**Supporting Information**

# Effects of *acuC* on the growth development and spinosad biosynthesis of ***Saccharopolyspora spinosa***

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**Table S1 Strains, plasmids used in this study**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Relative description | | Sources |
| Strains |  |  | |
| *E.coli* Top10 | Host for general cloning | Lab store | |
| *E.coli* S17-1 | Donor strains for conjugation | Lab store | |
| *S. spinosa* | The producer strains of *spinosad* | Lab store | |
| *S.spinosa-△acuC*  *S.spinosa-acuC* | *S. spinosa* harboring pOJ260-*acuC*  *S. spinosa* harboring pOJ260-*PermE-acuC* | This work  This work | |
| Plasmids |  |  | |
| pOJ260 | *E.coli* cloning vector, containing pUC18 replicon, oriT, ApraR | Lab store | |
| pOJ260-cm-*PermE* | Containing *PermE* sequence | Lab store | |
| pOJ260-*acuC*  pOJ260-PermE -*acuC* | *acuC* inserted into pOJ260 by *Eco*R Ⅰand *Hin*d Ⅲ  *PermE*-*acuC* inserted into pOJ260 by *Eco*RⅤ and *Hin* d Ⅲ | This work  This work | |

**Table S2 Nucleotide sequences of primers**

|  |  |
| --- | --- |
| Primer | sequence(5′→ 3′) |
| acuC-F | GGG*AAGCTT*GGGTCGATCTCGTCCTCCC (*Hin*dⅢ) |
| acuC-R  acuC-A  acuC-B | CCG*GAATTC*GTTCGTAGATGTCCGGTTCTG (*Eco*R Ι)  CCATGCAACGGAAACACC  GAGTCCGTGCTCGGCTAC |
| PermE-F | CG*AAGCT*TCTGGACTTCTAGAGCTAGCC (*Hin*dⅢ) |
| PermE-R | GCATGCCGGTCGACTCTA |
| PermE-*acuC*-F | CGGTTGGTAGGATCCTCTAGAGTCGACCGGCATGCCTTCCAGGTTGTCGATGACC |
| PermE-*acuC*-R  Apr-F  Apr-R | AGT*GATATC*CTGTACGAGTGCGTGAAGGA (*Eco*RⅤ)  GCTCATCGGTCAGCTTCTCAAC  CTTCGCATCCCGCCTCTG |
| *whiA-*F | CCGACGGGCTGAGGTTTC |
| *whiA-*R | GTGCCCGAACAGCTCGTG |
| *ssgA*-F | CGAGGGCGACGTGACGAT |
| *ssgA*-R | AGGTTCTCGTTGCCAGGCAC |
| *bldD-*F | TCGTCGGGTCCTATGAGCG |
| *bldD-*R | TCACAACTTTGGTGGCAGGC |

Note: Restriction enzyme sites were italic, overlaping sequences were underlined.

S1

**Figure S1. Construction and verification of *S. spinosa*-Δ*acuC***

A.Construction of the gene knockout vector pOJ260-**Δ***acuC*; B. 1.1 kb *acuC* Fragment amplification by using the *S. spinosa* genome as the template and the *acuC*-F and *acuC*-R as the primer pair; C. The restriction enzyme analysis and PCR amplification of pOJ260*-*Δ*acuC;* D. The schematic diagram of *acuC* gene knockout; E. PCR validation of 1.1 kb *acuC* fragment with the primer pair *acuC*-A and *acuC*-B; F. PCR validation of Apra resistance gene around 720 bp with the primer pair Apr*-*F and Apr*-*R.

S2

**Figure S2. Construction and verification of *S. spinosa*-*acuC***

A.Construction of the gene overexpression vector pOJ260-PermE-*acuC*; B. The complete *acuC* gene fragment around 1.3 kb was ampified by using the genome of *S. spinosa* as the template and the P*ermE*-*acuC*-F andP*ermE*-*acuC*-R were used as primer pair; C. The vector pOJ260-P*ermE***-***acuC*was confirmed via restriction enzyme analysis and PCR amplification;D. The schematic diagram of *acuC* geneoverexpression; E. PCR validation of the *PermE*-*acuC* fragment around 1.6 kb with the primer pair P*ermE*-F and P*ermE*-*acuC*-R; F. PCR validation of Apra resistance gene around 720 bp with the primer pair Apr*-*F and Apr*-*R.

Fig.S3

**Figure S3.** The insecticidal activity of the wild-type and mutant strainson *H. armigera.*

*Data 1*

**Figure S4.** The content of Acetyl-CoA between the wild-type and overexpression strains in 48h, 96h and 192h. Error bars represent the standard deviation of the mean. \*, \*\*and \*\*\* indicate P<0.05, P<0.01 and P<0.005, respectively.