A Novel *Aphis gossypii* Killing Surfactin Produced by *Bacillus australimaris* TRM82479 of Taklamakan Desert Origin

Yelin Wang , Zhibin Sun , Shiyu Wang , Feng Wen , Zhanfeng Xia *

Posted Date: 7 January 2025

doi: 10.20944/preprints202501.0560.v1

Keywords: Bacillus; Aphis gossypii; insecticidal activity; surfactin

Article

A Novel *Aphis gossypii* Killing Surfactin Produced by *Bacillus australimaris* TRM82479 of Taklamakan Desert Origin

Yelin Wang [1,2], Zhibin Sun [1,2], Shiyu Wang [1,2], Feng Wen [1,2] and Zhanfeng Xia [1,2,*]

[1] Key Laboratory of Conservation and Utilization of Biological Resources in the Tarim Basin, Alar 843300, China

[2] College of Life Science and Technology, Tarim University, Alar 843300, China

* Correspondence: fenge3721@163.com

Abstract: The cotton aphid *Aphis gossypii* poses a global, serious threat to cotton yield and quality. Although chemical pesticides are effective, pollution and resistance are increasingly prominent, making development of new biopesticides a priority in the context of green agricultural development. Given that reports on the activity of surfactins against *A. gossypii* are limited, here, 107 *Bacillus* strains isolated from the extreme environment of the Chinese Taklamakan Desert were screened for insecticidal activity against *A. gossypii* using the leaf-dip method. Active strains were characterized by morphological observation, 16S rRNA gene sequencing, and phylogenetic analysis. Secondary metabolite synthesis genes were identified by whole-genome sequencing and antiSMASH analysis. *B. australimaris* strain TRM82479 showed 75.00% 48-h mortality against *A. gossypii*. An antiSMASH analysis showed that this strain has several gene clusters associated with the synthesis of nonribosomal peptide (NRP) fengycin and lichenysin lipopeptide analogs; therefore, we isolated and characterized its NRPs. A surfactin was the primary insecticidal substance, with an aqueous LC_{50} of 0.857 mg/mL and LC_{95} of 4.350 mg/mL. This study not only provides new strain resources for *A. gossypii* control, but also demonstrates the potential of surfactin as a biopesticide, which will lay a foundation for its future agricultural application.

Keywords: *Bacillus*; *Aphis gossypii*; insecticidal activity; surfactin

1. Introduction

Cotton [1,2] is an important global cash crop in 80 countries with a total planted area of 32 million hectares and an estimated annual value of $5.7 billion, with China, India and the United States of America the largest producers, with more than half of global production [3]. Xinjiang, the largest cotton-growing region in China, had a total output of 5.1 million tons in 2023, accounting for more than 90.00% of total Chinese production. Pests are a serious threat to production; according to the United Nations Food and Agriculture Organization, annual global economic losses caused by pests are estimated to be billions of dollars [4].

There are a wide variety of cotton pests, including *Aphis gossypii*, *Tetranychus cinnabarinus*, and *Thrips tabaci.* Currently, pest control relies primarily on chemical pesticides, which achieve quick and effective control and reduce losses; for example, synthetic pyrethroids and organophosphates significantly reduce pest populations in the short term[5]. Although chemical pesticides remain important, their misuse has sparked widespread controversy [6]. Excessive use of insecticides can have a negative impact on beneficial insects and the environment [7]. Prolonged use of synthetic pesticides may also lead to the development of resistance [8]. For these reasons, biopesticides are becoming increasingly important in the context of rising demand for green agriculture [9]. Their use not only significantly reduces pollution and harm to agricultural products, but also counteracts the problem of resistance to traditional chemical pesticides [10].

 doi:10.20944/preprints202501.0560.v1

Bacillus produce a variety of biologically active substances with high bacteriostatic activities, antagonistic spectra, environmental friendliness, and other advantages. They provide a powerful alternative to chemical fertilizers and pesticides, as many bacteria themselves have strong resistance, high survival, and rapid reproduction; therefore, biological control is widely used [11]. *B. thuringiensis* produces δ-endotoxins, including Cry and Cyt proteins, which are toxic to a wide range of insects. Cry proteins are effective against specific insect orders, including Lepidoptera, Diptera, Hymenoptera, and Coleoptera; whereas Cyt proteins are toxic to Diptera [12]. *B. sphaericus* is capable of producing a variety of lipopeptides with antimicrobial activity and has been used to control pests. For example, lipopeptide metabolites produced by *B. subtilis* have been used to control *Spodoptera littoralis*, *Drosophila melanogaster*, *Culex quinquefasciatus*, *Anopheles stephensi* and Aedes aegypti [13]. Bora et al. identified compounds produced by *Bacillus* species using liquid chromatography-mass spectrometry (LC-MS), including Brevianamide A, Heptadecanoic acid, Thiolutin and Versimide, which showed toxicity against *Oligonychus coffeae* [14].

As the largest desert in China, the extreme environment of the Taklamakan Desert has created unique microbial resources, with *Bacillus* spp. the dominant bacteria [15]. In this study, we aimed to screen strains with efficient *A. gossypii* killing activity and to explore their insecticidal active components to provide strain resources and theoretical support for the development of insecticidal biopesticides. To this end, we screened 107 such strains from the Taklamakan Desert for *A. gossypii* killing activity. We further characterized them by multiple methods.

2. Materials and Methods

Experiments were conducted at Tarim University, College of Life Science and Technology, Xinjiang Production and Construction Corps, Key Laboratory of Conservation and Utilization of Biological Resources in the Tarim Basin.

2.1. Bacteria

All 107 strains of *Bacillus* spp. selected for this study were provided by the Microbial Strain Resource Bank of Tarim University and had been isolated from the Taklamakan Desert. They were preserved by freeze-drying at 5 °C. They were cultured in LB solid and liquid media.

2.2. Insects

A. gossypii was collected from the Horticultural Experiment Station of Tarim University (81°17 'E, 40°32 'N) during the infestation period. For their maintenance and expansion, cotton seeds were first soaked in pure water for 2–3 days until white shoots developed. Soil was prepared from ratio of nutrient soil:coconut bricks:vermiculite at a ratio of 5:3:2. Seeds were sown at 3–5 cm in this soil and kept moist. Seedlings were watered twice or thrice weekly after emergence. After 2–3 weeks, insects were placed onto the back of the leaves using a fine brush. Insects were reared at 25 °C, 50.00% humidity, and 12:12-h light:dark cycles in 50 × 50 × 65 cm (L×W×H) cages.

2.3. Screening

For shake-flask fermentation, bacteria were thawed at 4 °C, inoculated onto LB plates, and incubated at 28 ºC incubators for 2 days. Colonies were inoculated into liquid LB and shaken at 120 rpm at 30 °C for 2–3 days.

A. gossypii killing was measured from these cultures using the leaf-dip method [16]. LB without bacteria was used as a negative control and 0.1 g/mL of a 20.00% fludioxonil suspension (Jiangsu Keshen Group Co., Ltd.) was used as a chemical control. Assays were performed in triplicate. Twenty test insects were tested per replicate. Mortality and corrected mortality rates were calculated as:

Mortality rate = number of dead insects/total number of test insects × 100%

Corrected mortality (%) = Mortality in treatment (%) − Mortality in control (%)/100 − Mortality in control (%) × 100

 doi:10.20944/preprints202501.0560.v1

2.4. Strain Characterization

Strains with activity were observed on LB plates. Their microstructures were further observed using scanning electron microscopy. Their DNA was extracted using SDS-CTAB [17], amplified by PCR using primers 27F and 1492R for the 16S rRNA gene, and sequenced by Sangon Bioengineering Co. (Shanghai, China). The EzBioCloud database (www.EzBioCloud.net) was utilized for comparison. The 16S rRNA gene sequences of published strains with high similarity were used to construct phylogenetic trees using MEGA 5.05 software to determine the taxonomic status of the strains [18].

