Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

# Involvement of iturin and surfactin in inhibition of a post-harvest fungal pathogen on green bell pepper

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**DOI: 10.24425/jppr.2025.155786**

Received: September 30, 2024
Accepted: February 02, 2025
Online publication: September 23, 2025

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Responsible Editor:
Assunta Bertaccini

**Abstract**

This study aimed to investigate the potential of plant-associated bacteria as bio-control agents for the green bell pepper rot lesion caused by *Colletotrichum scovillei* 244830. A total of 378 bacteria strains isolated from stems and leaves of healthy red chili and tomato were tested for their antagonistic potential. Isolate TS001 associated with tomato stems was identified as *Bacillus* spp. It was found that TS001 showed remarkable inhibition to *C. scovillei* 244830 in *in vitro* and *in vivo* tests. TS001 significantly reduced rot lesions ($p < 0.05$) of fresh green bell pepper fruits by 71.43%. Furthermore, the result of the LC-ESI-MS/MS showed that the culture broth of the strain *Bacillus* sp. TS001 contained iturin and surfactin homolog in No. 3S medium. TS001 exhibited the strongest antagonistic activity that effectively suppressed *C. scovillei* 244830 rot lesion.

**Keywords:** *Bacillus* sp. TS001, bell pepper, iturin, rot lesion, surfactin

## Introduction

Chilli (*Capsicum annum* L.) or pepper is a primary vegetable and spice cultivated worldwide (Haq *et al.* 2022). The global fresh and dried chili production in 2019 reached approximately 38.1 million and 4.3 million tons, respectively. As the fourth largest chili producer in the world, Indonesia produced 2.59 M tons (FAOSTAT 2019). However, chili production also depends on several factors, including fungal disease. Anthracnose is a group of fungal diseases for chili caused by *Colletotrichum scovillei*. Based on its scientific and economic value, this fungal pathogen is categorized as the eighth most important group of phyto-pathogenic fungi globally (Kanto *et al.* 2013). Some fungicides have successfully handled chili fruit rot (Dubey *et al.* 2019). However, the toxic compounds found in fungicides are persistent in soil, contaminating the environment (Zubrod *et al.* 2019; Bendjedid *et al.* 2022). Recently, considerable attention has been paid to plant-associated bacteria, which can enhance host health through various mechanisms (Afzal *et al.* 2019). These bacteria can protect plants against pathogen colonization through direct inhibition of pathogen growth by nutrition and space competitions (Köhl *et al.* 2019), bioactive compounds production (Kaki *et al.* 2020), drought and stress protection (Kumar and Verma 2018; Albdaiwi *et al.* 2019), resistance enhancement (Figueredo *et al.* 2017), and other mechanisms. Plant-associated bacteria may become a new alternative fungicide to control the post-harvest disease caused by *Colletotrichum* in the green bell pepper fruit.

*Bacillus* spp. are ubiquitous bacteria in nature isolated from diverse environmental ecosystems (Pan *et al.* 2017) which are the major soil bacterium (Massadeh and Mahmoud 2019). *Bacillus* spp. possess endospores as a protective exterior, which protects them from extreme pH levels (Gabiatti *et al.* 2018). *Bacillus* spp. have been utilized as biological control agents against plant diseases because they enable survival under extreme environmental conditions with a wide range of temperatures and pH (Beladjal *et al.* 2018). *Bacillus*

spp. produces various lipoprotein antifungal metabolites, such as surfactin, iturin (Hussain *et al.* 2017), and fengycin. These peptides are cyclic compounds of 7 or 10 amino acid chains related to β-amino (iturin) or β-hydroxy fatty acids (surfactin and fengycin). Iturin and fengycin are effective antifungals that inhibit the growth of various types of phytopathogens. Surfactin has a synergistic effect as an antifungal when secreted with iturin (Setiaji *et al.* 2023).

Based on the above information, *Bacillus* has the potential to be a biological control agent that can be found in diverse environments. Thus, in this study, the bacterial biocontrol agent was isolated and screened from bacteria associated with tomato and chili plants for green bell pepper fruit rot lesions caused by *C. scovillei* 244830.

## Materials and Methods

### Isolation of plant-associated bacteria

Leaves and stems of healthy chili pepper (*Capsicum annum* L, cultivar Lembang 1) grown organically in Cibinong (6°29'41"S106°51'01"E) and tomato (*Solanum lycopersicum* L) grown organically in Bogor (6°36'29"S106°47'19"E), West Java, Indonesia, were used as sources of plant-associated bacteria in nature. The samples were collected and kept in a cooling bag for laboratory analysis in Tokyo. The plant samples were washed in running tap water and dried using sterilized tissue paper. One hundred mL of sterile distilled water (SDW) were added to each 5 g of leaves and stems of chili and tomato, and homogenized for 30 seconds using a blender. The homogenized samples were diluted to $10^{-1}$ and $10^{-2}$ using SDW, and 100 µl of the diluted samples were spread on King's B agar (2.0% proteose peptone, 0.15% $K_2HPO_4$, 0.15% $MgSO_4 \cdot 7H_2O$, 1.0% glycerol, 1.5% agar) plates. Plates were incubated at 25°C for 2–7 days. Each growing bacterial colony was purified by repeated transfer to a sterile fresh King's B medium.

