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Volatile Organic Compounds Produced by selected Antagonistic Rhizobacteria against soil-borne Phytopathogenic Fungi

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INTRODUCTION

Fungal pathogens cause most of the diseases occurring in agricultural and horticultural setups. The diseases cause by the fungal pathogens is the major causes of yield crop losses and diminished crop quality.

Fungal pathogens distribution


E. polygoni


U. appendiculatus


U. phaseoli


Ascochyta sp


Botrytis sp


Cercospora sp


C. gloeosporioides


Cercospora sp


Alternaria alternata


Uredo cajani


R. solani


Phaeoisariopsis griceola


R. solani


S. sclerotiorum


Fusarium oxysporum


Rizoctonia solani

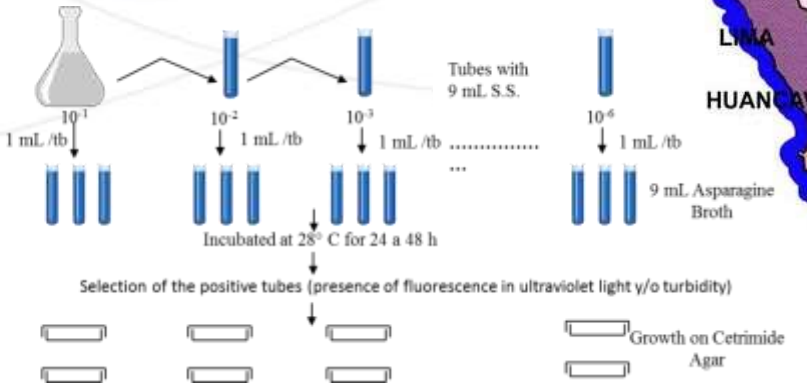

Cercospora sp

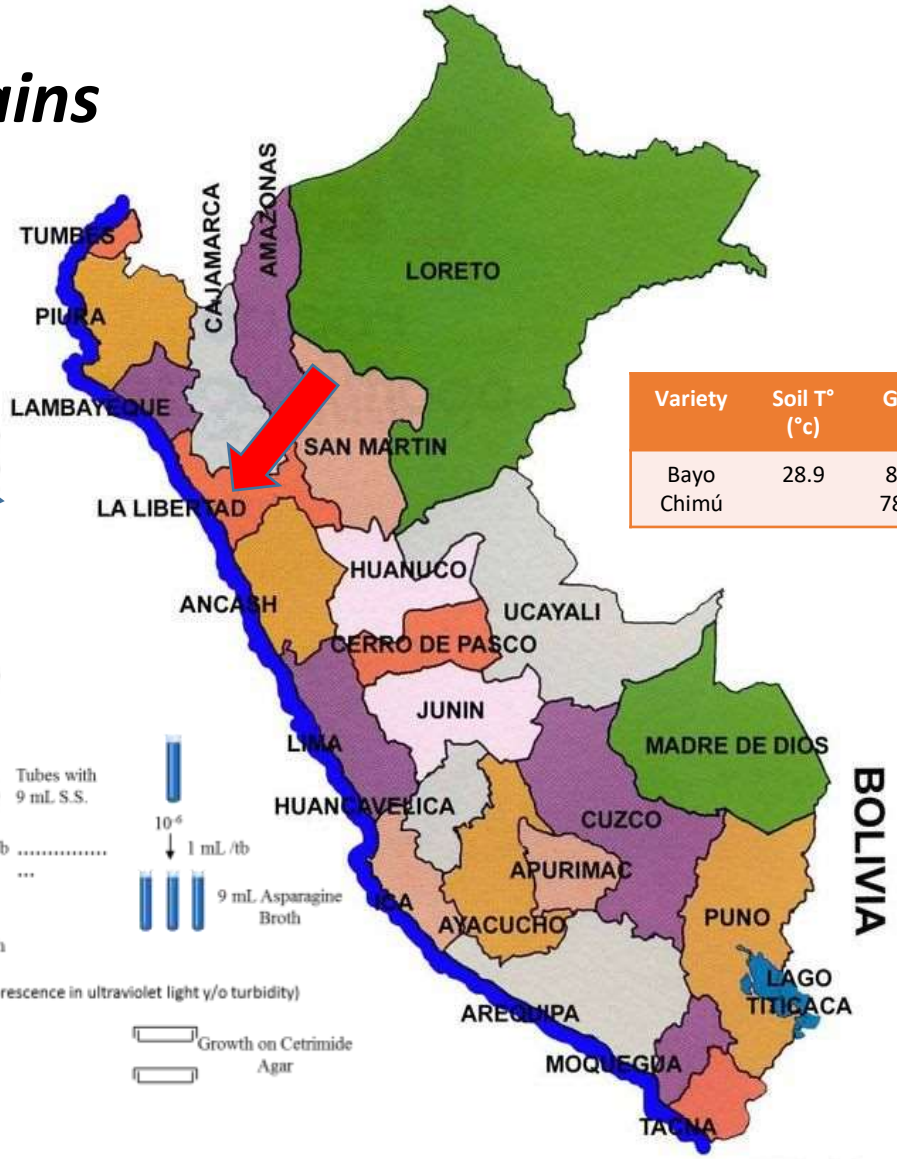
Piura
Amazonas
Loreto
Lambayeque
Cajamarca
San Martín
La Libertad
Ancash
Huánuco
Pasco
Lima
Cusco
Ayacucho
Arequipa
Moquegua

