

GC-MS analysis of antifungal metabolites secreted by two potent endophytes *Enterobacter cloacae* and *Achromobacter xylosoxidans*

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Two endophytic bacteria *Enterobacter cloacae* (ON955844) and *Achromobacter xylosoxidans* (ON955872), isolated from roots of *Musa paradisiaca* showed some plant growth promotion traits (PGP) and antifungal properties against leaf spot disease causing fungal pathogen *Curvularia lunata* (ON246070). *In vitro* assay of antagonist activities of these two bacterial isolates by using their culture filtrates showed positive results. *A. xylosoxidans* reduced pathogen mycelial growth significantly 80% and *Enterobacter cloacae* 75 % as compared to the control. The GC-MS analysis of culture filtrate of these two endophytic bacteria confirmed these finding. GC-MS analysis was carried by using two solvent ethyl acetate and chloroform and it showed several antifungal compounds. The identification of these bioactive secondary metabolites compounds was based on the peak area, retention time, molecular weight, molecular formula, and antimicrobial actions. GC-MS analysis result revealed the presence of major volatile compounds including, Cyclododecane, 1-Octanol, Cetene, Diethyl phthalate, n-Hexadecanoic acid, 1-Nonadecene, cetene, 5-Octadecene, 1-Tridecene, 2-Dodecanol.

Keywords: Antifungal metabolites, endophytes, banana, GC-MS analysis

INTRODUCTION

Banana plant (*Musa paradisiaca*) worldwide cultivated crop harbors many endophytic bacteria. Endophytic bacteria are those that live inside plant tissues without producing visible symptoms of infection or having an adverse effect on their hosts. The leaf spot disease in banana caused by *Curvularia lunata* from Sarai, Raiganj was first reported and Koch's postulates was established by Chowhan and Chakraborty (2022). Endophytic bacteria have potential to protect the host plant from different pathogens. The presents research has focused on the antagonist roles of the two endophytic bacteria *Enterobacter cloacae* (ON955844) and *Achromobacter xylosoxidans* (ON955872), isolated from banana roots against the fungal pathogen of the banana and GCMS analysis of those antifungal metabolites, secreted by the endophytic bacteria against *C. lunata*.

MATERIALS AND METHODS

In vitro* assay of antagonist activities of *E. cloacae* and *A. xylosoxidans* by using their culture filtrates against *C. lunata

The two endophytic bacteria *E. cloacae* and *A. xylosoxidans*-isolate number MRH-06 and MRH-11 respectively were inoculated separately in nutrient broth media, then after 4 days of incubation period the cultures were filtrated using Whatman Filter paper no- 1 and culture filtrates were taken. Then 1 ml of culture filtrate of each sample was transferred into two separate sterile Petridishes. Then 30ml of melted Potato Dextrose Agar was poured to both Petridishes, a control was taken where no culture filtrate was added on it. In all the Petridishes fungal inoculation was done by using a sterile cork borer. After that all the plates were incubated at 32 °C for 5 days.

GC-MS analysis of crude cell free extract

"Two solvent was used for GCMS analysis that were ethyl acetate and chloroform" 2ml sample

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of sterile culture filtrate was taken in a 10 ml of amber vial. 2ml of Ethyl acetate was added to the sample. The mixture was then extracted until the two layer separate clearly. The ethyl acetate layer was pipette out and passed through the anhydrous sodium sulphate layer. Finally the Ethyl Acetate layer was filtered through syringe filter and 1ul is subjected to GCMS. Identification of the antimicrobial metabolites was done by Gas Chromatography Mass Spectrometry (GCMS) analysis with AGILENT 7890B System (Agilent Technologies 7890B GC system for gas chromatography). The inlet temperature was 225C and initial temperature was 75C (Hold time zero). The Ramp 1 temperature was 25C/ min to 150C and Ramp 2 temperature 10C/min to 180C (Hold time 10 min) with MS transfer line 280C. Pure helium was used as a carrier gas at a flow rate of 1 ml/min. "Chloroform was used in place of ethyl acetate when the solvent was chloroform for GCMS analysis."

RESULTS AND DISCUSSION

In vitro assays showed that both bacterial isolates had an antagonist effect against *C. lunata*, by using their culture filtrates (Fig. 1), that indicates the presence of some antifungal metabolites in culture filtrates of bacteria. *Enterobacter cloacae* inhibited the growth of test pathogens viz. *Curvularia lunata*. *Achromobacter xylosoxidans* also checked the growth of the tested pathogens significantly. The tested fungus was inhibited to some degree, the percentage inhibition was 83.5% by *Achromobacter xylosoxidans* and 75% by *Enterobacter cloacae* (Table 1). *E. cloacae* isolated from *Ocimum sanctum* inhibited the growth of *Rhizoctonia solani* and *Pythium debaryanum* as reported by