2.5. Whole-Genome Sequencing (WGS) and antiSMASH

Strains were inoculated into LB grown with at 30 °C with shaking at 120 rpm for 24 h. Bacteria were pelleted at 10,000 rpm for 5 min at 4 °C and sent to Personal Bio (Shanghai, China, which used the shotgun, paired-end WGS using the Illumina NovaSeq platform. Data with removed splice sequences were assembled from scratch using A5-MiSeq and SPAdes software to construct contigs and scaffolds. Corrections were made using Pilon software [19–21]. Functional annotations were obtained by comparison with the NR, eggNOG, KEGG, and Swiss-Prot databases using BLAST software. Secondary metabolite synthesis genes were predicted using antiSMASH (antismash.secondarymetabolites.org).

2.6. Isolation and Characterization of Insecticidal Compounds

Strains exhibiting activity were fermented in large batches. Based on the antiSMASH data, small-molecule compounds were isolated and purified by macroporous resin adsorption, ODS column chromatography, gel column chromatography, and high-performance liquid chromatography (HPLC), then analyzed by ^{1H}NMR, ^{13C}NMR, HSQC, and HMBC spectroscopy [22,23]. Larger candidates, such as proteins, were precipitated using ammonium sulfate, purified using gel filtration, and sequenced by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [24,25]. Lipopeptides were ethanol precipitated and acid (HCl) precipitation was used to obtain lipopeptides [26], which were purified by three to five acid washes and deionized water rinses (at 5 °C) in small amounts and many times to pH 6–7. Powdered lipopeptides were obtained by freeze-drying and analyzed using ACQUITY UPLC/VION IMS QTOF MS Compositional testing was performed.

2.7. Insecticidal Assays

Based on the results of the preliminary experiments testing the activity of the products against A. gossypii, gradient concentrations were prepared to conduct insecticidal activity tests. Toxicity regression curves were used to calculate lethal median concentration (LC_{50}) values [27].

2.8. Statistical Analysis

Experimental data were subjected to analysis of variance (ANOVA) using DPS_V9.01 software and tested for the significance of differences using Duncan's new compound extreme deviation method ($p<0.05$). Virulence equations and LC_{50} were calculated using IBM SPSS Statistics 25 software. Graphs and charts were produced using Origin 2021 software.

3. Results

3.1. Screening Identified Two Active Bacillus Strains

The leaf-dip method was used to determine the virucidal activity of shake flask fermentation broth of 107 strains of *Bacillus* spp. against *A. gossypii*. The corrected mortality rate of *A. gossypii* ⩾ 60.00% after 48 h of treatment was used as the criterion for the evaluation of active strains. Two strains with high insecticidal activity were identified. Of the two, strain TRM82479 had higher activity,

 doi:10.20944/preprints202501.0560.v1

causing 75.00% mortality with a corrected mortality rate of 73.21% (Table 1). Data from both strains are shown in Figure 1.

As strain TRM82479 had higher insecticidal activity, it was subjected to species identification, WGS, antiSMASH, and isolation insecticidal active components.

Table 1. Cotton aphidicidal active strains obtained by screening[1]

Strain	Mortality rate (%)	Corrected mortality rate (%)
TRM82529	(50.00±5.00) c	(46.43±5.36) c
TRM82467	(71.67±7.6) b	(69.64±8.19) b
TRM82479	(75.00±5.00) b	(73.21±5.36) b
Negative control	(6.67±2.89) d	——
Chemical control	(76.67+2.89) a	(75.00+3.09) a

[1] The data in the table are mean ± standard deviation. Different lowercase letters indicate significant differences at $P < 0.05$ using Duncan's new complex range method.

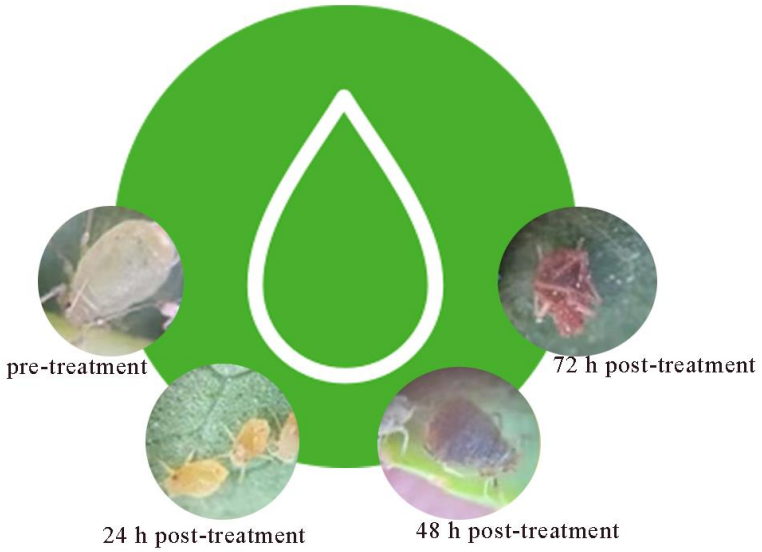

Figure 1. Photographs of antibacterial efficacy

3.2. Species Identification

Strain TRM82479 formed milky-white, nearly round colonies on LB plates with a glossy surface (Figure 2). By scanning electron microscopy, its cells were long and cylindrical, 2–4 μm in length, with a smooth cell surface (Figure 3). The 16S rRNA sequence of strain TRM82479 was compared with those in the EzBioCloud database. We observed a similarity of 99.72% with the 16S rRNA sequence of *B. australimaris* NH71_1; other 16S rRNA sequences similar to this strain were retrieved and downloaded from the EzBioCloud database, and used to construct a phylogenetic tree. The tree suggests that TRM82479 is most closely related to *B. australimaris* NH71_1; therefore, the strain was preliminarily identified as *B. australimaris* TRM82479 (Figure 4). The 16S rRNA sequence of this strain has been uploaded to NCBI under GenBank accession number PQ423094.

 doi:10.20944/preprints202501.0560.v1

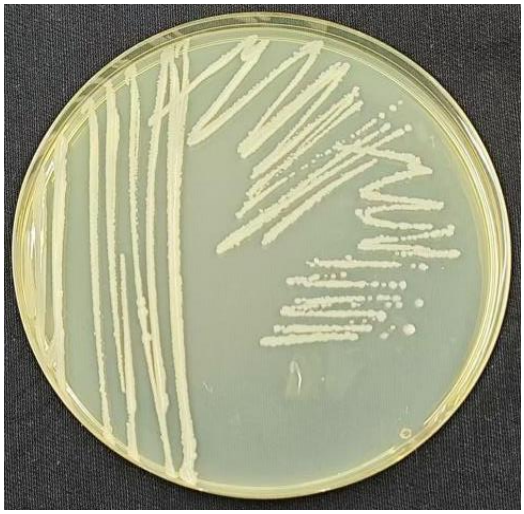

Figure 2. Strain TRM82479 growth on solid substrate.