### Inhibition test of plant-associated bacteria against *Colletotrichum scovillei* 244830

*Colletotrichum scovillei* 244830 was inoculated into the center of sterile King's B agar plates, and each of the four plant-associated bacterial strains was inoculated at an equidistance from the center of the medium where *C. scovillei* was first inoculated. The plates were kept at 25°C for 3–7 days, followed by the observation of growth inhibition. Each plate treatment was performed in three replications for each isolate. The inoculation of the fungal pathogen and the bacterial test were done using a sterilized toothpick.

### TS001 strain identification

The genomic DNA was extracted from the colonies on the King's B agar plates with the PrepMan Ultra DNA extraction kit (Applied Biosystems, Foster City, CA, USA). The PCR amplification of 16S rRNA was performed using primers F27 and 1492R as described by Frank *et al.* (2008). The purification of PCR products was conducted with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Duren, Germany) and were used for the DNA sequencing. A partial sequence of 16SrRNA gene was examined using the EzBioCloud 16S Identify (https://www.ezbiocloud.net).

### Efficacy of TS001 strain in *Colletotrichum scovillei* rot lesion suppression in green bell pepper

### Preparation of *Colletotrichum scovillei* culture suspension

About 5 ml of SDW was pipetted onto the vegetable medium agar plate (200 ml vegetable juice, 800 ml distilled water, 15 g agar), which was overgrown with 7-day-old *C. scovillei*. The fungal pathogen mycelia were gathered from the surface of the vegetable media using a glass rod and applied to a 14 ml plastic centrifuge tube, then centrifuged at 2300 ×g for 10 minutes. Subsequently, the precipitated cells were re-suspended in 1% Plus One TritonX-100 to a final density of approximately $4 \times 10^6$ conidia $ml^{-1}$ (Koley and Bard 2010).

### Preparation of TS001 strain culture suspension

About 5 ml of SDW was pipetted onto a 2-day-old TS001 strain on the King's B agar plate. The bacterial colonies were rubbed using a glass rod, transferred to a 14 ml plastic centrifuge tube, and centrifuged at 2300 × g for 10 minutes. The precipitated cells were re-suspended in 1% of Plus One TritonX-100 to a final density of approximately $31 \times 10^6$ cell $ml^{-1}$ (Koley and Bard 2010).

### Inoculation of green bell pepper with fungal pathogen *Colletotrichum scovillei* and TS001 strain

Nine fresh green bell pepper fruits were surface-sterilized with a household commercial bleaching solution containing 5% sodium hypochlorite, diluted in SDW to a ratio of 1:100 (v/v) for 3 minutes, followed by three washes with SDW. Any remaining water attached to the fruits was wiped using sterilized tissue paper. Each bell pepper was cut into two sections, obtaining 18 half-fruits that were grouped into

three groups, namely "No inoculum" as the control, "*C. scovillei* infection", and treatment group (pathogen and TS001). Each group contained six half-fruits; and was moved to plastic boxes (25 × 17 cm), covered with sterilized tissue paper at the bottom of the plastic boxes, and sprayed with SDW to maintain relative humidity. Three uniform holes were made in each bell pepper using the tip of a sterile needle. In the "No inoculum" group, the holes were filled with 20 µl of sterile distilled water. Meanwhile, in the "*C. scovillei* infection" group, the holes were injected with only the *C. scovillei*. For the treatment group, namely "*C. scovillei* pathogen+TS001", the holes were injected with 20 µl of *C. scovillei* and 20 µl culture suspension of TS001 strain. Thus, there were 18 inoculation replications for each treatment. The plastic boxes containing the green bell pepper were closed and stored in an incubator at 25℃. The infection rate and lesion diameter of fruits were measured 3 days after inoculation.

### Cultivation and extraction of the bioactive compounds produced by TS001 strain

One hundred µl of overnight pre-cultivation of TS001 strain in No. 3S medium was inoculated into 10 ml No. 3S medium in a test tube, and then incubated for 4 days using an incubator shaker at 25°C with 150 rpm. One liter of No.3S medium contained 10 g of polypeptone S (Nihon Pharmaceutical, Tokyo, Japan), 10 g of glucose, 1 g of $K_2HPO_4$, and 0.5 g of $MgSO_4 \cdot 7\ H_2O$ (Zohora *et al.* 2009). Then, 10 ml of bioactive compounds in the 4-day-old TS001 culture broth was extracted using the butanol extraction method (Yuliar *et al.* 2015). Ten ml of bioactive compounds in the 4-day-old TS001 culture broth was extracted using the butanol extraction method. First, 10 ml of the bacterial culture was centrifuged at 2000 × g for 5 min, then 1 ml of butanol was added to the supernatant. After centrifugation, the organic layer was transferred to a 2 ml microtube. After the organic layer was transferred, 0.5 ml of butanol was added to the remaining sample solution before vortexing and centrifuging. The organic layer was recollected into the previous 2 ml microtube. This step was performed twice. The collected butanol fraction was dried using a centrifugal concentrator (VC-36R, TAITEC CO., Ltd., Saitama, Japan). After drying, 240 µl of MeOH (100%) was added. When it became homogenous, 160 µl of super pure water (SPW) was added. Finally, the sample was filtered through a 0.45 µm membrane filter, and the filtrate sample was used for the LC-ESI-MS/MS analysis (Thongjun *et al.* 2016).

