



Supplementary File

Toxicity of *Bacillus thuringiensis* Strains Derived from the Novel Crystal Protein Cry31Aa with High Nematicidal Activity against Rice Parasitic Nematode *Aphelenchoides besseyi*

Zhao Liang^{1, 2†}, Qurban Ali^{1, 2†}, Yujie Wang², Guangyuan Mu³, Xuefei Kan³, Yajun Ren², Hakim Manghwar⁴, Qin Gu^{1, 2}, Huijun Wu^{1, 2} and Xuewen Gao^{1, 2*}

¹ The Sanya Institute of Nanjing Agricultural University, Sanya 572024, Hainan, China.

² Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China; zhaol0619@163.com (Z.L.); 2020202068@stu.njau.edu.cn (Q.A.); yjiewang57@163.com (Y.W.); ren-yajun0719@163.com (Y.R.); guqin@njau.edu.cn (Q.G.); hjwu@njau.edu.cn (H.W.)

³ Shenzhen Batian Ecotypic Engineering Co., Ltd., Shenzhen 518057, China; muguangyuan1@sina.com (G.M); xavier2014@163.com (X.K.)

⁴ Lushan Botanical Garden, Chinese Academy of Sciences, Jiujiang 332000, China; hakim@lsbg.cn (H.M).

* Correspondence: gaoxw@njau.edu.cn; Tel.: +86-025-8439-5268

† These authors are contributed equally to this study.

Table S1. Bacterial strains used in this study.

Strains	Characteristics	Source or reference
Top10	<i>Escherichia coli</i>	TaKaRa Bio Inc.
BL21	<i>Escherichia coli</i>	TaKaRa Bio Inc.
GBAC46	<i>Bacillus thuringiensis</i>	This study
NMTD81	<i>Bacillus thuringiensis</i>	This study
FZB42	<i>Bacillus amyloliquefaciens</i>	Stored in the lab

Table S2. Defense related genes primers used in this study.

No.	Gene	Code	Primer(5'–3')
1	<i>PR1a</i>	<i>PR1a</i> -F	TCTTCATCACCTGCAACTACTC
		<i>PR1a</i> -R	ATTCATCGGATTTATTCTCACC
2	<i>PR4</i>	<i>PR4</i> -F	TCTAACGTGCGAGCCACATA
		<i>PR4</i> -R	CTGGATACACTTGCCACACG
3	<i>LOX1</i>	<i>LOX1</i> -F	GCATCCCCAACAGCACATC
		<i>LOX1</i> -R	AATAAAGATTTGGGAGTGACATATTGG
4	<i>PBZ1</i>	<i>PBZ1</i> -F	CTACTATGGCATGCTCAAGAT
		<i>PBZ1</i> -R	ATAGAAAGGCACATAAACACAA
5	<i>PAL1</i>	<i>PAL1</i> -F	ACTACCCGCTGATGACCAAG
		<i>PAL1</i> -R	GCTCTCCTGATCCTGTTGG

Table S3. Proteins primers used in this study.

No.	Protein	Code	Primer(5'–3')
1	Cry31Aa	<i>cry31Aa</i> -F	TGCAGGATCCATGACTAAAAACCACAAGAAAATA
		<i>cry31Aa</i> -R	CGAAGCTTTCACCTTATCGTCGTCATCCTTGTAATCTTTTTTCGCACCGATAACTT
2	Cry40ORF	<i>cry40ORF</i> -F	GCGGGATCCATGTTCACTAATAATGCGAAAA
		<i>cry40ORF</i> -R	GCGAAGCTTTCACCTTATCGTCGTCATCCTTGTAATCACTAAGCTGAGTAGTTACTC
3	Cry73Aa	<i>cry73Aa</i> -F	TGCAGGATCCATGACCATAACAGTTTATAGATA
		<i>cry73Aa</i> -R	CGGGTACCTCACTTATCGTCGTCATCCTTGTAATCATTTATAAATAAACTATTCA