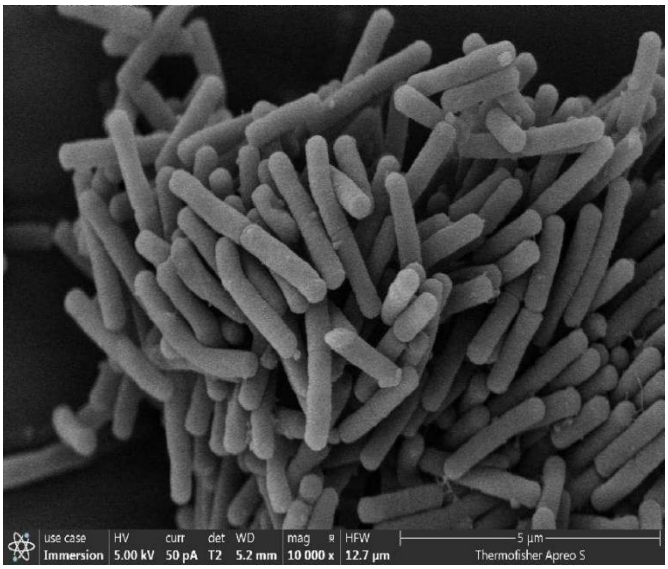

Figure 3. Scanning electron micrograph of strain TRM82479.

 doi:10.20944/preprints202501.0560.v1

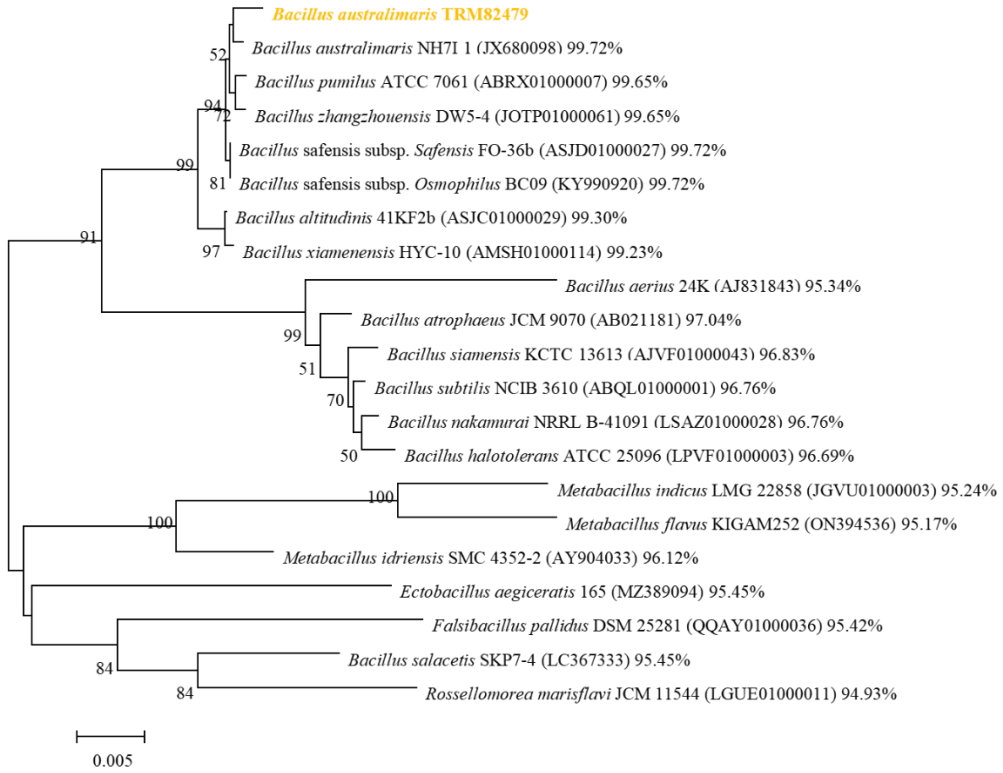

Figure 4. Phylogenetic tree of strain TRM82479

3.3 Strain TRM82479 Genome Contains Clusters of Genes Associated with Lipopeptide Analog Synthesis

Based on WGS, 12 clusters of secondary metabolite synthesis genes were predicted using the antiSMASH database. Of these, six clusters had higher similarity to known gene clusters, with two associated with fengycin and lichenysin synthesis (Table 2). In a comparison of Cluster 8 with the lichenysin, 92.00% similarity was found. This cluster of includes three NRPS genes, lchAA, lchAB, and lchAC; it is responsible for the synthesis of a lipopeptide containing seven modules: Gln, Leu, D-Leu, Val, Asp, D-Leu, and Ile. In contrast, the cluster of *B. australimaris* TRM82479 includes multiple gene fragments (ctg1_7, ctg1_8, ctg1_9, ctg1_10, and ctg1_11) and contains nine modules: Glu, Ile, D-Leu, Ile, Asp, D-Leu, Ile, D, and Val. In modules 8 and 9, there were modifier enzyme domains such as CAL and KR, but the last putative esterase gene in the lichenysin biosynthesis gene cluster was not observed. These differences suggest that the TRM82479 lipopeptides are significantly different structurally and functionally from lichenysin (Figure 5). In summary, *B. audiculmaris* TRM 82479 may produce lipopeptides that are distinct from fengycin and lichenysin, and lipopeptides have been reported in recent years to have insecticidal activity, we isolated lipopeptides from this strain.

Table 2. Putative gene clusters for the synthesis of secondary metabolites in the *B. australimaris* TRM82479 genome.

Clusters	Type	From	To	Most similar known clusters	Similarity
Cluster 1	betalactone	6,442	34,091	Fengycin biosynthetic gene cluster from B. velezensis FZB42	53%
Cluster 2	terpene	102,467	124,341	None	
Cluster 3	T3PKS	163,679	204,779	None	
Cluster 4	RiPP-like	513,619	523,945	None	
Cluster 5	betalactone	663,643	696,094	None	
Cluster 6	NI-siderophore,	184,353	212,695	Schizokinen biosynthetic gene cluster from Nostoc sp.	60%

 doi:10.20944/preprints202501.0560.v1

	terpene			PCC 7120 = FACHB-418r	
Cluster 7	RRE-containing	357,579	378,424	None	
Cluster 8	NRPS	165,368	249,092	Lichenysin biosynthetic gene cluster from B. licheniformis DSM 13 = ATCC 14580	92%
Cluster 9	RRE-containing, LAP	97,071	120,235	Plantazolicin biosynthetic gene cluster from B. pumilus ATCC 7061	91%
Cluster 10	lanthipeptide-class-iii	25,195	47,885	None	
Cluster 11	other	17,685	59,052	Bacilysin biosynthetic gene cluster from B. velezensis FZB42r	85%
Cluster 12	NRP-metallophore, NRPS	26,276	63,648	Bacillibactin biosynthetic gene cluster from B. subtilis subsp. subtilis str. 168	100%