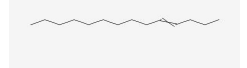

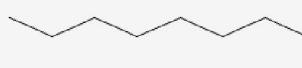
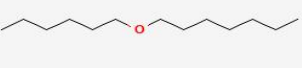
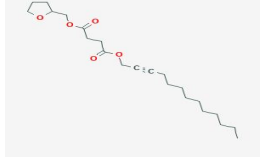
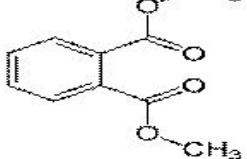
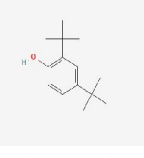
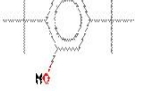
Panigrahi (2021). *A. xylosoxidans* was reported previously for producing inhibitory metabolites having significant effect to inhibiting *Aspergillus flavus* and *Aspergillus parasiticus* (Ren et al. 2020). This finding was confirmed by GC-MS analysis of the culture filtrate of *E. cloacae* and *A. xylosoxidans*, which identified presence of several antifungal compounds. Two solvents was used for GC-MS analysis- chloroform and ethyl acetate and the Chromatogram of GC-MS analysis where each separated substance was represented by a peak and retention times are shown for *Enterobacter cloacae* (Figs. 2, 3) and for *Achromobacter xylosoxidans* (Figs. 4, 5). The major volatile antifungal compound identified in culture filtrate of *Enterobacter cloacae* are Cyclododecane, n-Tridecan-1-ol, 1-Dodecanol, Tetradecene, Cyclododecane, Octane, Dimethyl phthalate, 2,4-Di-tert-butylphenol, Cetene, Diethyl phthalate, 1-Nonadecene, 1-Octadecene, 3,5-Dimethoxyphenol, n-Hexadecanoic acid, 1-Docosene, n-Tetracosanol-1, 1-Octacosanol, Cyclopentane 1-ethyl-2-methyl- cis-, 5-Tridecene (Z), Azetidine, 1,2-dimethyl, Hexane, 2,3,4-trimethyl-, 1-Pentanol 2-methyl-, p-aminobenzamide, 2,4-Di-tert-butylphenol, 5-Octadecene, 1-Tridecene, 2-Dodecanol, 5-Octadecene, n-Hexadecanoic acid (Tables 2 and 3). The major volatile antifungal compounds identified in culture filtrate of *Achromobacter xylosoxidans* are Cyclododecane, 2-Tetradecene, 3-Tetradecene, 4-Tetradecene, Tritetracontane, 2,4-Di-tert-butylphenol, Cetene, Sulfurous acid, dodecyl 2-propyl ester, Diethyl phthalate, 1-Octadecene, E-15-Heptadecenal, 3,5-Dimethoxyphenol, n-Hexadecanoic acid, 1-Nonadecene, 1-Heneicosanol, 1-Heptadecanol, 1-Octanol, Ethyl Acetate 1-Tridecene, Phenol 3,5-Bis(1,1-Dimethylethyl)-, 5-Octadecene (E) -, 2-Dodecanol, Diethyl phthalate, 2-Tetradecanol,


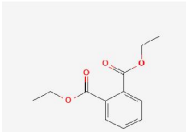




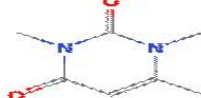

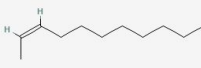

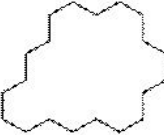
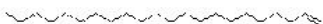
Table1: *In vitro* antagonism of *Enterobacter cloacae* and *Achromobacter xylosoxidans* against *Curvularia lunata*

Test fungi	Paired with bacterium	Dia. of fungal growth* (cm)	Zone of inhibition (cm)	% of Inhibition
<i>Curvularia lunata</i>	-	8.8±1.2	-	-
	<i>Enterobacter cloacae</i>	2.1±0.22	1.7±0.09	75.0±2.91
	<i>Achromobacter Xylosoxidans</i>	1.5±0.09	2.0±0.24	83.5±2.65

*After 7 days; ±= SE

Table 2: Major volatile antifungal compounds identified from the culture filtrate of *Enterobacter cloacae* in solvent chloroform

Compound name	Molecular Formula	Mol. weight (g/mol)	RT	Area %	Structure	Bioactivity
Cyclododecane	C ₁₂ H ₂₄	168.32	3.821	2.56		Adulaimi <i>et al.</i> (2022)
n-Tridecan-1-ol	C ₁₃ H ₂₈ O	200.3608	3.821	2.56		Abdulrahman <i>et al.</i> (2023)
1-Dodecanol	C ₁₂ H ₂₆ O	186.3342	3.821	2.56		Okoye <i>et al.</i> (2012)
4-Tetradecene	C ₁₄ H ₂₈	196.3721	5.269	7.82		Ara <i>et al.</i> (2013)
Cyclododecane	C ₁₂ H ₂₄	168.32	5.269	7.82		Adulaimi <i>et al.</i> (2022)
Octane	C ₈ H ₁₇	114.23	5.326	0.52		Wang <i>et al.</i> (2022)
Ether, heptyl hexyl	C ₁₃ H ₂₈ O	200.36	5.326	0.52		-
Succinic acid, tridec-2-yn-1-yl tetrahydrofurfuryl ester	C ₂₂ H ₃₆ O ₅	380.5	5.326	0.52		-
Dimethyl phthalate	C ₁₀ H ₁₀ O ₄	194.18	5.910	0.50		Premjanu and Jayanthy (2014); Shafikova <i>et al.</i> (2020)
2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32	6.339	23.52		Seenivasan <i>et al.</i> (2022)
Phenol, 2,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206.3239	6.339	23.52		