Isolation of the strains

Virú – La Libertad

APHA Standard Methods (1998).





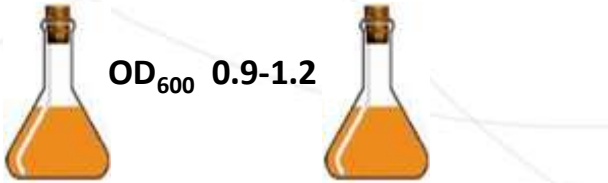
Variety	Soil T° (°c)	Geographical location	Altitute (masl)
Bayo Chimú	28.9	8°23'11.54''S 78°43'9.74''W	76

OBJECTIVE

The aim of this study was to elucidate the antagonistic potential activity of TvPs1.6 and TvPs2.4 strains isolated from rhizosphere common bean plant, based on the identification of volatile organic compounds produced to control mycelial growth of *S. sclerotiorum*, *F. oxysporum* and *R. solani*.

Methodology

Volatile compounds production by sealed plate method



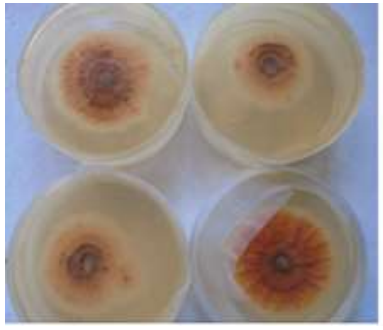
TvPs1.6 TvPs2.4

TSA medium were inoculated with strains

S. sclerotiorum *F. oxysporum* *R. solani*

PDA were inoculated whit a agar discs of test fungi

Plates with only the agar discs of test fungi were used as controls



25° C for 4 to 5 days

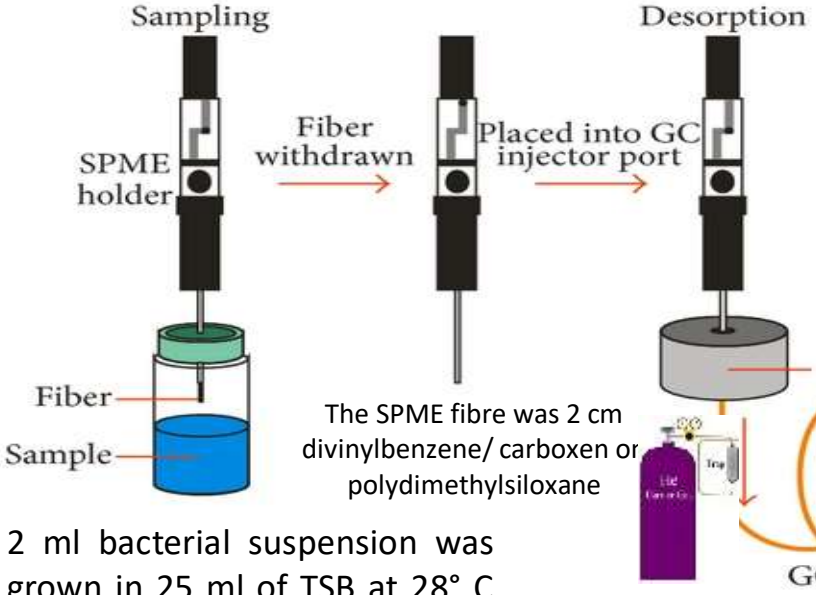
Then fungi plates were immediately inverted over the bacteria plates and sealed with Parafilm