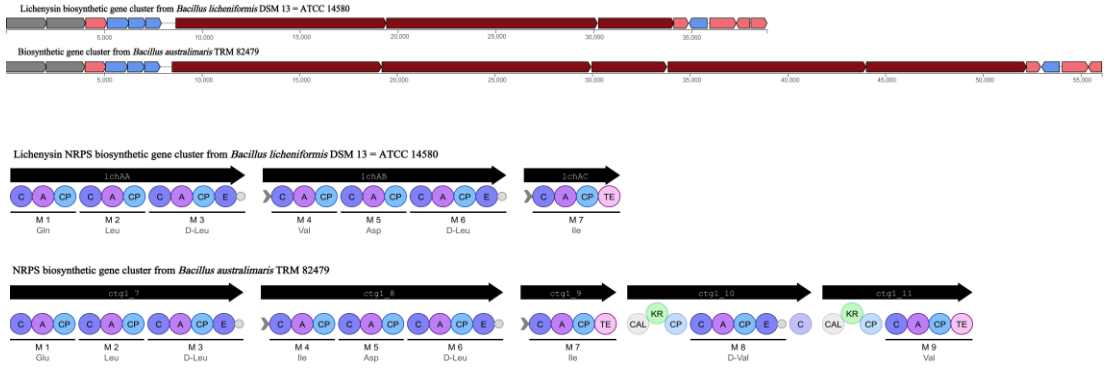

Figure. 5 Cluster 8 vs. lichenysin synthesized gene clusters

3.4 Identification of Insecticidal Components

The lipopeptide compounds obtained by isolation and purification were detected by ACQUITY UPLC/VION IMS QTOF MS (Positive ion mode scanning, mass scanning range of 50–2000 m/z, Capillary voltage: 3.00 kV; Source temperature: 100 °C; Desolvation temperature: 500 °C; Cone gas: 50 L/h; Desolvation gas: 800 L/h), and ion peaks with regularity of m/z of 994.63592, 1008.65235, 1022.66552, 1036.68148, and 1050.69834 appeared in the primary mass spectra, which were presumed to be the fatty acid chains differing by one substituent methyl (-CH_2) homologue of Surfactin [28]. Secondary MS was performed on the above five ion peaks. Both 685.4 and 699.4 fragments were present in the secondary mass spectra; the former corresponds to a known characteristic fragment of surfactin, corresponding to the fatty acid chain +Glu (N-terminal fragment after ring-opening) that frequently appears in the MS/MS spectra of Surfactin as a B_1 ion [29]. The latter fragment likely corresponds to an increase in fatty acid chain length by one CH_2 of the B_1 ion, or the replacement of Val with Leu or Ile. Together, these data strongly suggest that the lipopeptide analog belongs to the surfactin series (Figures 6–11).

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 January 2025 doi:10.20944/preprints202501.0560.v1

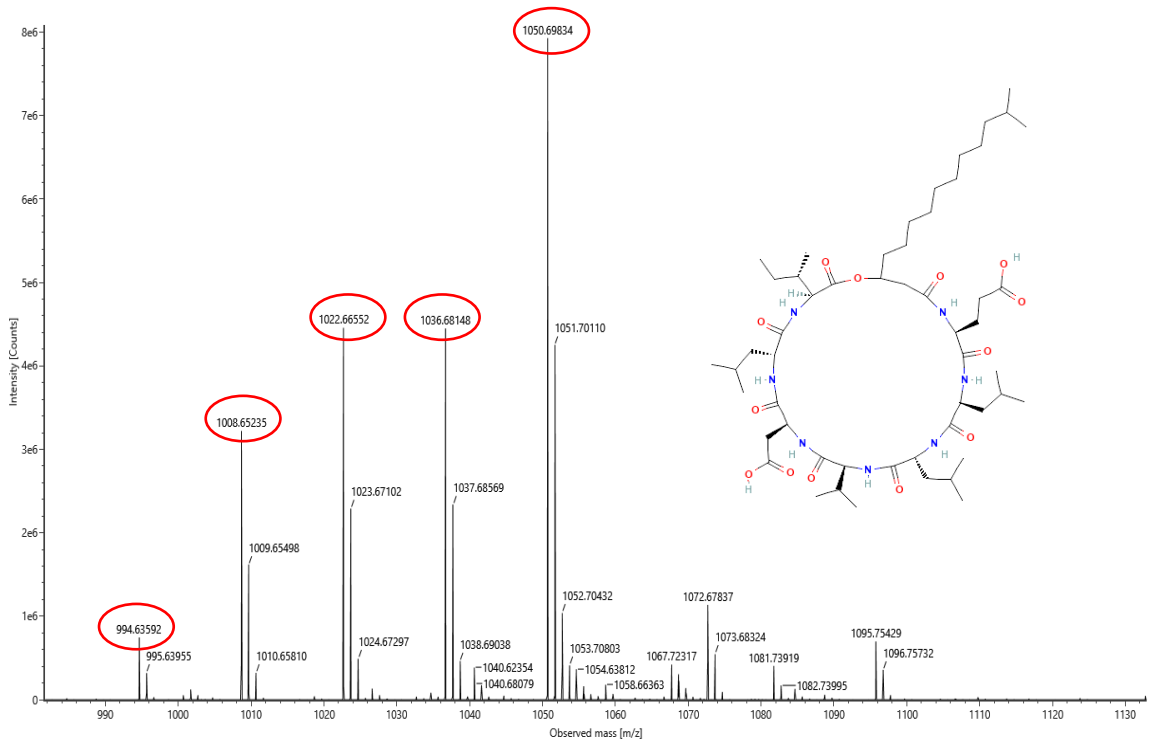

Figure 6. Primary mass spectra and chemical structure of surfactin.

