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ORIGINAL PAPER

Impacts of *Streptomyces lavendulae* on the Survival of *Pectobacterium atrosepticum*, the Causal Agent of Potato Blackleg Disease, in Two Soil Types

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ABSTRACT

The aim of the study was to evaluate the impact of *Streptomyces lavendulae* HHFA1 (Acc. No. HQ702485) on the extent of survival of the blackleg potato pathogen, *Pectobacterium atrosepticum* P3 (Acc. No. PQ588453), in sandy and clay soil types, along with characterization of the metabolites secreted by *S. lavendulae* that may be detrimental for the pathogen. The short term survival of *P. atrosepticum* was significantly recorded in clay as compared to sandy soils. A significant decrease in the survival of *P. atrosepticum* was observed in *S. lavendulae*-treated soil compared to non-treated ones, with a strong evidence on long term survival of the pathogen in sandy soil. Meanwhile, the survival of *S. avendulae* was higher in sandy compared to clay soils. The most abundant metabolites in *S. lavendulae* (PQ588453) trials were determined by Gas Chromatography-Mass Spectrometry (GC–MS). The results showed Isochiapin B (39.67%), Octadecenoic acid derivatives (9-Octadecenoic acid (Z)-, methyl ester (Isomers), and 9-Octadecenoic acid (Z)- (9.15%)), 1,2-Benzenedicarboxylic acid and their derivatives (1,2-Benzenedicarboxylic acid, and Benzenedicarboxylic acid butyl octyl ester (7.51%)), Retinoic acid, 5,6-epoxy-5,6-dihydro- (Isomers) (7.03%), 1-Dodecanamine, N,N-dimethyl (5.90%), n-Hexadecanoic acid (4.69%), 1-Chloroeicosane (3.09%) and 1-Tetradecanamine, N,N-dimethyl- (3.04%).In retrospect , it could be concluded that, *S. lavendulae* may be recommended for controlling soil-borne pathogens similar to *P. atrosepticum*, especially in sandy soils.

Keywords: Blackleg, biocontrol, metabolites, gas chromatography-mass spectrometry (GC-MS), isochiapin B.

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INTRODUCTION

The negative impacts of plant diseases on agriculture, the economy, and food security have been well documented. Soft rot diseases, primarily caused by various species belonging to Pectobacteriaceae family, specifically *Pectobacterium* spp. and *Dickeya* spp., are among the most destructive bacterial diseases affecting many crops (Mansfield *et al*., 2012). Approximately 32% loss in seed potatoes, about 43% in table potatoes, and 25% in processing potatoes are attributed to *Pectobacterium* spp. and *Dickeya* spp. (Dupuis *et al*., 2021). The main virulence factor for *Pectobacterium* spp. and *Dickeya* spp. is plant cell wall-degrading enzymes (Van Gijsegem *et al*., 2021). Toth *et al*., (2015) demonstrated the existence of *P. atrosepticum* in several weeds, similar to its survival in the potato rhizosphere, which increases the risk of potato contamination with the pathogen in the field. Unlike *Dickeya* spp., *P. carotovorum* and *P. atrosepticum* can survive in irrigation water with full viability for over 150 days (Van Doorn*et al*., 2011). *Pectobacterium* spp. can survive in soil at 6°C and 50% soil moisture capacity for 42 days (Van der Wolf *et al*., 2009).The survival of *Pectobacterium* spp. is affected by soil biotic and abiotic factors. For example, the pathogen was below the detection level in sandy soil within one month and for about 50 days in loamy soil (Armon *et al*., 1995). *P. atrosepticum* was found to survive in loam soil for two months at 2–10 °C and only for two weeks at 20 °C (Ficke*et al*., 1973). Meanwhile, survival in soil for six months was recorded during winter in many regions (Anilkumar and Chakravarti, 1970).

Actinomycetes are known to play an important role in protecting plants against phytopathogens by secreting bioactive metabolites, including growth-promoting

substances, enzymes, antioxidants, fatty acids, and antibiotics (Doumbou *et al*., 2002; Rajan and Kannabiran,2014; Barka *et al*., 2015; Rajaram *et al*., 2020). *S. lavendulae* HHFA1 (Acc. No. HQ702485) has been proven effective in controlling onion bacterial diseases, specifically *Pectobacterium carotovorum* subsp. *carotovorum* and *Burkholderia cepacia* (Abdallah *et al*., 2013). The ethyl acetate extract of *S. lavendulae* demonstrated antimicrobial and antioxidant potential, as reported by Saravana Kumar *et al*., (2014). Six antibiotics, namely ileumycin, mitomycin C, eurymycin, glomecidin, SL-1 pigment, and saframycin A, are being produced by *S. lavendulae* (Rizk*et al*., 2007), as well as streptothricin (Waksman *et al*., 1951). A notable advantage of *S. lavendulae* over other antimicrobial agents is the stability of the antibiotic after 15 years of preservation on artificial media, with only a slight decrease (Rifaat, 2009).

GC-MS is described as a simple, sensitive, and efficient technique for either quantitative or qualitative separation of mixed components (Medeiros, 2018). The approach is effective at determining the biological activity of actinomycetes isolated from various soil samples (Ibnouf*et al*., 2022).

The objective of this study was to evaluate the influence of *S. lavendulae* on the survival of *P. atrosepticum* in bare absence of the host in two soil types. The most abundant metabolites in *S. lavendulae* (PQ588453), which may correlate with its biological activity, were determined as Isochiapin B, Octadecenoic acid derivatives, Benzenedicarboxylic acid and their derivatives, 1-Dodecanamine, N,N-dimethyl, and n-Hexadecanoic acid.