### LC-ESI-MS/MS analysis

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) coupled with collision-induced dissociation was performed to identify the bioactive compounds in extracts from TS001. The selected precursor ions were obtained by the auto LC–ESI–-MS/MS modalities. The data were analyzed using an accurate-mass quadrupole time of- flight LC/MS with 6530 (Agilent Technologies, Santa Clara, CA, USA). The separation was conducted using an Inert Sustain AQ-C18 (1.0 × 150 mm, 3 µm, GL Sciences, Tokyo, Japan) at 40°C with a constant flow rate of 0.07 ml/min using the following gradient: i) 30% B for 1 min; ii) a gradient of 30-100% B; iii) 1005B for 12 min. Mobile phase A consisted of ultrapure water (UPW) containing 0.1% (v/v) formic acid, B consisted of acetonitrile containing 0.1% (v/v) formic acid. The injection volume was set to 3 µl. These data were analyzed by the Agilent Mass Hunter Qualitative Analysis Software (Version B.06.00).

### Data analysis

Data on the inhibitory ability of *Bacillus* sp.strain TS001 to reduce *C. scovillei* rot lesion were determined statistically by analysis of variance (ANOVA) using Minitab 16 software. The significance of mean differences was determined using the Duncan's test. The significance responses were assessed based on a 5% confidence level.

## Results

### Isolation of red chili and tomato plant--associated bacteria

A total of 378 bacterial strains were isolated from the leaves and stems of healthy red chili and tomato plants. The leaves yielded more bacterial strains than the stems, with 220 isolates from leaves and 158 isolates from stems, respectively (Fig. 1).

### Inhibition test of plant-associated bacteria against *Colletotrichum scovillei* 244830

Based on the 378 bacterial strains tested, the TS001 strain isolated from the tomato stem showed the highest antagonistic activity against *C. scovillei* on the King's B agar plates (Fig. 2).

### Identification of TS001 strain

A partial sequence of 16S rRNA gene of TS001 strain showed high homology (>98%) with several strains of *Bacillus* spp. (data not shown). The result of 16S rRNA sequence in the TS001 strain indicated that the strain was *Bacillus* sp.

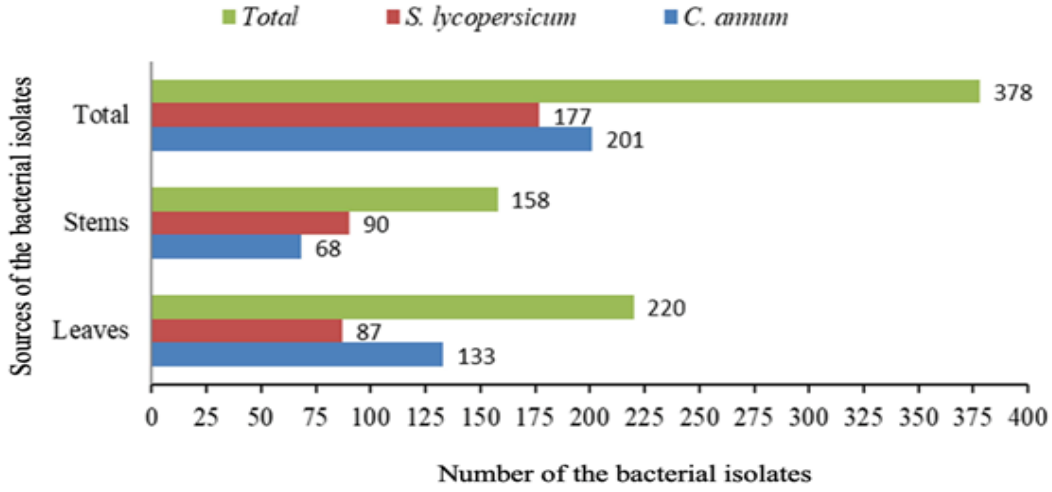


**Fig. 1.** Number of plant-associated bacterial strains isolated from *Capsicum annum* (chili pepper) and *Solanum lycopersicum* (tomato)

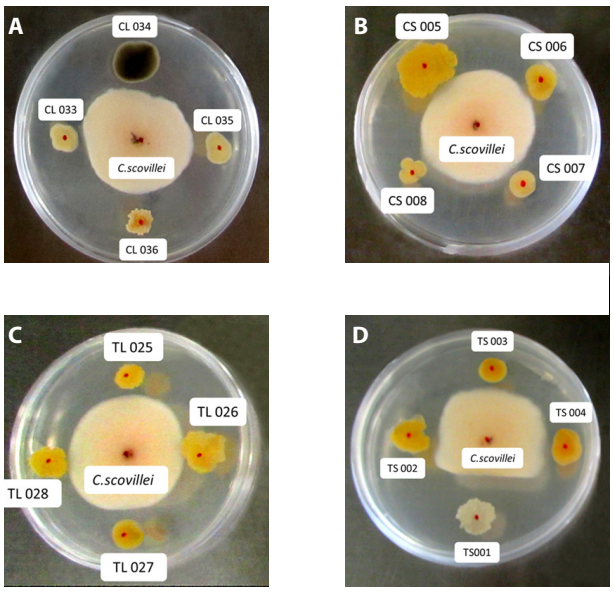


**Fig. 2.** Inhibition test of A – chili leaves (CL); B – chili stems (CS); C – tomato leaves (TL), and D – tomato stems (TS) plant-associated bacteria against *Colletotrichum scovillei* 244830

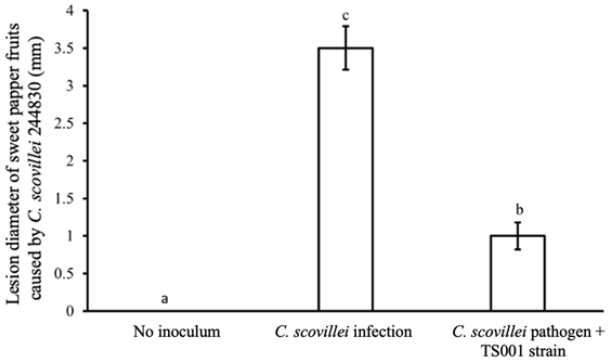


**Fig. 3**. Suppressive ability of *Bacillus* sp. TS001 strain to reduce rot lesion diameter of green bell pepper caused by *Colletotrichum scovillei* 244830