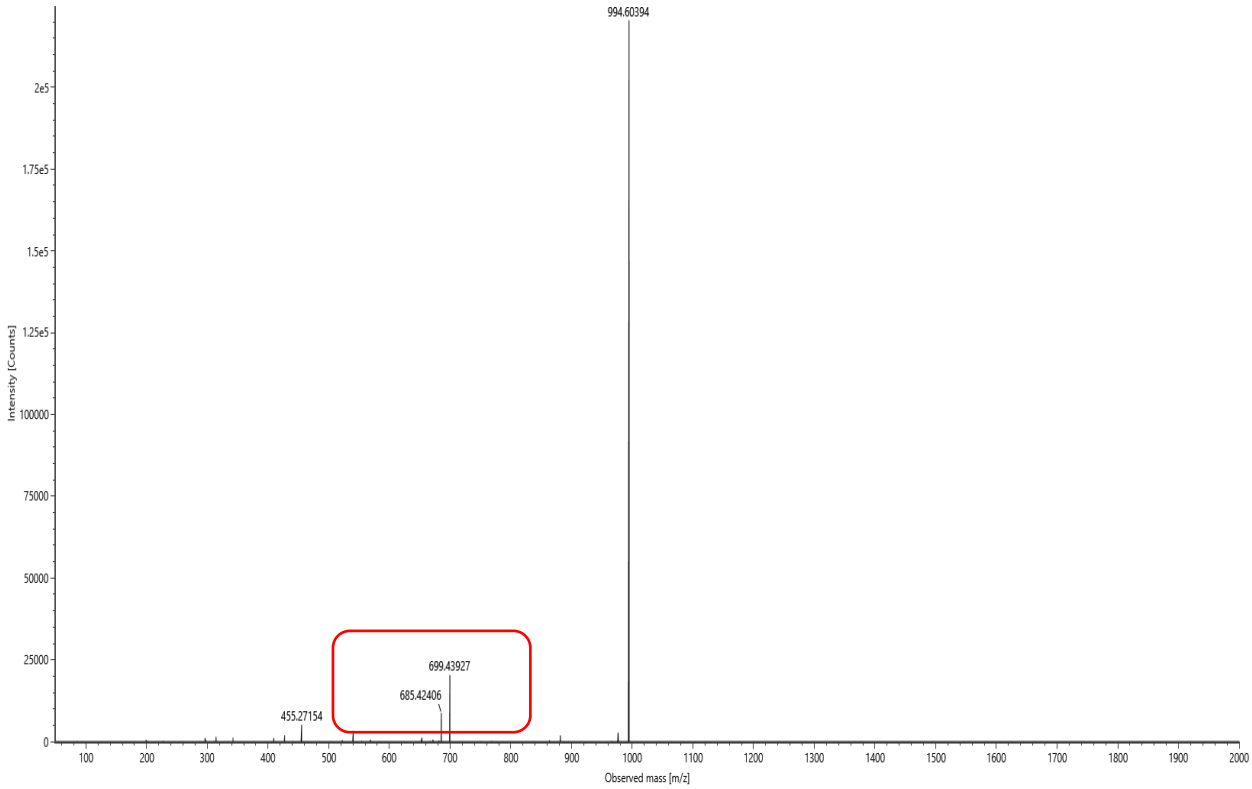

Figure 7. Mass:charge ratio (m/z) of 994.6 secondary mass spectra

 doi:10.20944/preprints202501.0560.v1

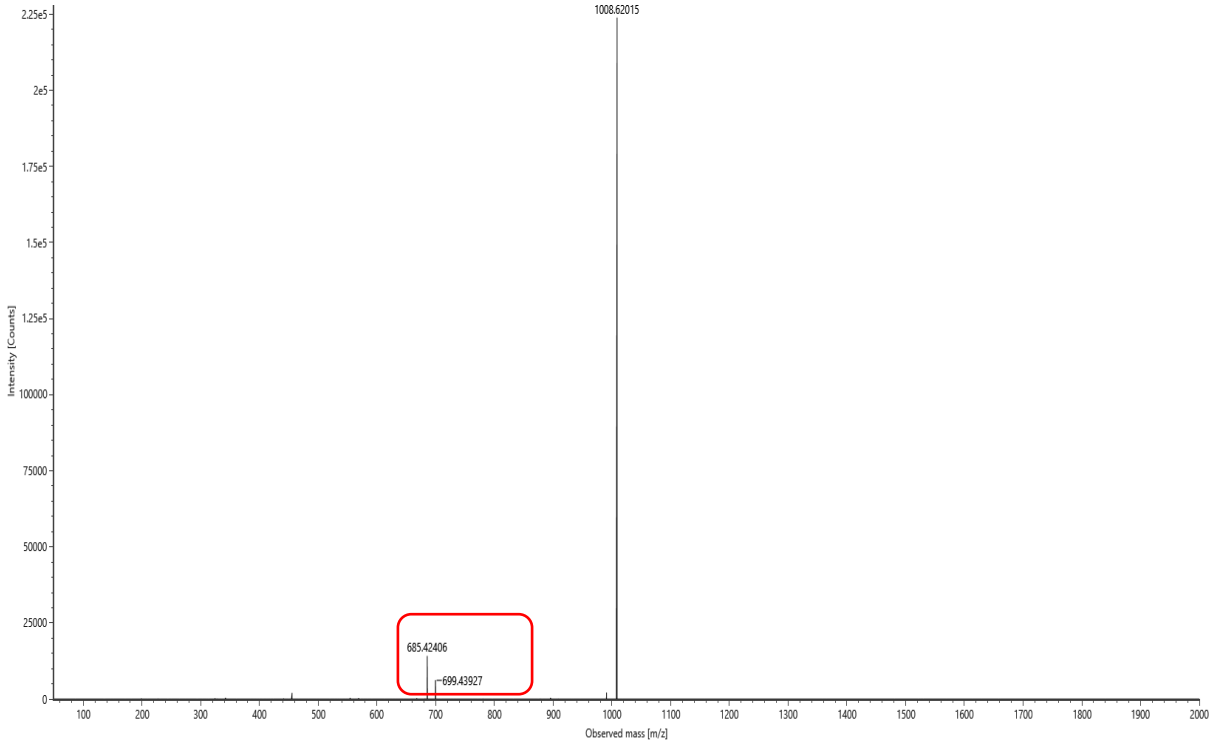

Figure 8. Mass:charge ratio (m/z) of 1008.6 secondary mass spectra

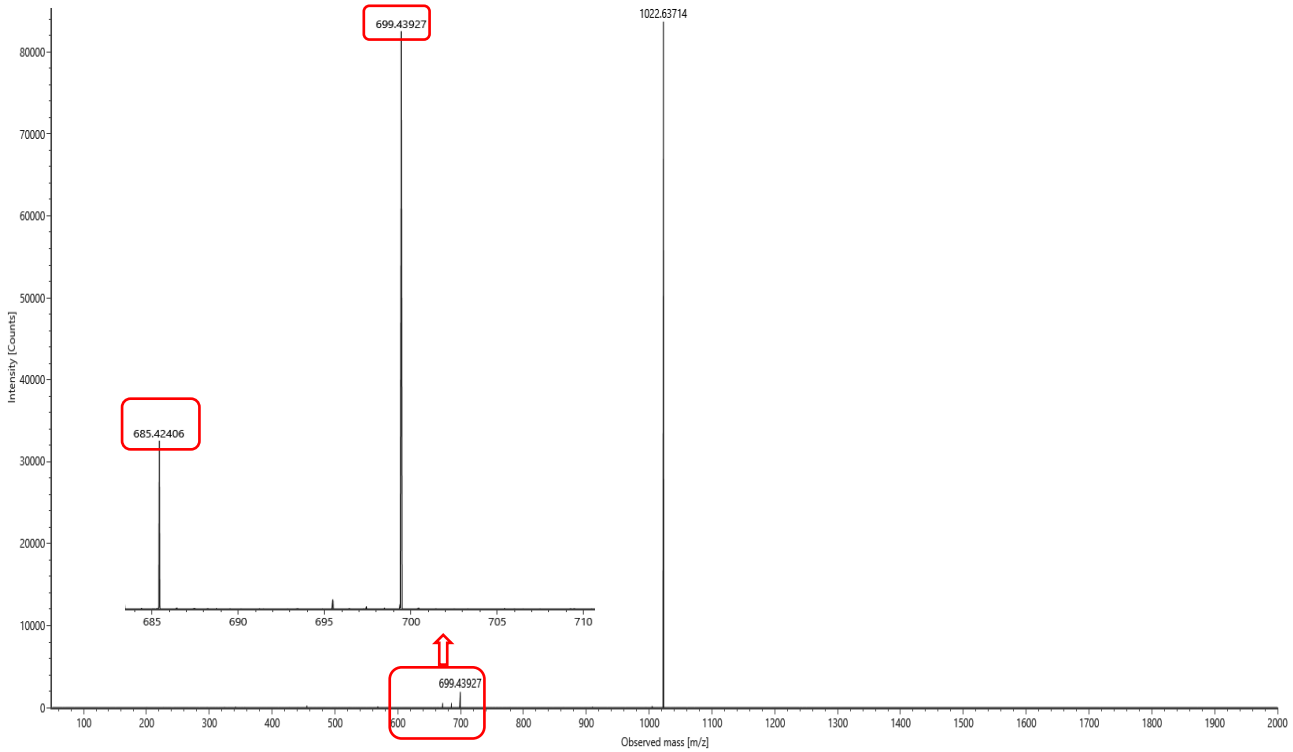

Figure 9. Mass:charge ratio (m/z) of 1022.6 secondary mass spectrometry

 doi:10.20944/preprints202501.0560.v1

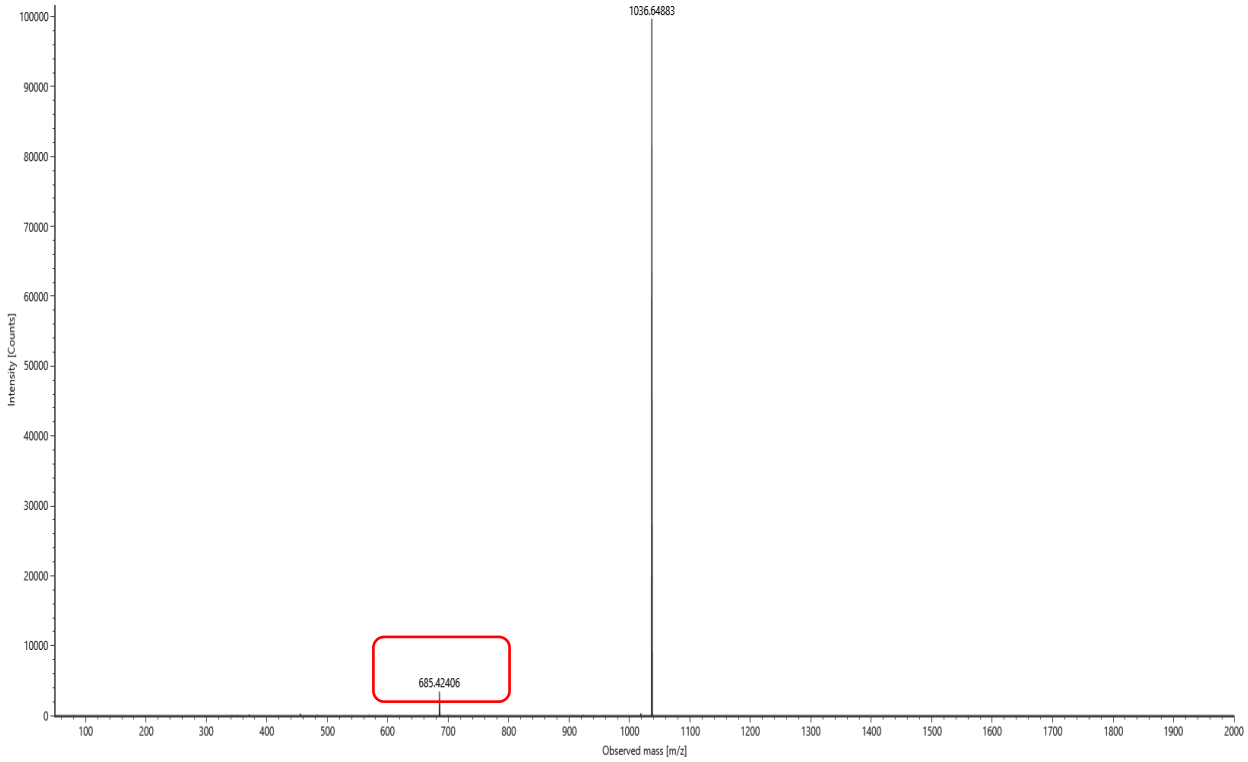

Figure 10. Mass:charge ratio (m/z) of 1036.6 secondary mass spectra

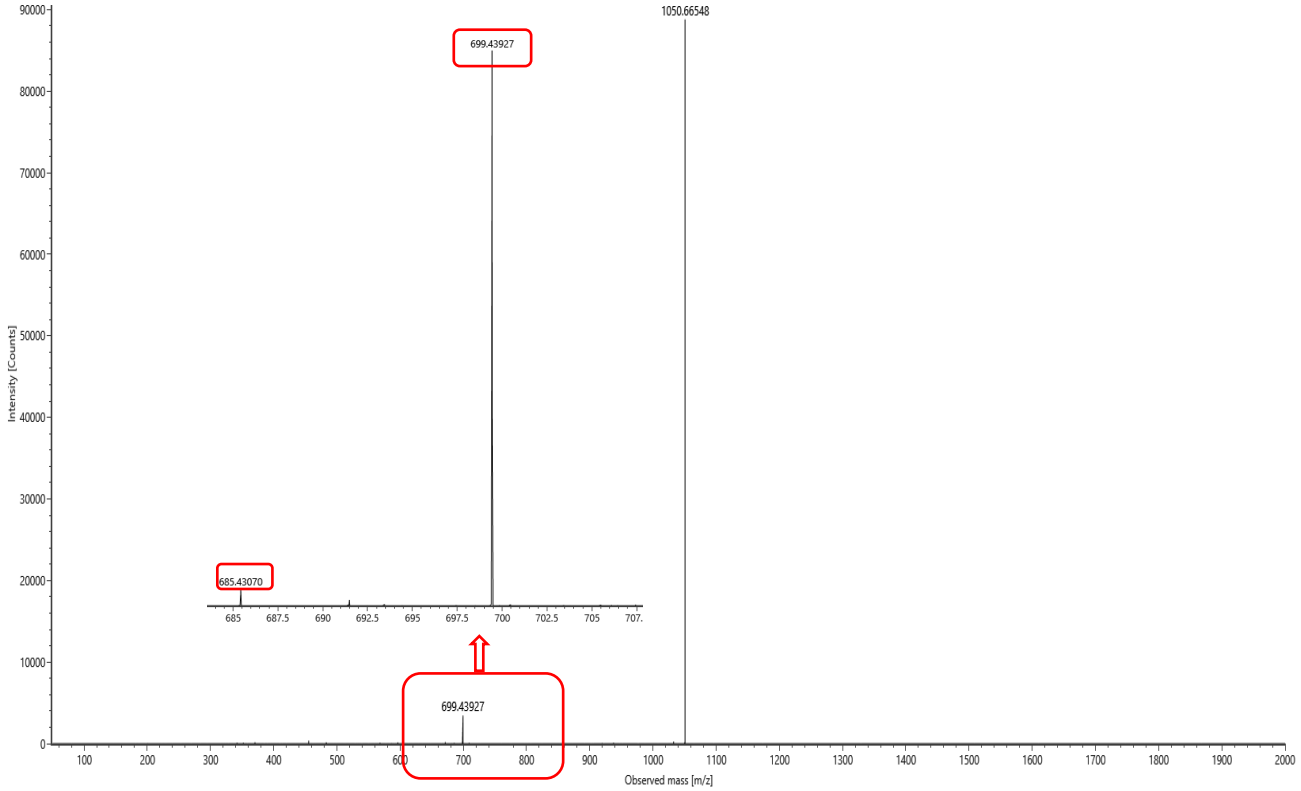

Figure 11. Mass:charge ratio (m/z) of 1050.6 secondary mass spectra

3.5 Surfactin Insecticidal Activity

The aqueous surfactant was dissolved in deionized water with ultrasound assistance at a concentration of 4 mg/mL and tested for *A. gossypii* killing activity using foliar sprays [30], which yielded an *A. gossypii* mortality rate of 93.33% at 48 h. Surfactin at concentrations of 2 mg/mL, 1

 doi:10.20944/preprints202501.0560.v1

mg/mL, 0.5 mg/mL, and 0.25 mg/mL yielded mortalities of 80.00%, 53.33%, 40.00%, and 5.00% respectively (Figure 12). The toxicity regression equation was calculated as Y = 2.331X + 0.156, with an LC_{50} of 0.857 mg/mL and an LC_{95} of 4.350 mg/mL.