MATERIALS AND METHODS

Streptomyces lavendulae HHFA1 (Acc. No. HQ702485)

S. lavendulae (HHFA1) was previously isolated and identified from Egyptian soils and showed an antagonistic activity against *Pectobacterium carotovorum* subsp. *carotovorum* and *Burkholderia cepacia* of onion bulbs (Badr 2011; Abdallah *et al*., 2013). *S. lavendulae* (HHFA1) was kept in 20% glycerol at -20°C.

Isolation, and pathogenicity test.

Potato plants with typical blackleg symptoms were obtained from a potato farm located in Nubaria, Behera Governorate. Stems were washed, and the surfaces disinfected by flaming. Crown areas with typical symptoms were macerated in sterilized phosphate buffer (PB 0.05M). The resulting suspension was plated onto Logan media (Logan, 1963, 1966; Schaad *et al*., 2001) and incubated for 24 hours at 28°C. Colonies that remained colorless after 24 hours, then turned pink with white margins after further incubation for another 24 hours and reached approximately 0.5 mm in diameter were selected for subsequent pathogenicity and identification. Pathogenicity on potato tubers was conducted for the developed colonies as described by Badr *et al*., (2024).

Identification of the pathogenic isolates

DNA extraction from the suspected colonies developed on Logan medium was conducted using lysis buffer, following the methodology outlined by Farag *et al*., (2017).

a. Identification by conventional PCR

For the PCR assay, oligonucleotide primers Y45 (5'-TCACCGGACGCCGAACTGTGGCGT-3') and Y46 (5'-TCGCCAACGTTCAGCAGAACAAGT-3') were utilized, as described by Frechon *et al*., (1998). The reactions were set up in a 25-µl PCR mixture using Cosmo PCR RED MMIX (WF-10203001-M, Willowfort, UK). PCR products were separated on a 1.5% agarose gel in tris-acetate-EDTA (TAE) buffer and visualized by staining with RedSafe^{TM} Nucleic Acid Staining Solution. A Molecular 100-1,500 bps DNA Ladder was employed (Gen BIO-HELIX - DM001-R500).

b. **Identification by DNA-sequencing**

DNA purification and sequencing were conducted as outlined in Badr *et al*., (2024). The evolutionary history was inferred using the Neighbor-Joining method Saitou and Nei, (1987). The evolutionary history was

inferred using the Neighbor-Joining method (Saitou *et al*., 1987). The optimal tree is shown (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. The analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 438 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021).

In vitro* inhibition assay of *S. lavendulae* against *P. atrosepticum

The inhibitory potential of *S. lavendulae* was evaluated against *P. atrosepticum* (PQ588453) using the cross-streaking method (Oskay *et al*., 2004 and Messiha *et al*., 2007). The distance free from the pathogen near the antagonist line was measured.

GC-MS analysis of Ethyl Acetate Extract of *S. lavendulae*

Solid-state fermentation was employed to identify the active ingredients from *S. lavendulae*. An ethyl acetate solvent-based extract was used. Twenty SNA plate cultures, seven days old, of *S. lavendulae* were mixed with ethyl acetate solvent in a ratio of 1/10 (w/v). The mixture was kept for complete extraction for 7 days at ambient room temperature, then filtered, and the ethyl acetate fraction was evaporated using a rotary evaporator. The crude ethyl acetate extract was analyzed using GC-MS. (Badr 2011).

Survival of *S. lavendulae* in two textured soil types and its influence on survival of *P. atrosepticum*

The survival of both the pathogen and the antagonist was assessed using the method described by Messiha *et al*., (2007) with certain adjustments. A spontaneous mutant of *P. atrosepticum*, resistant to bacitracin and chloramphenicol, was selected and was adapted to determine the duration of its survival in different textured soils. This mutant strain was selected from the wild-type strain (PQ588453) on Logan medium supplemented with increasing doses of bacitracin and chloramphenicol (0, 20, 40, 60, 80, and 100 ppm) for each antibiotic. The mutant's virulence was checked by testing pathogenicity on potato intact tubers to be similar to those of the wild-type strain. The mutant was subsequently cultured on NA supplemented with 100 ppm of each antibiotic for 48 hours. *S. lavendulae* was grown on SNA plates for seven days. Suspensions of each bacterium were prepared in phosphate buffer (PB, 0.01 M). The density was standardized using a spectrophotometer, with optical readings at OD600 = 1.7 (equivalent to 10^9 CFU ml^{-1}) for *P. atrosepticum* and OD600 = 0.7 (equivalent to 10^9 CFU ml^{-1}) for *S. lavendulae*. One milliliter of each suspension was mixed with 100 g of soil in plastic bags according to the experimental design. The treatments for each soil type comprised: a negative control (soil inoculation with phosphate buffer (PB) only), soil only infested with the pathogen, soil only infested with *S. lavendulae*, and soil infested with both the pathogen and the biocontrol agent together. All experimental treatments received the same amount of PB (2 ml in total). Each treatment comprised of 100 g of soil, divided into three equal quantities within 50-ml Greiner tubes. The tubes were loosely closed to allow air exchange during incubation at 28°C. The actual soil moisture content was maintained at 10.5% in sandy and 25.7% in clay soils. The tubes were weighed, and water loss was compensated by adding sterile tap water to maintain the soil fixed moisture standard throughout the experiment. The survival of *P. atrosepticum* and *S. lavendulae*, along with the total actinomycetes densities, was weekly assessed. For bacterial enumeration, 1 g of soil (one sample among one replicate tube) was suspended in 9 ml of sterile 0.05 M phosphate buffer, and were shaken at 100 rpm for 2 hours at 20 °C, then 10-fold serial dilutions were prepared. The enumeration of bacteria was run on three Logan-medium

plates supplemented with 100 mg/l chloramphenicol and 100 mg/l bacitracin to detect the mutant *P. atrosepticum*, and onto SNA medium to count *S. lavendulae* and total actinomycetes. Colonies of *S. lavendulae* were identified and counted after seven days'incubation, while *P. atrosepticum* were counted after two days incubation at 28 °C. The physical and chemical properties of each soil type are presented in Table 1. Physical and chemical soil properties were determined at the Central Analysis Lab, Faculty of Agriculture's, Mansura University (Badr *et al*., 2024).