Methodology

Analysis of volatiles by Solid Phase Microextraction-Gas Chromatography–Mass Spectrometer (SPME-GC–MS)

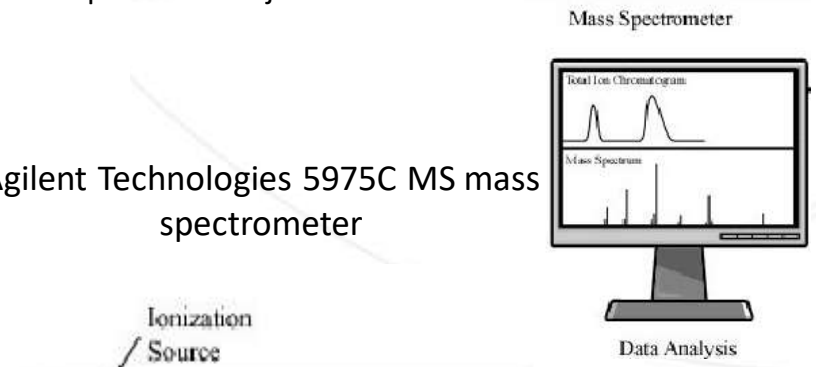
The SPME fibre was inserted into the headspace vial for 30 min

The SPME fibre was inserted and desorbed at 70° C for 1 min into the injection port of the injector



The SPME fibre was 2 cm divinylbenzene/ carboxen or polydimethylsiloxane

Agilent Technologies 5975C MS mass spectrometer



NIST08 MS-libraries

2 ml bacterial suspension was grown in 25 ml of TSB at 28° C for 24 H.

Agilent Technologies 7890-GC gas chromatograph

The working temperature of the column was set as follows: 35° C for 3 min at the beginning, increased to 110° C for 10 min and finally to 300° C at 30° C for 1 minute.