## Efficacy of TS001 strain in *Colletotrichum scovillei* rot lesion suppression in green bell pepper

TS001 strain significantly ($p < 0.05$) suppressed *C. scovillei* rot lesion in fresh green bell pepper 3 days after incubation by 71.43%. Furthermore, green bell pepper treatment without the TS001 strain formed 3.5 mm rot diameter, while green bell pepper treatment with the TS001 strain could reduce the rot diameter to 1 mm (Fig. 3 and S1).

## LC–ESI-MS/MS analysis of bioactive compounds

The elution programs for putative lipopeptides were specifically designed for surfactins, iturins, and fengycins. The LC–ESI-MS/MS chromatograms of the TS001 extract displayed main peaks between 9.5 and 21 min (Fig. 4). The precursor ions were assigned as the proton ion adductor homologous to surfactin and iturin lipopeptides, as shown in the LC-ESI-MS/MS (Fig. 5).

## LC-ESI-MS/MS analysis of iturin lipopeptides

The LC–ESI–MS/MS spectrum of the protonated molecule [M+H]+ at m/z 1043.6 revealed a set of fragment ions (Fig. 6A). Contiguous fragmentation of both ends of m/z of 1043.6 occurred b-type ions

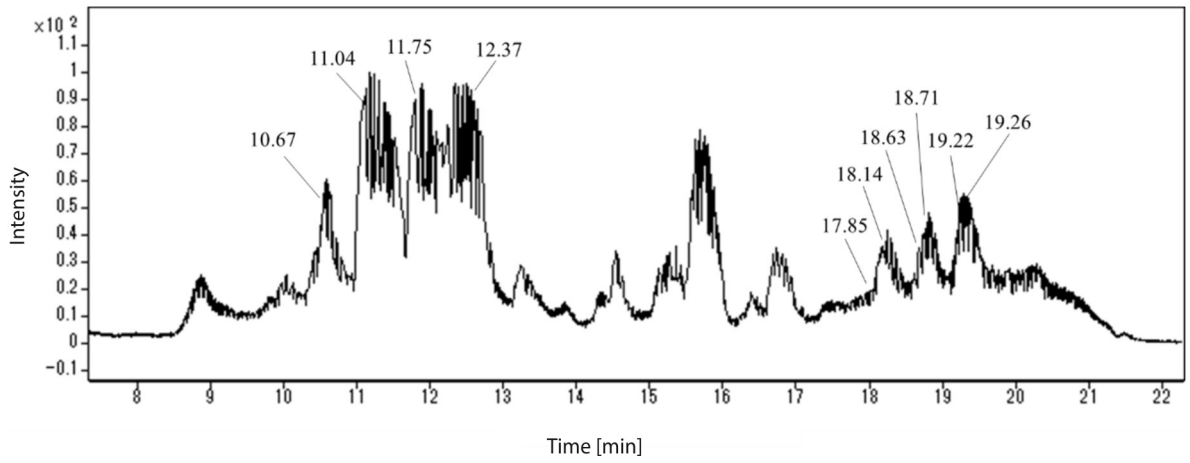


**Fig. 4.** LC–ESI-MS/MS chromatograms of the extract from the TS001 in positive ion mode, TIC

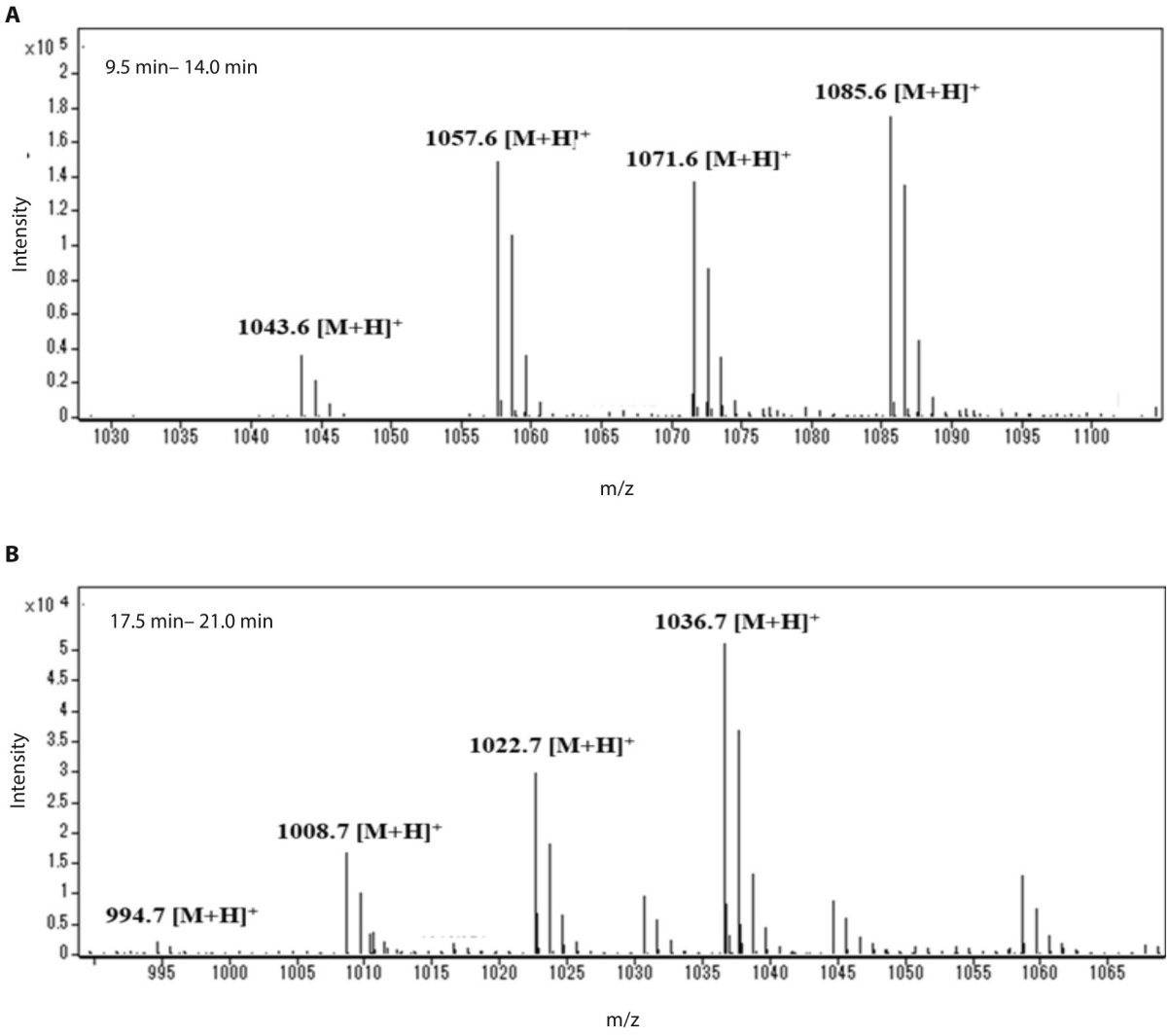


**Fig. 5.** LC-ESI-MS/MS spectrum in positive ion mode: A – cluster of iturin family; B – cluster of surfactin family

at m/z 915.4(+$H_2O$, 933.4), 801.4, 638.3, 524.3 (-$NH_3$, 507.3), 299.1, 212.1, and 70.1 and the corresponding y-tipe ion detected at m/z 832.4, 745.4,406.3, 243,1 and 101.1. The results showed that the sequence can be deduced as Pro-Asn-Ser-C14β- -Asn-Thr-Asn-Gln. In addition, LC–ESI–MS/MS spectra of protonated molecules detected m/z of 1057.6 at a retention time of 11.04 min (Fig. 6B), 1071.6 with a retention time of