Table1. Physical, chemical characteristics of the different soils

Soil type	pH	(ds/m)	%
		EC	OM
Sandy	8.81	0.25	0.29
Clay	7.9	0.8	1.70
Soil type	Ppm		
	N	P	K
Sandy	7.72	17.56	155
Clay	202	6.25	319
Soil type	%		
	Sand	Silt	Clay
Sandy	89.1	7.5	3.4
Clay	30.8	35	34.2

Statistical analysis

The log of microbial population of the pathogen and the antagonist per soil type and treatment fitted to an exponential survival model as described by Franz *et al*., (2005) to be : ct = am / (1+exp (-d*(t-c))). Where C_t = log10 (CFU) of bacteria, am = initial count of the pathogen (asymptote), d = decline rate (days^{-1}), t = time (days), and c = 50% decrease of microbial population in days. The estimated parameter values, c and d, for the two types treated soils with both the pathogen and the antagonist separately were subjected to multivariate analysis of variance (MANOVA) using SPSS v23 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

***Streptomyces lavendulae*HHFA1**

Figure 1 illustrates the growth of *S. lavendulae* HHFA1(Acc. No. HQ702485) on SNA medium. The observed cultural characteristics include well-developed aerial and substrate mycelia. Isolate HHFA1 displayed reddish-brown aerial mycelium, brownish-yellow substrate mycelium, and an absence of diffusible pigment production.

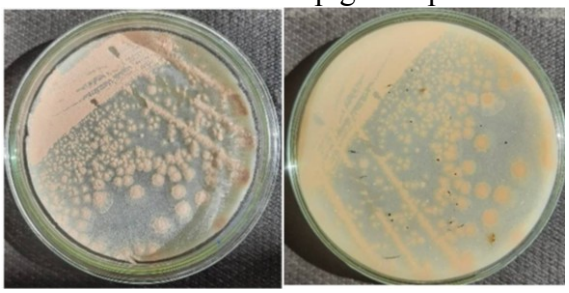

Fig.1. Culture growth of *S. lavendulae* HHFA1 on SNA medium.

Isolation and pathogenicity test

White colonies (isolates) developed on Logan medium plates were selected. These white colonies after 24 hours incubation were turned entirely pink with white margins after 48 hours. They were tested for pathogenicity after propagation on the NA medium. Two isolates, P2 and P3, produced typical soft rot symptoms on potato tubers, as shown in Fig.(2) .

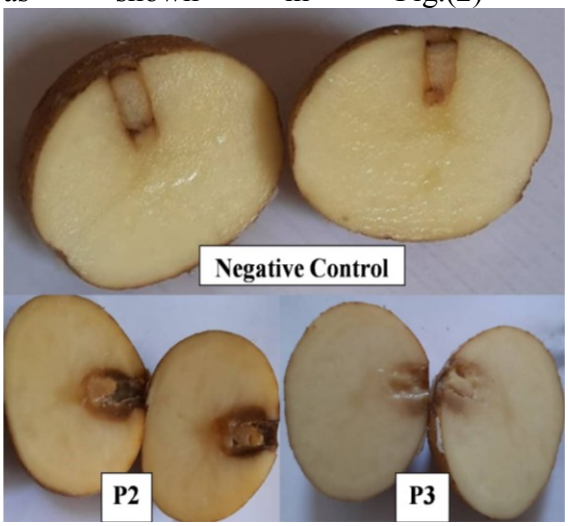

Fig. 2. Pathogenicity test by artificial inoculating potato tubers with the two isolates, P2 and P3, seven days' post-inoculation (10^6 CFU/ml) in a sterile 0.01 M phosphate buffer (PB).

Identification of the pathogenic isolates

Isolates which showed pathogenic potential on potato tubers were subjected to identification methods.

a. Identification by conventional PCR

Fig (3) shows a confirmative specific band (439 bp) for isolates in concern (P2 and P3) as well as a positive control.

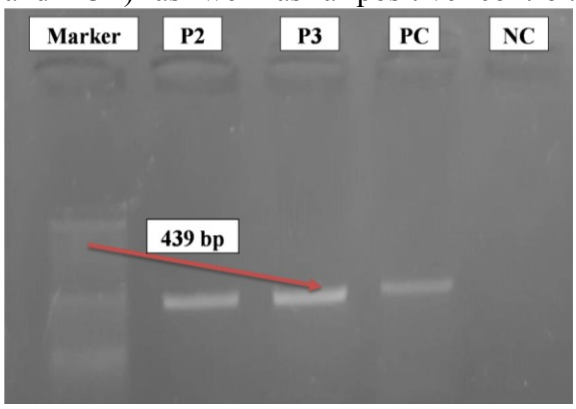

Fig. 3. The identification of different suspected isolates developed on Logan medium (Logan, 1966) using specific primers Y45 and Y46 (Frechon *et al*., 1998). The typical band observed corresponds to a size of approximately 439 bp as detected by the Molecular marker 100-1,500 bps (Gen BIO-HELIX - DM001-R500).

b. Identification by DNA-sequencing

One isolate, P3, showed 99.54% similarity with *P. atrosepticum*. The isolate was deposited into the NCBI as *P. atrosepticum* (PQ588453). Figure (4) reveals the evolutionary analysis for *P. atrosepticum* (PQ588453) using the Neighbor-Joining method (MEGA11).