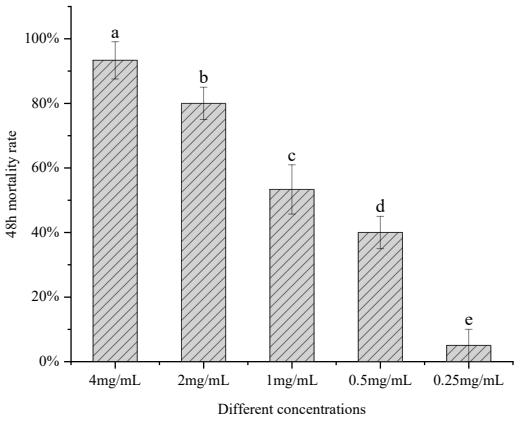

Figure 12. Effect of different concentration treatments on 48h mortality of A. gossypii (48 h)

4. Discussion

A. gossypii is one of multiple pests that pose a serious threat to cotton crops. Bacteria provide safer and more environment-friendly alternatives to commercially available synthetic aphidicides [31]. Numerous studies have shown that certain *Bacillus* species exert insecticidal effects against multiple pests and diseases in a wide range of crops [32–34]. In this study, we used 107 *Bacillus* strains isolated from the Taklamakan Desert to explore the *A. gossypii* killing potential. We identified two *A. gossypii*-killing strains, TRM82467 and TRM82479, under laboratory conditions, with strain TRM82479 showing a 48-h lethality of 75.00% against *A. gossypii* (Table 1). In addition, morphological observations revealed that *A. gossypii* was morphologically complete before the activity test, with a yellowish body color, smooth body surface, and clear antennae and foot structures; 24 h after the activity test, the color of *A. gossypii* became slightly darker, and the body surface appeared slightly wrinkled or discolored; 48 h after the activity test, the morphological changes of *A. gossypii* were more obvious, with significant wrinkling or discoloration of the body surface; severe crumpling (twisting of the abdomen and folding of the legs) and discoloration of *A. gossypii* worms occurred 72 h after the activity test (Figure 1). It has been hypothesized that these changes are caused by a combination of microbial lipopeptides and extracellular cuticle-degrading enzymes (chitinases and proteases), which degrade the chitin and protein components of the cuticle and disrupt the basic function of the exoskeleton [35].

Previous studies have confirmed the insecticidal efficacy of multiple *Bacillus* species. Ruiu et al. found that the spores of *Brevibacillus laterosporu* infect *Musca domestica* and that this group of bacteria adsorbs secreted laterosporamine toxin to the spores, producing insecticidal activity [36]. Fathy et al. isolated 200 strains of *B. subtilis* from mangrove ecosystems in Egypt and tested their activity against *Spodoptera frugiperda*. Among these, *B. subtilis* Esh73 had the highest larval mortality (80.00%) [13]. Ma et al. identified eight *B. thuringiensis* strains with activity against *Culex pipiens pallens* larvae and adults, with the spore-crystal mixture of strain A4 showing significant activity against larvae with an LC_{50} of 1.4±0.5 μg/mL [37]. Al-Azzazy et al. tested *B. subtilis* (2.470×10^8 cfu/ml) and *B. qassimus* (3.320×10^8 cfu/mL) under laboratory conditions on eggplant infested with *Tetranychus urticae* and reductions of 72.22% and 70.74% respectively after seven days of treatment [38]. Liu et al. investigated the insecticidal activity of lipopeptides isolated from *B. velezensis* ZLP-101 against *Acyrthosiphon pisum*. The LC_{50} of the crude extract of ZLP-101 against bean aphids was 411.535 mg/L. The active components in the crude extract, included iturins, engycins, surfactins, and spergualins [39].

 doi:10.20944/preprints202501.0560.v1

This study analyzed the secondary metabolite synthesis genes of *B. australimaris* TRM82479 using antiSMASH and isolated lipopeptide analogs. The lipopeptides obtained from the isolation and purification were detected by ACQUITY UPLC/VION IMS QTOF MS and were confirmed to be a surfactin series by GNPS database comparison and literature research. Xia et al. reported that surfactin produced by *B. subtilis* YZ-1 was significantly toxic to *Tenebrio molitor* by Xia (2023) et al [40]. Moreover, there are few reports on the toxicity of surfactin against *A. gossypii*. Therefore, the findings of this study not only expand our understanding of the bioactive substances produced by the *B. australimaris* TRM82479 strain but also provide new scientific evidence for surfactin as a potential biopesticide.

5. Conclusions

Our findings indicate that *B. australimaris* TRM82479 and the surfactin series it produces have significant potential for application in the green control of *A. gossypii*, for which little has been reported about the toxicological activity of Surfactin against *A. gossypii.* Although this study achieved remarkable results under laboratory conditions, its effectiveness in field applications needs to be further verified. In addition, the development of surfactin as a biopesticide requires further research on its mechanism of action, effects on non-target organisms, and technical issues for large-scale production. This study provides new strain resources and active substances for the biological control of *A. gossypii* and lays the foundation for the development of surfactin as a biopesticide. Future studies should explore its potential application in the field.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Package S1: antiSMASH analysis packet; Package S2: Secondary mass spectral raw data of Surfactin; Table S1: Confidence level of surfactin in killing *A. gossypii*.

Author Contributions: Data curation, Yelin Wang and Zhibin Sun; Methodology, Yelin Wang and Zhanfeng Xia; Resources, Feng Wen and Zhanfeng Xia; Software, Yelin Wang and Zhibin Sun; Supervision, Zhanfeng Xia; Validation, Yelin Wang and Shiyu Wang; Writing – original draft, Yelin Wang; Writing – review & editing, Zhanfeng Xia.

Funding: This work was supported by the Third Xinjiang Scientific Expedition Program (2022xjkk150307), Open Project of the State Key Laboratory of Microbial Metabolism (MMLKF22-01), and Tarim University Graduate Innovation Program (TDGRI2024007).

Data Availability Statement: All data are presented in the article.

Acknowledgments: We thank the Xinjiang Production and Construction Corps Key Laboratory for the protection and utilization of biological resources in the Tarim Basin and for providing the experimental site. We thank the Analysis and Testing Center and the Supercomputing Center of Tarim University for technical support.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

Abbreviations

FAO	Food and Agriculture Organization
LB	Luria-Bertani
PCR	Polymerase Chain Reaction
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
NMR	Nuclear Magnetic Resonance

