in GbL202 (lane 1); two bands at 1506 bp and 831 bp in single crossover mutant strain (lane 2); and a 1506 bp band (*genL* anaplerosis of GbL202) in GbHL202 (lane 3). The DL5000 DNA marker is in lane M.

**Figure S5. MS and TLC analysis of metabolites from GbHL202**



(A) TLC of metabolites from GbHL202 (*genL* anaplerosis of GbL202): Lane st: Standard gentamicin C complex including C1, C1a, C2, C2a and C2b (C2a and is C2 stereoisomer, they have the same Rf); Lane 1: Metabolites of GbHL202; Lane 2: Metabolites of the wild-type strain *M.purpurea* Gb1008 producing C1, C2, C2a, C2b and C1a.

(B) Mass spectrum analysis of metabolites from GbHL202.