In vitro* inhibition assay of *S. lavendulae* against *P. atrosepticum

The inhibition zone between *S. lavendulae* and *P. atrosepticum* was determined as 32.7±1.4 mm (mean±SE) (Fig 5).

GC-MS analysis of ethyl acetate extract of *S. lavendulae*

GC-MS analysis of *S. lavendulae* ethyl acetate extract (Table 2) reveals the abundance of 17 volatile components based on a comparison of their EI-MS spectra with those reported in the NIST database spectral library, along with the published activities. Figure (6) represents the most abundant components, listed from highest to lowest: Isochiapin B (39.7%), Retinoic acid, 5,6-epoxy-5,6-dihydro- (Isomers) (7.03%), 9-Octadecenoic acid (Z)-, methyl ester (isomers) (6.55%), 1-Dodecanamine, N,N-dimethyl (5.9%), n-Hexadecanoic acid (4.69%) ,1,2-Benzenedicarboxylic acid, butyl octyl ester (3.79%), 1,2-Benzenedicarboxylic acid (3.72%), 1-Chloroeicosane (3.09%), and 1-Tetradecanamine, N,N-dimethyl- (3.04%). GC-MS analysis was made using a Varian GC interfaced to a Finnegan SSQ 7000 Mass selective Detector (SMD) with an ICIS V2.0 data system for MS characterization of the GC components. The column utilized was the DB-5 (J&W Scientific, Folsom, CA) cross-linked fused silica capillary column (30 m. long, 0.25mm. internal diameter) covered with (0.5µm. film thickness) poly dimethyl-siloxane. The ionization energy was fixed at 70 eV (Abdurazakov *et al*., 2021).

Survival of *S. lavendulae* in two textured soil types and its influence on survival of *P. atrosepticum*

The log of microbial population of the pathogen (*P. atrosepticum*) per soil type and treatment fitted to an exponential survival model with as described by (Franz *et al*., 2005). $C_t = a_m / (1+\exp(-d*(t-c)))$. Where $C_t = \log_{10}$ (CFU) of bacteria, a_m = initial count of the pathogen (asymptote), d = decline rate ($days^{-1}$), t = time (days), and c = 50% decrease of microbial population in days. Figure (7) shows a significant decrease in the *P. atrosepticum* densities in *S. lavendulae*-treated soil (Wilks' Lambda, $P< 0.001$). A significant interaction between soil type and treatment was observed ($P = 0.001$). In sandy soil, a highly significant decrease in pathogen survival was clear, as indicated by a marked decrease in c ($F = 256.5$, $P< 0.001$) in *S. lavendulae*-treated soil compared to non-treated soil. Additionally, a trend toward a significant higher d (decline rate) was noted ($F = 5.4$, $P< 0.08$). In clay soil, however, the impact of *S. lavendulae* amendments was less pronounced but still significant, with a decrease in both c ($F = 18.7$, $P = 0.012$) and d ($F = 14.4$, $P = 0.019$). The survival of *P. atrosepticum* was limited in clay soil compared to sandy soil in non-amended soil, both in c ($F = 113.9$, $P< 0.001$) and d ($F = 42.8$, $P = 0.003$), without significant difference between both soil types in *S. lavendulae*-amended treatments.

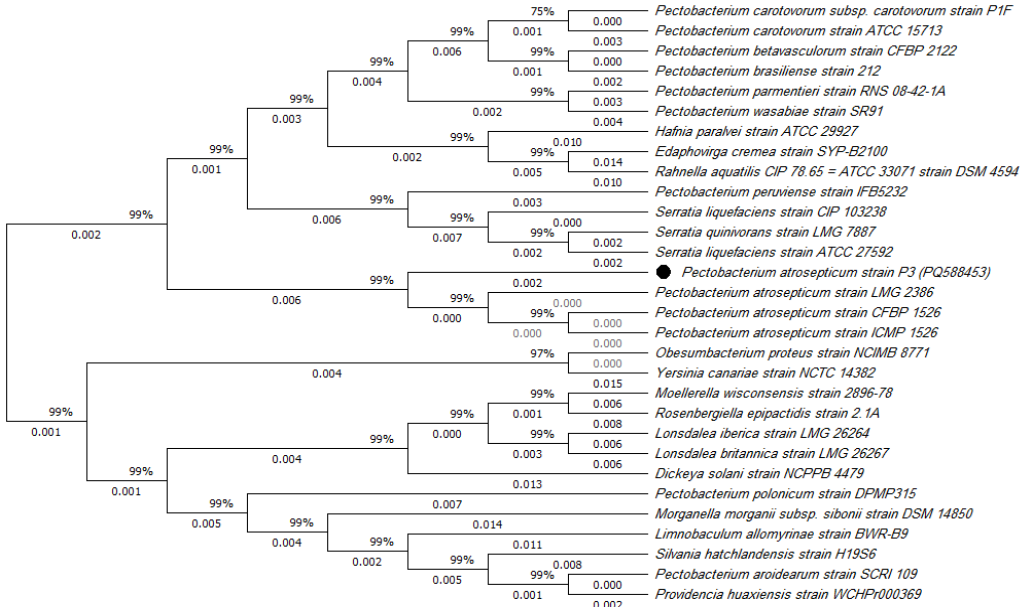

Fig. 4. The phylogenetic tree of *P.atrosepticum* strain P3 (PQ588453) using the Neighbour-Joining method.