11.75 min (Fig. 6C) and 1085.6. at a retention time of 12.37 min (Fig. 6D). Several 14 Da ($-CH_2$) differences proved to be homologous amino acid sequences with differences in the C14, C15, C16 and C17 β-amino fatty acids, respectively (Table 1).

### LC–ESI–MS/MS analysis of surfactins

Figure 7A displays the LC–ESI–MS/MS spectrum of the protonated molecule at m/z of 994.7 with the fragmentation appearance at a retention time of 17.85 min. Consecutive fragmentations from the two ends of the m/z of 994.7 yielded b-type ions at m/z of 895.6, 782.5, 667.4, 568.4, 455.3, and 342.2, together with the matching y-type ions recognized at m/z of 796.5, 667.4 ($+H_2O$, 685.5), 554.4, 441.3, 342.2, and 227.2. The results indicate that the sequence can be deduced as C12 β-hydroxyl -Glu-Leu-Leu-Val-Asp-Leu-Leu/Ile, namely surfactin A. Moreover, the LC–ESI–MS/MS spectra of protonated molecules detected m/z of 1008.7 at a retention time of 18.14 min (Fig. 7B), 1022.7 at a retention time of 18.70 min (Fig. 7C), and 1036 at a retention time of 19.26 min (Fig. 7D). Various 14 Da ($-CH_2$) distinctions proved to be surfactin A with homologous amino acids arrangement, but different in C12, C13, C14 and C15 β-OH fatty acids (Table 1).

**Table 1**. Assignment of the structure of lipopeptides by LC-ESI-MS/MS

| Mass [m/z] | Rt [min] | Family | Assignment |
|---|---|---|---|
| 1043.6 | 10.67 | Iturin | $C_{14}$ iturin A $[M + H]^+$ |
| 1057.6 | 11.04 | | $C_{15}$ iturin A $[M + H]^+$ |
| 1071.6 | 11.75 | | $C_{16}$ iturin A $[M + H]^+$ |
| 1085.6 | 12.37 | | $C_{17}$ iturin A $[M + H]^+$ |
| 994.7 | 17.85 | Surfactin | $C_{12}$ Surfactin A/C $[M + H]^+$ |
| 1008.7 | 18.14 | | $C_{13}$ Surfactin A/C $[M + H]^+$ |
| 1022.7 | 18.71 | | $C_{14}$ Surfactin A/C $[M + H]^+$ |
| 1036.7 | 19.26 | | $C_{15}$ Surfactin A/C $[M + H]^+$ |
| 1008.7 | 18.63 | | $C_{14}$ Surfactin B $[M + H]^+$ |
| 1022.7 | 19.22 | | $C_{15}$ Surfactin B $[M + H]^+$ |