Results

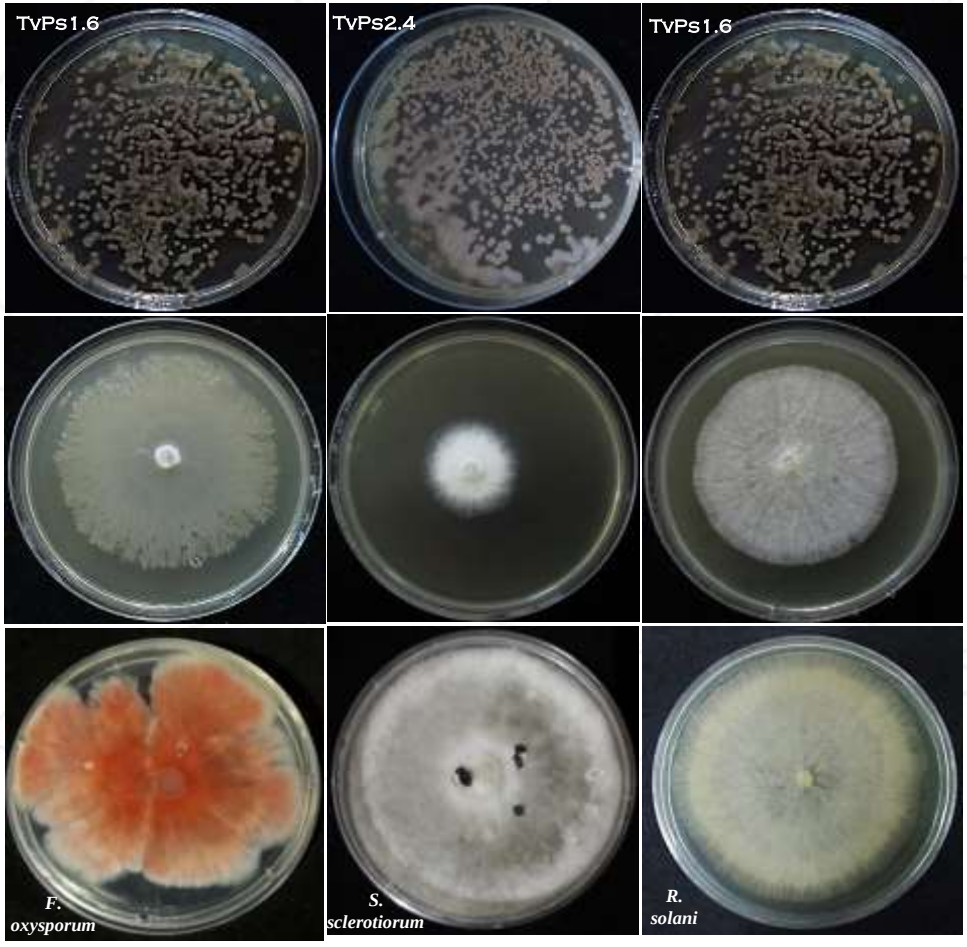
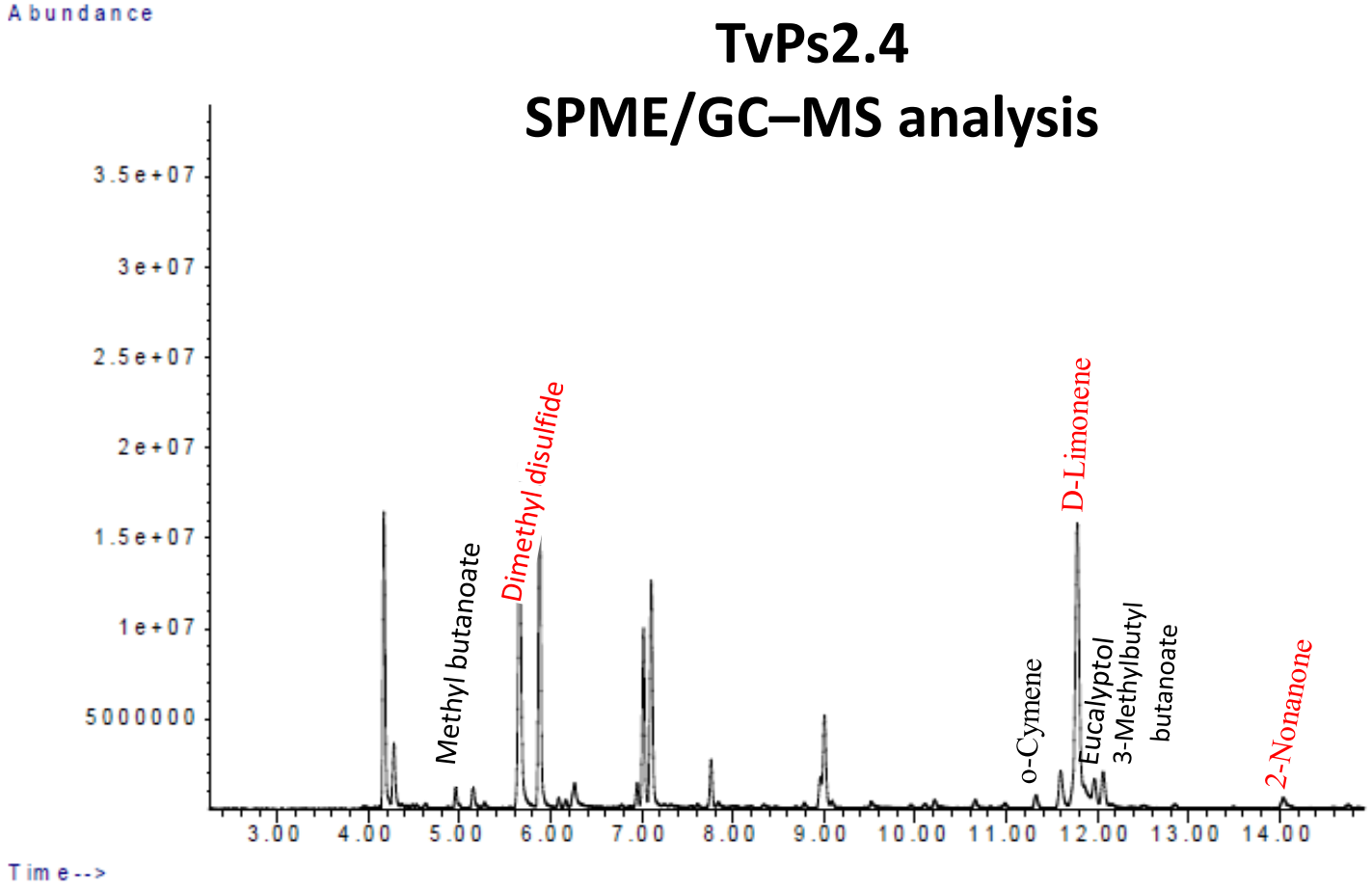