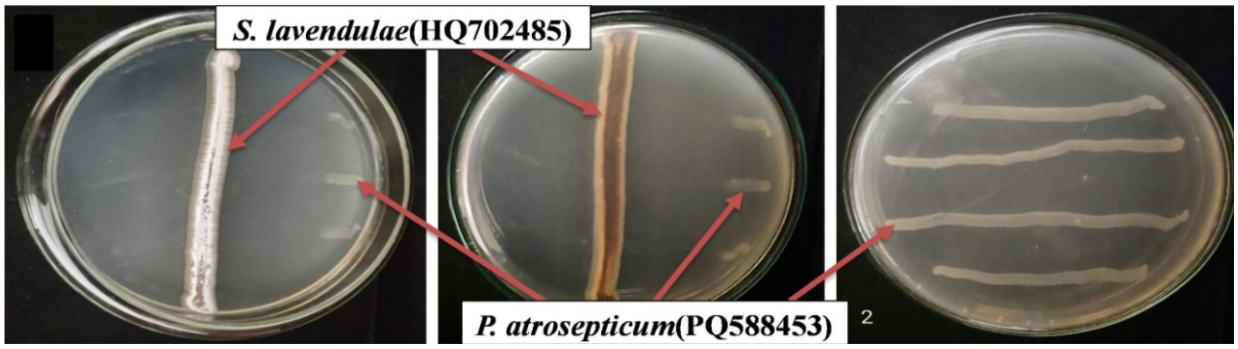

Fig. 5. Antagonistic potential of *S. lavendulae* against *P. atrosepticum* on NA medium. *S. lavendulae* was 7 days old when *P. atrosepticum* was streaked perpendicular to it, and the plates were incubated further at 28°C for 24 hours. Growth inhibition of the pathogen was recorded.

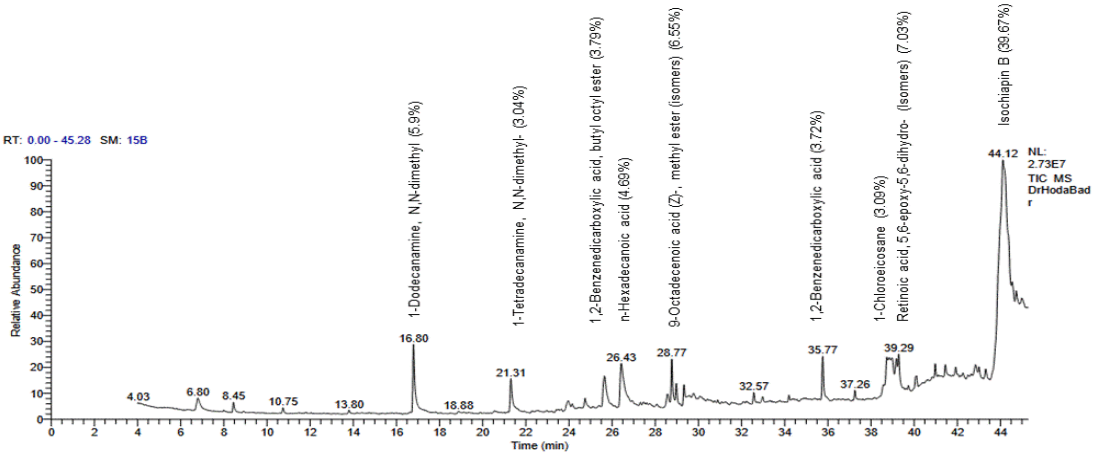

Fig. 6. GC-MS analysis for the isolate *S. lavendulae* HHFA1 (HQ702485) revealing the most abundant volatile components.

Table 2. GC-MS chemical profiling of ethyl acetate extract of *S. lavendulae* metabolites

No.	RT	Name of the compound (Peak name)	MF	MW	Peak Area %	Activity reported
1	6.8	Isophorone	$C_9H_{14}O$	138	1.73	Antimicrobial (Kiran *et a*l., 2013)
2	16.79	1-Dodecanamine, N,N-dimethyl	$C_{14}H_{31}N$	213	5.90	Antibacterial (Mokhtar *et al*., 2023)
3	21.31	1-Tetradecanamine, N,N-dimethyl-	$C_{16}H_{35}N$	241	3.04	antimicrobial (Birnie*et al*., 2000)
4	23.96	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	1.14	
5	25.64	1,2-Benzenedicarboxylic acid, butyl octyl ester[3]	$C_{20}H_{30}O_4$	334	3.79	Antibacterial (Garba, 2016)
6	26.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.69	Antibacterial (Shaaban et al 2021)
7	28.56	Oleic Acid	$C_{18}H_{34}O_2$	282	1.25	
8	28.77	9-Octadecenoic acid (Z)-, methyl ester (isomers)[1]	$C_{19}H_{36}O_2$	296	6.55	Antimicrobial (Al-Askar*et al*., 2024)
9	28.98	N-Methyl-N-benzyltetradecanamine	$C_{22}H_{39}N$	317	1.65	
10	32.57	Ethyl 2-[(4-methylphenyl)amino]propanoate	$C_{12}H_{17}NO_2$	207	1.09	
11	35.76	1,2-Benzenedicarboxylic acid[4]	$C_{24}H_{38}O_4$	390	3.72	
12	38.73	1-Chloroeicosane	$C_{20}H_{41}Cl$	316	3.09	
13	39.18	Retinoic acid, 5,6-epoxy-5,6-dihydro- (Isomers)	$C_{20}H_{28}O_3$	316	7.03	
14	39.28	9-Octadecenoic acid (Z)-[2]	$C_{18}H_{34}O_2$	282	2.60	
15	44.11	Isochiapin B	$C_{19}H_{22}O_6$	346	39.67	Antibacterial characteristics (Cartagena *et al*., 2008).
16	44.39	Dotriacontane	$C_{32}H_{66}$	450	1.16	
17	44.74	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	498	1.28	