References

 doi:10.20944/preprints202501.0560.v1

1. Zhang Z, Huang J, Yao Y, Peters G, Macdonald B, La Rosa AD, Wang Z, Scherer LJNRE, Environment: **Environmental impacts of cotton and opportunities for improvement**. 2023, **4**(10):703-715.
2. Quan Y, Wu KJI: **Managing practical resistance of Lepidopteran pests to Bt cotton in China**. 2023, **14**(2):179.
3. Vitale GS, Scavo A, Zingale S, Tuttolomondo T, Santonoceto C, Pandino G, Lombardo S, Anastasi U, Guarnaccia PJA: **Agronomic strategies for sustainable cotton production: A systematic literature review**. 2024, **14**(9):1597.
4. Sharma S, Kooner R, Arora RJBircfsa: **Insect pests and crop losses**. 2017:45-66.
5. Malinga L, Laing MJAACS: **A Review of Pesticides for the Control of Some Cotton Pests**. 2024, **9**(1):1148.
6. Aktar W, Sengupta D, Chowdhury AJIt: **Impact of pesticides use in agriculture: their benefits and hazards**. 2009, **2**(1):1-12.
7. Machado AV, Potin DM, Torres JB, Torres CSSJE, Safety E: **Selective insecticides secure natural enemies action in cotton pest management**. 2019, **184**:109669.
8. Kone PWE, Didi GJR, Ochou GEC, Kouakou M, Bini KKN, Mamadou D, Ochou OG: **Susceptibility of cotton leafhopper Jacobiella facialis (Hemiptera: Cicadellidae) to principal chemical families: implications for cotton pest management in Côte d'Ivoire**. 2018.
9. Samada LH, Tambunan USFJOJBS: **Biopesticides as promising alternatives to chemical pesticides: A review of their current and future status**. 2020, **20**(2):66-76.
10. Fenibo E, Ijoma G, Matambo T: **Biopesticides in sustainable agriculture: A critical sustainable development driver governed by green chemistry principles. Frontiers in Sustainable Food Systems 5: 619058**. In.; 2021.
11. Prasad B, Sharma D, Kumar P, Dubey RCJP, Pathology MP: **Biocontrol potential of Bacillus spp. for resilient and sustainable agricultural systems**. 2023:102173.
12. Rajashekhar M, Shahanaz E, Vinay KJJEZS: **Biochemical and molecular characterization of Bacillus spp. isolated from insects**. 2017, **5**(5):581-588.
13. Fathy HM, Awad M, Alfuhaid NA, Ibrahim E-DS, Moustafa MA, El-Zayat ASJB: **Isolation and Characterization of Bacillus Strains from Egyptian Mangroves: Exploring Their Endophytic Potential in Maize for Biological Control of Spodoptera frugiperda**. 2024, **13**(12):1057.
14. Bora P, Gogoi S, Deshpande MV, Garg P, Bhuyan RP, Altaf N, Saha N, Borah SM, Phukon M, Tanti NJM: **Rhizospheric Bacillus spp. exhibit miticidal efficacy against oligonychus coffeae (Acari: tetranychidae) of tea**. 2023, **11**(11):2691.
15. Wen F, Wu S, Luo X, Bai L, Xia ZJB: **Microbial Community Structure in the Taklimakan Desert: The Importance of Nutrient Levels in Medium and Culture Methods**. 2024, **13**(10):797.
16. da Silva Queiroz O, Nyoike TW, Koch RLJCP: **Baseline susceptibility to afidopyropen of soybean aphid (Hemiptera: Aphididae) from the north central United States**. 2020, **129**:105020.
17. Mesapogu S, Jillepalli CM, Arora DK: **Microbial DNA extraction, purification, and quantitation**. In: *Analyzing microbes: manual of molecular biology techniques.* Springer; 2012: 1-16.
18. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun JJIjos, microbiology e: **Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies**. 2017, **67**(5):1613-1617.
19. Coil D, Jospin G, Darling AEJB: **A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data**. 2014, **31**(4):587-589.
20. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski ADJJocb: **SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing**. 2012, **19**(5):455-477.
21. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SKJPo: **Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement**. 2014, **9**(11):e112963.
22. Wu J, Huang C, Wang J, Hu D, Jin L, Yang S, Song BJJoss: **Separation, interconversion, and insecticidal activity of the cis-and trans-isomers of novel hydrazone derivatives**. 2013, **36**(3):602-608.

23. CHAI Y-q, JIN Y-w, CHEN G-j, LIU Y-g, LI X-l, WANG G-eJASiC: **Separation of insecticidal ingredient of Paecilomyces cicadae (Miquel) Samson**. 2007, **6**(11):1352-1358.
24. Koteshwara A, Philip NV, Aranjani JM, Hariharapura RC, Volety Mallikarjuna SJSR: **A set of simple methods for detection and extraction of laminarinase**. 2021, **11**(1):2489.
25. Li J, Zhu H-JJM: **Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics of drug-metabolizing enzymes and transporters**. 2020, **25**(11):2718.
26. Alajlani M, Shiekh A, Hasnain S, Brantner AJC: **Purification of bioactive lipopeptides produced by Bacillus subtilis strain BIA**. 2016, **79**:1527-1532.
27. Paramasivam M, Selvi CJJoE, Studies Z: **Laboratory bioassay methods to assess the insecticide toxicity against insect pests-A review**. 2017, **5**(3):1441-1445.
28. Mnif I, Grau-Campistany A, Coronel-León J, Hammami I, Triki MA, Manresa A, Ghribi DJES, Research P: **Purification and identification of Bacillus subtilis SPB1 lipopeptide biosurfactant exhibiting antifungal activity against Rhizoctonia bataticola and Rhizoctonia solani**. 2016, **23**:6690-6699.
29. Bochynek M, Lewińska A, Witwicki M, Dębczak A, Łukaszewicz MJFiB, Biotechnology: **Formation and structural features of micelles formed by surfactin homologues**. 2023, **11**:1211319.
30. Paliwal D, Hamilton AJ, Barrett GA, Alberti F, Van Emden H, Monteil CL, Mauchline TH, Nauen R, Wagstaff C, Bass CJMB: **Identification of novel aphid-killing bacteria to protect plants**. 2022, **15**(4):1203-1220.
31. Ayilara MS, Adeleke BS, Akinola SA, Fayose CA, Adeyemi UT, Gbadegesin LA, Omole RK, Johnson RM, Uthman QO, Babalola OOJFiM: **Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides**. 2023, **14**:1040901.
32. Islam MS, Ahmad I, Ali MJBJoB: **Biocontrol studies on rizpspheric microorganisms against black rot disease of tea caused by Corticium theae Bernard**. 2018, **47**(4):985-991.
33. Choi S-I, Ajuna HB, Won S-J, Choub V, Kim C-W, Moon J-H, Ahn YSJCP: **The insecticidal potential of Bacillus velezensis CE 100 against Dasineura jujubifolia Jiao & Bu (Diptera: Cecidomyiidae) larvae infestation and its role in the enhancement of yield and fruit quality of jujube (Zizyphus jujuba Miller var. inermis Rehder)**. 2023, **163**:106098.
34. Pappas ML, Samaras K, Koufakis I, Broufas GDJA: **Beneficial soil microbes negatively affect spider mites and aphids in pepper**. 2021, **11**(9):1831.
35. Akpor OB, Akinwusi OD, Ogunnusi TAJH: **Production, characterization and pesticidal potential of Bacillus species metabolites against sugar ant (Camponotus consobrinus)**. 2021, **7**(11).
36. Ruiu L, Satta A, Floris I: **Emerging entomopathogenic bacteria for insect pest management**. 2013.
37. Ma X, Hu J, Ding C, Portieles R, Xu H, Gao J, Du L, Gao X, Yue Q, Zhao LJBm: **New native Bacillus thuringiensis strains induce high insecticidal action against Culex pipiens pallens larvae and adults**. 2023, **23**(1):100.
38. Al-Azzazy MM, Alsohim AS, Yoder CEJA: **Biological effects of three bacterial species on Tetranychus urticae (Acari: Tetranychidae) infesting eggplant under laboratory and greenhouse conditions**. 2020, **60**(3):587-594.
39. Liu Q, Zhao W, Li W, Zhang F, Wang Y, Wang J, Gao Y, Liu H, Zhang LJBm: **Lipopeptides from Bacillus velezensis ZLP-101 and their mode of action against bean aphids Acyrthosiphon pisum Harris**. 2024, **24**(1):231.
40. Xia M, Munir S, Li Y, Ahmed A, He P, Wu Y, Li X, Tang P, Wang Z, He PJPMS: **Bacillus subtilis YZ-1 surfactins are involved in effective toxicity against agricultural pests**. 2024, **80**(2):333-340.