Figure 1. Inhibition of micelial growth of *F. oxysporum*, *S. sclerotiorum* and *R. solani* (second row) by volatile organic compounds produced by TvPs1.6 and TvPs2.4 strains (top row). Control plates with the mycelium of the fungus grown on PDA medium (bottom row).

Results

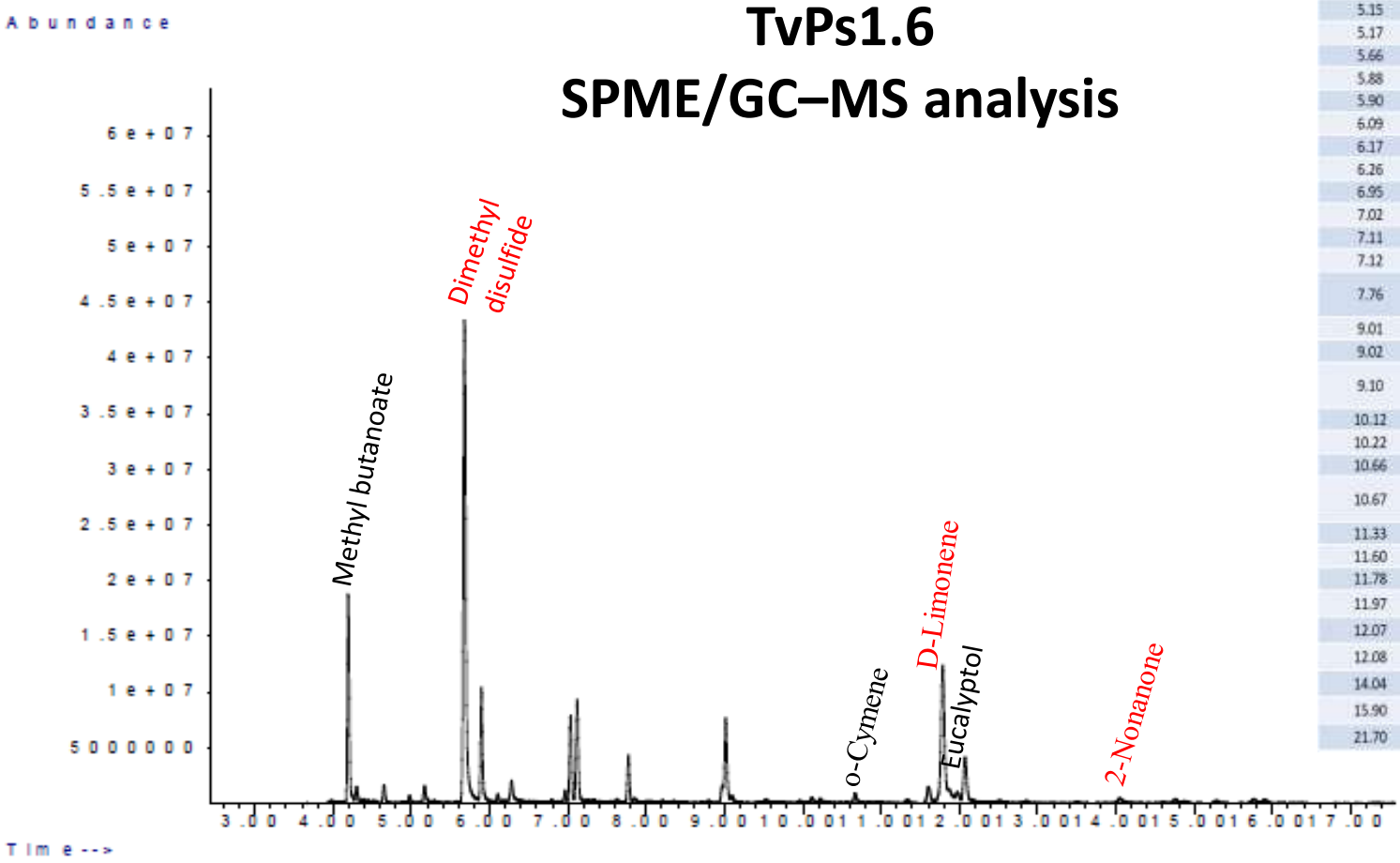
TvPs2.4
SPME/GC–MS analysis



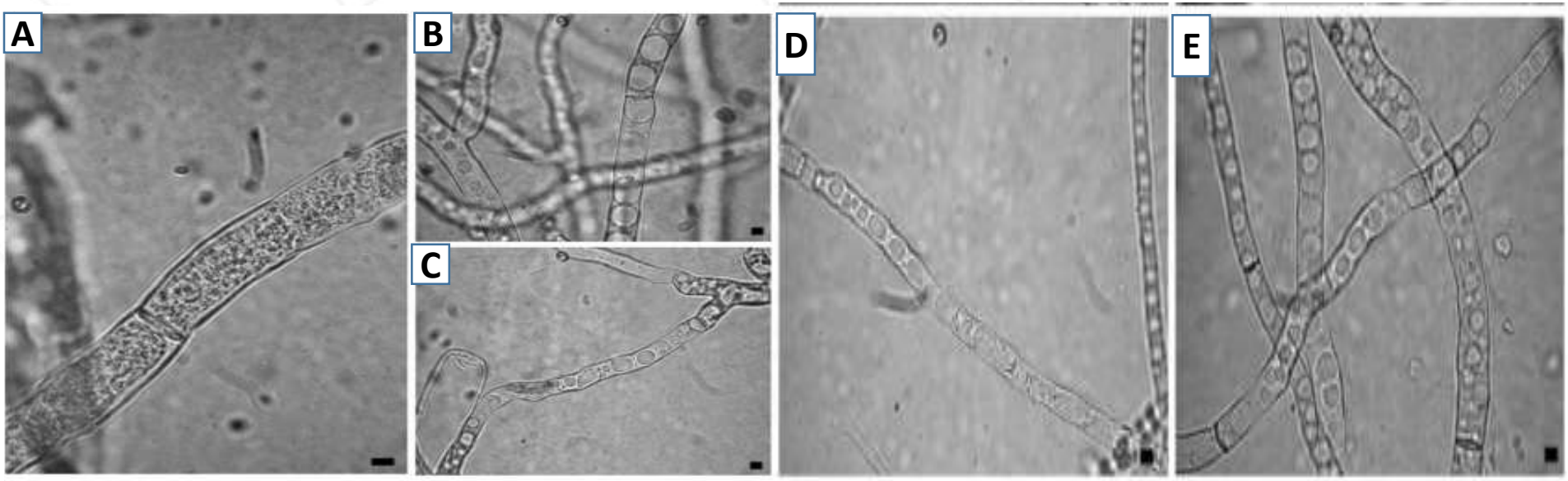
Retention time (min)	Compound	Abundance (Relative)* TvPs2.4
4.96	methyl butanoate	1.02
5.15	Unknown ($C_4H_{10}S$)	1.08
5.17	Unknown (C_3H_6S)	
5.66	Dimethyl disulfide	18.65
5.88	methyl isovalerate	15.41
5.90	methyl 3-methylbutanoate	
6.09	Unknown ($C_7H_{14}O_2$)	0.38
6.17	ethyl butyrate	0.30
6.26	Unknown ($C_6H_{12}S$)	1.98
6.95	ethyl 2-methylbuttyrate	0.98
7.02	ethyl 3-methylbutanoate	7.84
7.11	Unknown	10.77
7.12	Unknown ($C_5H_{10}OS$)	
7.76	3-methyl-1-methylethyl butanoate	2.01
9.01	Unknown	5.86
9.02	Unknown ($C_6H_{12}OS$)	
9.10	propionic acid 2-methyl-hexyl ester	
10.12	Unknown ($C_9H_{18}O_2$)	
10.22	β-Pinene	0.55
10.66	Unknown	0.55
10.67	2-methylpropyl 3-methylbutanoate	
11.33	Terpinolene	0.84
11.60	o-Cymene	2.65
11.78	D-Limonene	22.57
11.97	eucalyptol	1.93
12.07	3-methylbutyl butanoate	2.15
12.08	Unknown ($C_9H_{18}O_2$)	
14.04	2-Nonanone	1.03
15.90	α-Thujone	0.83
21.70	Unknown ($C_{35}H_{60}O_4$)	0.62

