**Supporting Information**

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

Zhudong Liu#, Jie Xiao#, Jianli Tang, Yang Liu, Ling Shuai, Li Cao, Ziyuan Xia, Xuezhi Ding, Jie Rang and Liqiu Xia\*

State Key Laboratory of Development Biology of Freshwater Fish, Hunan Provincial Key Laboratory for Microbial Molecular Biology, College of Life Science, Hunan Normal University, Changsha, China.

**\*Correspondence:** Liqiu Xia, Hunan Provincial Key Laboratory for Microbial Molecular Biology, State Key Laboratory of Development Biology of Freshwater Fish, College of Life Science, Hunan Normal University, Lushan Road 36, Changsha 410081, China. Tel/ Fax: +86 073188872298.

 E-mail address: xialq@hunnu.edu.cn

**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 workThis 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 workThis work |

**Table S2 Nucleotide sequences of primers**

|  |  |
| --- | --- |
| Primer | sequence(5′→ 3′) |
| acuC-F | GGG*AAGCTT*GGGTCGATCTCGTCCTCCC (*Hin*dⅢ) |
| acuC-RacuC-AacuC-B | CCG*GAATTC*GTTCGTAGATGTCCGGTTCTG (*Eco*R Ι)CCATGCAACGGAAACACCGAGTCCGTGCTCGGCTAC |
| PermE-F | CG*AAGCT*TCTGGACTTCTAGAGCTAGCC (*Hin*dⅢ) |
| PermE-R | GCATGCCGGTCGACTCTA |
| PermE-*acuC*-F | CGGTTGGTAGGATCCTCTAGAGTCGACCGGCATGCCTTCCAGGTTGTCGATGACC |
| PermE-*acuC*-RApr-FApr-R | AGT*GATATC*CTGTACGAGTGCGTGAAGGA (*Eco*RⅤ)GCTCATCGGTCAGCTTCTCAACCTTCGCATCCCGCCTCTG |
| *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.



**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.



**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.



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

**

**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.