[1,2]Octadecenoic acid derivatives (9.15%) [3,4]Benzenedicarboxylic acid and their derivatives (7.51%)

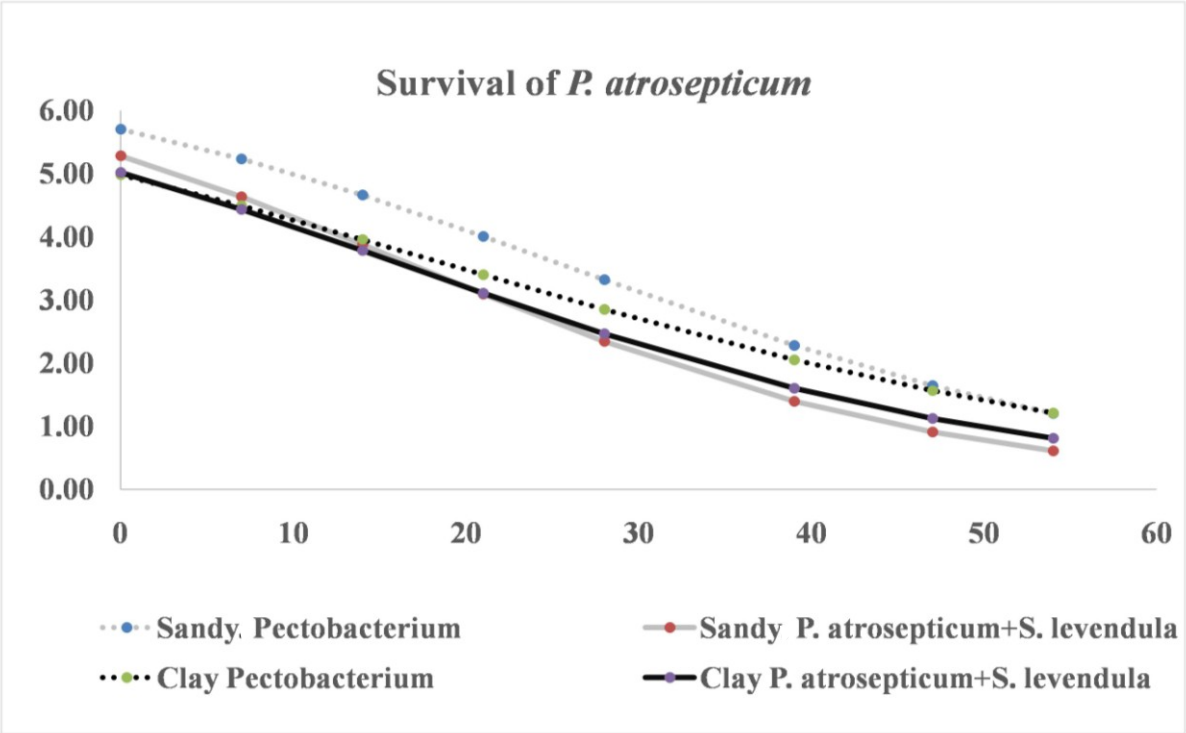

Fig. 7. Decline in *P. atrosepticum* density (CFU g^{-1} dry soil) of in different soil types with different management regimes at 28°C. The dots are the observed values. The lines are the predicted values from the logistic decline model: $C_t = a_m/(1 + \exp(-d*(t-c)))$. Where C_t = log transformed number of bacteria, a_m = initial density of the pathogen, d = decline rate ($days^{-1}$), and t = time (days) and c = length of the 50%-reduction-time in days (Franz *et al*., 2005). The first letter represents.

Figure (8) shows a significant slower decline of *S. lavendulae* in sandy soil treated with *S. lavendulae* compared to clay soil inoculated with *S. lavendulae*, as shown by a longer c (F=112.8, *P*<0.001) and an increased slope (d) (F=8.4, *P*=0.044). This indicates better survival of *S. lavendulae* in sandy soil over clay ones.

Figure (9) reveals significant longer duration in survival of total actinomycetes in clay as compared to sandy soil only inoculated with with *P. atrosepticum*as shown with an increase in c (F=6.5, *P*=0.019). However, there was a significant increase in the survival duration of actinomycetes in sandy soil treated with *S. lavendulae* compared to sandy soil non-inoculated with *S. lavendulae*, as expressed by an increase in c (F=6.5, *P*=0.043) (Figs 8 and 9). On the other hand, there was no significant difference in the survival of actinomycetes between treatments of clay soil.

In conclusion, the survival duration of *P. atrosepticum* was lower in clay compared to sandy soils. Moreover, the survival of *S. lavendulae* was also lower in clay as compared to sandy soil. The impact of *S. lavendulae* in decline of *P. atrosepticum* was significantly higher in sandy soil as compared to clay soils.