Results

TvPs1.6
SPME/GC–MS analysis



Retention time (min)	Compound	Abundance (Relative) *
4.96	methyl butanoate	0.46
5.15	Unknown ($C_5H_{10}S$)	
5.17	Unknown (C_3H_6S)	1.17
5.66	Dimethyl disulfide	38.89
5.88	methyl isovalerate	
5.90	methyl 3-methylbutanoate	7.52
6.09	Unknown ($C_7H_{14}O_2$)	0.48
6.17	ethyl butyrate	
6.26	Unknown ($C_5H_{12}S$)	2.14
6.95	ethyl 2-methylbuttyrate	0.67
7.02	ethyl 3-methylbutanoate	5.28
7.11	Unknown	
7.12	Unknown ($C_5H_{10}OS$)	6.73
7.76	3-methyl-1-methylethyl butanoate	2.82
9.01	Unknown	
9.02	Unknown ($C_6H_{12}OS$)	7.42
9.10	propionic acid 2-methyl-hexyl ester	0.62
10.12	Unknown ($C_9H_{18}O_2$)	0.36
10.22	β-Pinene	
10.66	Unknown	
10.67	2-methylpropyl 3-methylbutanoate	0.80
11.33	Terpinolene	
11.60	o-Cymene	1.65
11.78	D-Limonene	16.10
11.97	eucalyptol	1.10
12.07	3-methylbutyl butanoate	
12.08	Unknown ($C_9H_{18}O_2$)	4.83
14.04	2-Nonanone	0.66
15.90	α-Thujone	
21.70	Unknown ($C_{15}H_{24}O_4$)	0.31



Light observations of *Sclerotinia sclerotiorum* hyphae exposed to bacterial volatiles

A) control; (B,C) *Pseudomonas* sp TvPs1.6 (D,E); *Alcaligenes* sp TvPs2.4

Strong vacuolization with internal residues of membranes and cytoplasmic matrix in the cytoplasm

Conclusions

- TvPs1.6 and TvPs2.4 produced 42 volatile organic compounds detected by SPME/GC–MS analysis. From them, Dimethyl disulfide, D-Limonene and 2-Nonanone and have been reported their antagonistic activity against different soil-borne pathogens.
- The results of this preliminary screening of PGPR from the rhizosphere of the common bean plants in Peru form a starting point to optimize the production of bioactive extracellular compounds that inhibit the mycelial growth and sporulation of harmful phytopathogens.

Acknowledgements

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Thank you for your attention