Figure 7E showcases the LC–ESI–MS/MS spectrum of the protonated molecule at m/z of 1008.7 with the fragment ions at a retention time of 18.63 min. Sequential fragmentations from the two terminations of the m/z of 1008.7 produced the b-type ions at m/z of 909.6, 796.5, 582.4, 469.3, 356.3, and 227.2 in company with corresponding y-type ions formed at m/z of 653.4 ($+H_2O$, 671.4), 540.3, 427.3, 328.2, and 213.1. The results indicate that the arrangement can be firm as C14 β-hydroxyl -Glu-Leu-Leu-Val-Asp-Leu-Val, namely surfactin B. Furthermore, the LC–ESI– MS/MS spectra of protonated ions found at m/z of 1022.7 at a retention time of 19.22 min (Fig. 8F) with multiple 14 Da (–CH2) dissimilar proved to be surfactin B with a homologous amino acid chain but different in C14 and C15 β-OH fatty acids, respectively (Table 1).

## Discussion

### Isolation of red chili and tomato plants--associated bacteria

The results of this study demonstrate that leaves contained more bacterial isolates than stems, with 220 isolates from leaves and 158 from stems (Fig. 1). These results align with a previous report that bacterial isolates are more abundant in leaves than in stems of mangrove and sugarcane plants (Rezamahalleh *et al.* 2019). The differences can be caused by the type of tissue structure of leaves and stems, as reported by Hong *et al.* (2018), Jose and Christy (2013), and Rezamahalleh *et al.* (2019). Hong *et al.* (2018) stated that the tissue type and plant age influence the structure and composition of bacteria in *Panax ginseng*. The leaf tissue has a higher bacterial diversity and distribution than stem and flower stalk tissues. In addition, bacterial colonization in the leaves occurs through natural openings, such as hydathodes and stomata (Hong *et al.* 2018). This may also be the reason why there were more bacterial strains in leaves than in stems.

### Inhibition test of plant-associated bacteria against *Colletotrichum scovillei* 244830

All the tested bacterial isolates exhibited various antagonistic effects on the mycelial growth of the fungal pathogen. TS001 strain isolated from tomato stems showed the highest antagonistic activity against the *C. scovillei* on King's B agar plates. Figure 2 shows the inhibition interactions between the chili and tomato-associated bacterial colonies and fungal pathogen. A distinct interaction between the antagonistic bacteria and the fungal pathogen led to a distant mutual inhibition due to the antifungal production or competition of nutrients and space.

### Efficacy of TS001 strain in *Colletotrichum scovillei* rot lesion suppression in green bell pepper

It is well known that antagonistic microorganisms are capable of producing a wide variety of antimicrobial metabolites (Li *et al.* 2020). In this study, the antagonistic bacterial isolates that suppressed *C. scovillei* in vitro and in the bioassay test on the fresh green bell pepper were screened from chili pepper and tomato plants associated with bacteria. The screening result helped to develop pathogen control. The highest

