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

C1, C2X, C1a
C2X=C2a, C2b, C2b

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 RI); 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.