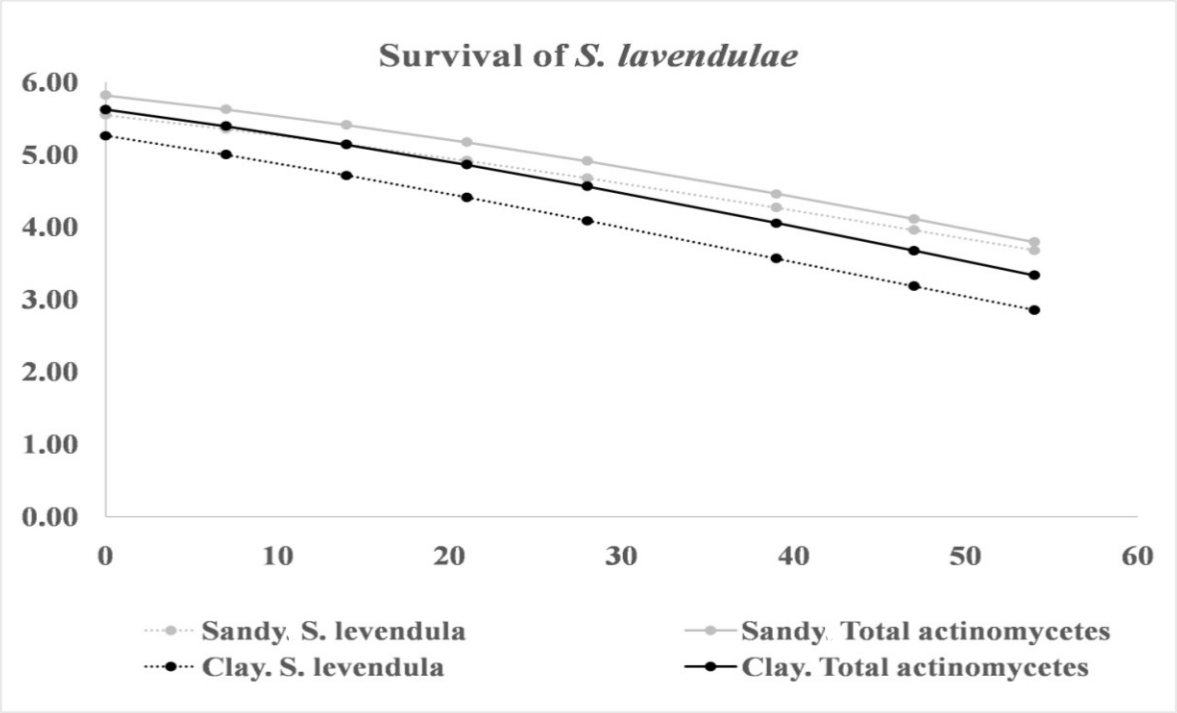

Fig. 8. Decline in *S. lavendulae* HHFA1 (HQ702485) density (CFU g^{-1} dry soil) of in different soil types with different management regimes at 28°C.