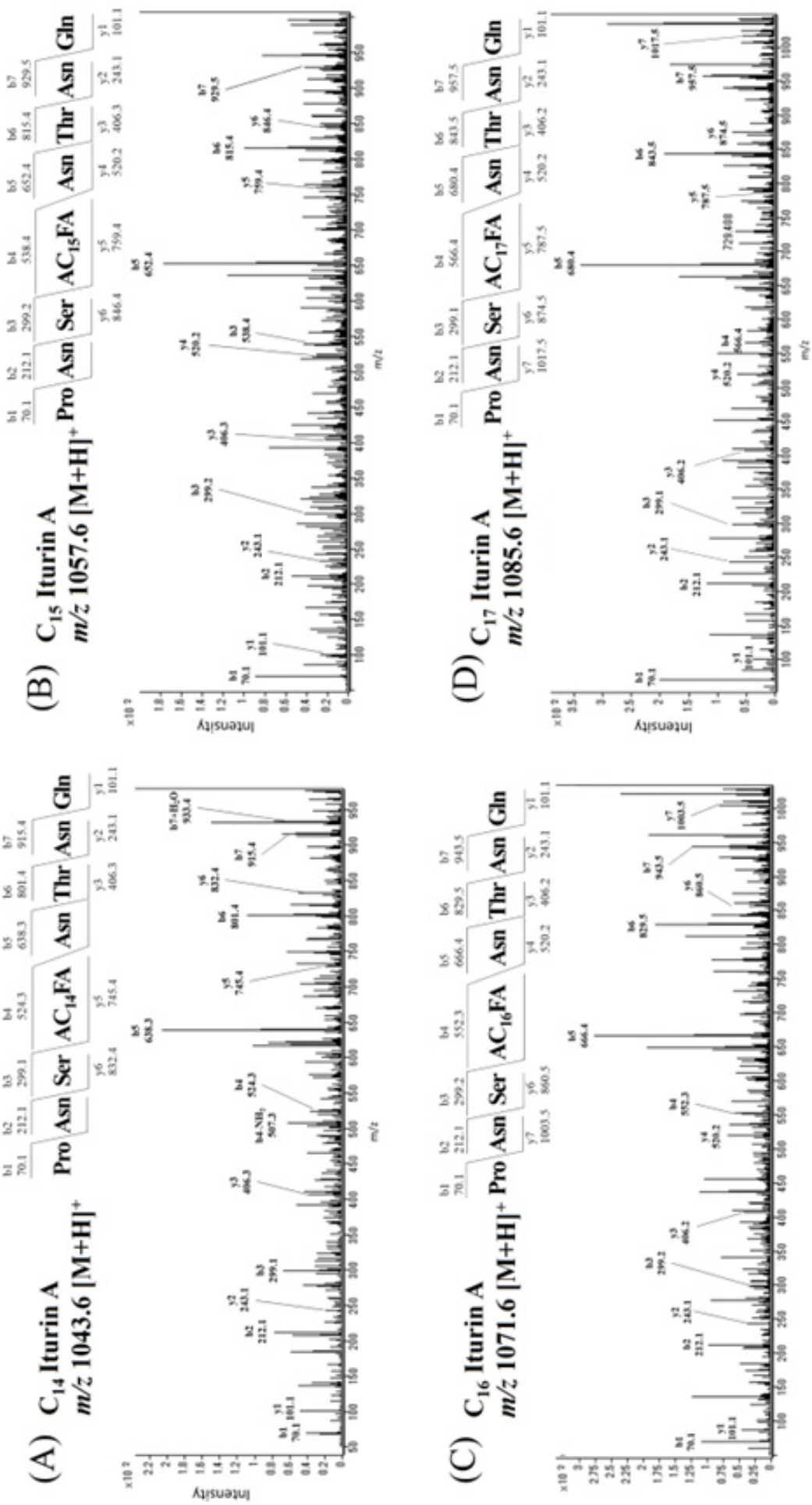


**Fig. 6.** Product ions spectra obtained by the LC-ESI-MS/MS of the protonated molecules [M+H]+ of homologous iturins at m/z of 1043.6 – A; 1057.6 – B; 1071.6 – C and 1085.6 – D

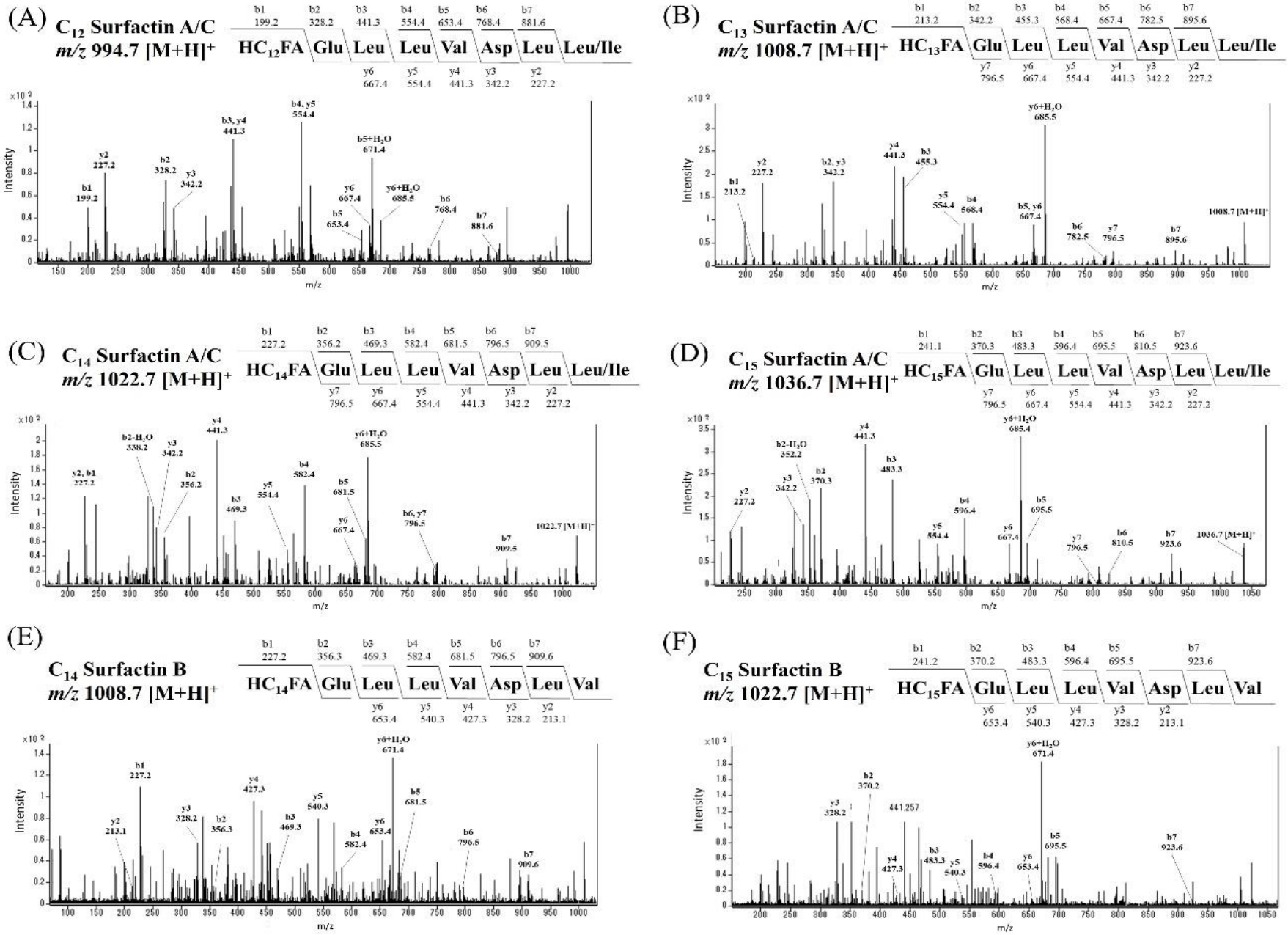


**Fig. 7**. Product ions spectra obtained by LC-ESI-MS/MS of the protonated molecules [M+H]+ of homologous surfactin at m/z of 994.7 – A; 1008.7 – B; 1022.7 – C; 1036.7 – D; 1008.7 – E, and 1022.7 – F