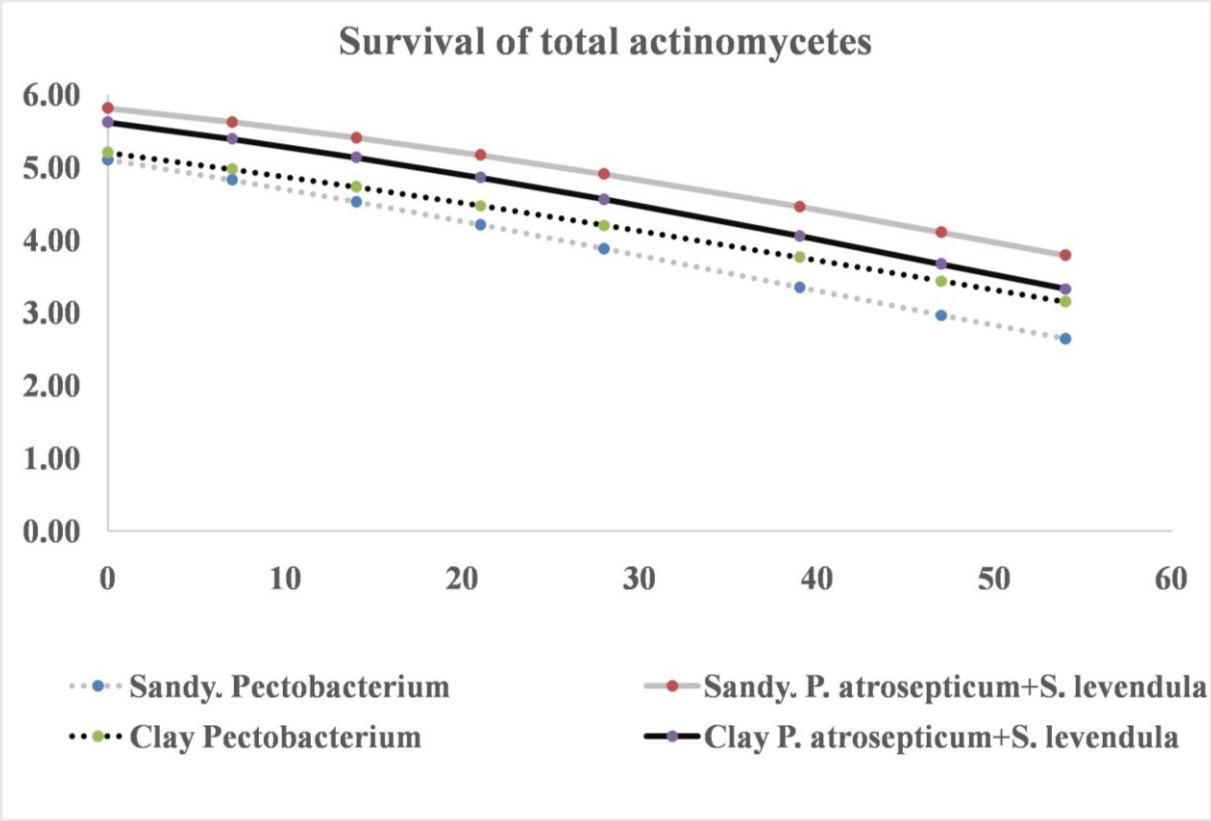

Fig. 9. Survival of total actinomycetes (CFU g^{-1} dry soil) in sandy and clay soils infested with *P. atrosepticum*in presence and absence of *S. lavendulae* HHFA1 (HQ702485) at 28°C

DISCUSSION

Due to the increasing population and limited resources, significant efforts are being made to increase crop yield using safe, sustainable methods that avoid the hazardous effects of chemical fertilizers and pesticides. *Streptomyces* spp. are known as promising sustainable biocontrol agents that can survive active in soil for a long period, producing antibiotics, along with bioactive compounds, and extracellular enzymes. These secreted metabolites can combat pathogens in the soil as well as promote plant growth (Olanrewaju and Babalola, 2019). *Streptomyces* spp. can be added directly to the soil, or their populations can be enhanced indirectly by altering microbial biodiversity through specific fertilizers or crops (Messiha *et al*., 2019, 2021, 2023). Using *S. lavendulae* HHFA1 (Acc. No. HQ702485) has proven effective in controlling onion bacterial diseases, specifically *P. carotovorum* subsp. *carotovorum* and *B. cepacia* (Abdallah *et al*., 2013). This study approves its efficacy against *P. atrosepticum,* the causal agent of potato blackleg. The used *P. atrosepticum* strainP3 (PQ588453) in this study was identified by conventional PCR (Frechon *et al*., 1998), DNA sequencing and pathogenicity test (Badr *et al*., 2024). The GC-MS chemical profiling of ethyl acetate extract of *S. lavendulae* metabolites characterized 17 compounds. Isochiapin B is a particular sort of sesquiterpene lactone group. Sesquiterpene lactone has several biological influences, including antibacterial characteristics (Cartagena *et al*., 2008). The Octadecenoic acid derivatives (9- Octadecenoic acid (Z)-, methyl ester (Isomers), and 9-Octadecenoic acid (Z)-) was recovered as 9.15% in the same extract. The 9-Octadecenoic acid (Z)-methyl ester, also known as methyl oleate, is an ester of oleic acid. Oleic acid is known for its antimicrobial and antibacterial activities (Dilika *et al*., 2000; Al-Askar*et al*., 2024). The 1-Dodecanamine, N, N-dimethyl was recovered at 5.9%. The antibacterial activity of this metabolite was previously proven (Mokhtar *et al*., 2023). Other metabolites recorded as antibacterial and antimicrobial were recovered in considerable ratios, namely n-hexadecanoic acid (4.69%) (Shaaban *et al*., 2021), 1,2-Benzenedicarboxylic acid, butyl octyl ester (3.79%) (Garba, 2016), and 1-Tetradecanamine, N, N-dimethyl- (3.04%) (Birnie *et al*., 2000).These compounds may explain why *S. lavendulae* decreased the survival of *P. atrosepticum* in both soil types. The *S. lavendulae* was more effective in decreasing the pathogen survival in sandy than in clay soils. Meanwhile, *S. lavendulae*'s survival was longer in sandy than in clay soils, that might explain its greater impact in decreasing the pathogen survival in sandy compared to clay soils. The *Streptomyces* spp. have described as soil health indicators (van Bruggen and Semenov, 2000). Additionally, *Streptomyces* spp. are recommended as sustainable biocontrol agents because they can survive under harsh conditions, such as water and nutrient stresses, by producing spores (Pacios-Michelena *et al*., 2021). Their ability to endure such harsh conditions gives them an advantage over other microorganisms. Most *Streptomyces* spp. prefer aerated environments, as proven by Kukharenko *et al*., (2010) that may explain the longer survival of *S. lavendulae* in sandy soil. Both the pathogen and the biocontrol agent have adequately survived in sandy soil, characterized by lower EC, indicating lower salinity compared to clay soil. Higher salinity may decrease bacterial densities and activities (Li *et al*., 2021). The employed sandy soil had significantly lower nutrient content, especially OM, N, and K. Lower nutrient content supports a smaller microbial community, which may favor the growth and survival of *S. lavendulae*. Also, the employed sandy soil was characterized by significantly higher P content (about three times) compared to clay soil. Higher soil phosphorus content is known to support the growth of *Streptomyces* spp. and their ability to

produce antibiotics, antimicrobial compounds, and other metabolites (Chouyia *et al*., 2022).

CONCLUSION

S. lavendulae may be highly recommended as a biocontrol agent against bacterial pathogens inducing soft rot and blackleg diseases. Sandy soils are generally more appropriate for selecting the biocontrol agents. Further studies are required to select an appropriate carrier for the biocontrol agent for commercial, large-scale use.

Author contributions

Nevein A.S. Messiha: Methodology, investigations, DNA sequencing for the pathogen, statistical analysis, and writing - original draft. Huda H. Badr: Methodology, providing the pathogen and the biocontrol agent, experimental setup, investigations, writing - review & editing.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statements and Declarations

Competing Interests: The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

Conflict of Interest

The authors declare no conflict of interest.

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