suppression of *C. scovillei* rot lesion in fresh green bell pepper was recorded in the *Bacillus* sp. TS001 strain. The *Bacillus* sp. TS001 strain significantly decreased the *C. scovillei* rot lesion in fresh green bell pepper 3 days after incubation ($p < 0.05$) by 71.43%. In contrast, green bell pepper without the TS001 strain inoculation formed a 3.5 mm rot diameter, and with the TS001 strain inoculation could reduce the rot diameter to 1 mm (Fig. 2 and Fig. 3). A similar finding was also reported by Guerrero-Barajas *et al.* (2020), who evaluated the *Bacillus mycoides* A in avocado fruit against the *Colletotrichum gloeosporoides*. The culture broth of *B. mycoides* produced bioactive compounds to compete with the *C. gloeosporoides* (Guerrero-Barajas *et al.* 2020). Previous studies have shown that iturin and surfactin exhibit antifungal activity (Bakker *et al.* 2024; Sreelakshmi *et al.* 2024). As reported by several researchers, *Bacillus* produces bioactive compounds of lipopeptide antibiotics, such as fengycin, iturin, and surfactin (Sun *et al.* 2019). Cyclic lipopeptides consisting of the iturin, surfactin, and fengycin families are known to be the main factors in suppressing fungal diseases by *Bacillus* spp. (Romero *et al.* 2007; Penha *et al.* 2020). Iturin plays a role in antifungal activity by inhibiting spore germination and mycelial growth, causing disruption of cytomembrane integrity, oxidative stress, lipid peroxidation, metabolic pathways including the TCA cycle, glycolysis/gluconeogenesis, and mitochondrial energy metabolism, as well as mitochondrial damage (Bakker *et al.* 2024; Wang *et al.* 2024).

The *Bacillus* genome contains specific biosynthetic gene clusters (BGCs) responsible for the production of cyclic lipopeptide antibiotics such as iturin and surfactin (Franco-Sierra *et al.* 2020). These genes encode enzymes involved in non-ribosomal peptide synthesis (NRPS) and polyketide synthesis, which are essential for lipopeptide biosynthesis. Around 4-5% of the *B. subtilis* genome contains genes suitable for antibiotic synthesis, which have produced over two dozen structurally diverse antimicrobial compounds. The sequenced genomes of *B. subtilis* EA-CBo575 brought about entire genomes of 4.09 Mb with 4332 genes associated with producing indoles, siderophores, lipopeptides, volatile compounds, phytase, bacillibactin, and nitrogenase.

### Bioactive compounds produced by *Bacillus* sp. TS001 strain

The use of antagonistic microorganisms for pre- and post-harvest disease control is gaining popularity as a sustainable alternative to agrochemicals. Biological management practices play a crucial role in protecting plants from diseases while ensuring food safety. Several studies have provided strong evidence that the *Bacillus* species, including *B. amylolyquefaciens,* exhibit significant biocontrol potential (Jinal and Amaresan 2020). The following could significantly reduce pre- and post-harvest disease severity on various hosts: *B. flexus* (Tran *et al.* 2019), *B. licheniformis* (Peng *et al.* 2017), *B. megaterium* (Baek *et al.* 2020), *B. mojavensis*, *B. mycoides* (Moghaddam *et al.* 2014), *B. oryzicola*, *B. pumilus* (Wang *et al.* 2020), *B. valenzensis* (Shabanamol *et al.* 2021), *B. sphaericus* (Khedher *et al.* 2020), *B. subtilis* (Sun *et al.* 2019), *B. tequilensis* (Bhattacharya *et al.* 2019), and *B. valezensis* (Wang *et al.* 2020).

The present study isolated plant-associated bacteria from chili and tomato plants, obtaining *Bacillus* sp. TS001 to discover the potential of antagonistic bacteria against *C. scovillei*. *Bacillus* sp. TS001 performed the highest antagonistic activity. In addition, the result of LC-ESI-MS/MS of the bioactive compounds of *Bacillus* sp. TS001 strain indicated that the TS001 strain produced several antibiotic cyclic lipopeptides (CLPs), namely iturin and surfactin in No. 3S medium. The bioactive compounds of iturin and surfactin produced by TS001 were assumed to be a possible mechanism for suppressing the pathogenic fungi of *C. scovillei* in fresh green bell pepper fruits. The ability of *Bacillus* sp. TS001 to produce several polypeptide antibiotic compounds makes it a potent bio-control agent against post-harvest *Colletotrichum* rot lesion in green bell pepper. Another study identified lipopeptides of fengycin, iturin, and surfactin from the *Bacillus megaterium* CGMCC7086 isolates using methods of twain-step ultrafiltration and LC-ESI-MS/MS (Ma *et al.* 2016). Meanwhile, iturin, surfactin, and fengycin produced by *B.subtilis* THY-7 were refined by RP-HPLC, and then the isolipopeptides were identified by MALDI-TOF-MS/MS (Yang *et al.* 2015).

## Conclusions

The plant-associated bacterium *Bacillus* sp. TS001, isolated from tomato stems, exhibited strong antagonistic activity, effectively suppressing *C. scovillei* 244830 rot lesions in fresh green bell pepper fruits due to the production of iturin and surfactin. However, a little lesions on green bell pepper were still visible, indicating that while TS001 is effective, further optimization may be needed to achieve complete disease suppression.

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