Cetene	$C_{16}H_{32}$	58.08	7.031	11.87		Femi-Adepoju, (2018)
Diethyl phthalate	$C_{12}H_{14}O_4$	222.24	7.163	24.40		Premjanu <i>et al.</i> (2014)
1-Octadecene	$C_{18}H_{36}$	252.5	8.943	12.45		Orchard <i>et al.</i> (2023)
1-Nonadecene	$C_{19}H_{38}$	266.5	8.943	12.45		Adhikari <i>et al.</i> (2023)
3,5-Dimethoxyphenol	$C_8H_{10}O_3$	154.16	9.538	0.56		Sánchez-Hernández <i>et al.</i> (2023)
Pyrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-.	$C_{11}H_{18}N_2O_2$	210.2728	10.453	0.49		Nas <i>et al.</i> (2021)
2,4(1H,3H)Pyrimidinedione, 1,3,6-trimethyl	$C_7H_{10}N_2O_2$	154.1665	10.453	0.49		-
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	10.453	0.49		Aldarhami <i>et al.</i> (2023)
2-Undecene	$C_{11}H_{22}$	154.29	10.453	0.49		-
1-Nonadecene	$C_{19}H_{38}$	266.5	10.842	9.55		Ghavam <i>et al.</i> (2021)
Cycloeicosane	$C_{20}H_{40}$	280.5	10.842	9.55		-
1-Docosene	$C_{22}H_{44}$	308.6	12.645	3.53		Albratty <i>et al.</i> (2021)

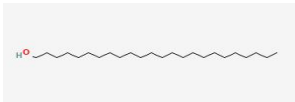
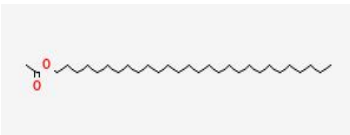



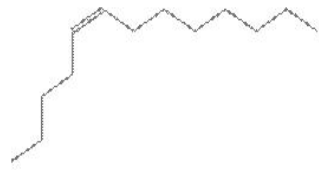

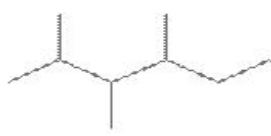
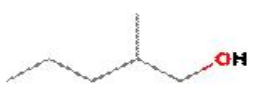
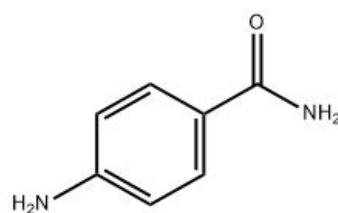
n-Tetracosanol-1	C ₂₄ H ₅₀ O	354.6532	12.645	3.53		Sánchez-Hernández <i>et al.</i> (2021)
Octacosyl Acetate	C ₃₀ H ₆₀ O ₂	452.8	14.327	0.79		-
Heptacosyl acetate	C ₂₉ H ₅₈ O ₂	438.7696	14.327	0.79		-
1-Octacosanol	C ₂₈ H ₅₈ O	410.76	14.327	0.79		Zavala-Sánchez <i>et al.</i> (2020)

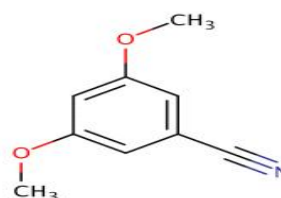
Table 3: Major volatile antifungal compounds identified from the culture filtrate of *Enterobacter cloacae* in solvent ethyl acetate

Compound name	Molecular Formula	Mol. weight	RT	Area %	Structure	Bioactivity
Cetene	C ₁₆ H ₃₂	58.08	5.258	9.00		Femi-Adepoju <i>et al.</i> (2018)
Cyclopentane, 1-ethyl-2-methyl- cis-	C ₈ H ₁₆	112.21	5.258	9.00		Rahmawati <i>et al.</i> (2020)
5-Tridecene, (Z)	C ₁₃ H ₂₆	182.35	5.258	9.00		Kavitha <i>et al.</i> (2010)
Azetidine, 1,2-dimethyl-	C ₅ H ₁₁ N	85.15	5.904	1.29		Asif (2018)
Hexane, 2,3,4-trimethyl-	C ₉ H ₂₀	128.25	5.315	1.29		Anbu <i>et al.</i> (2022)
1-Pentanol, 2-methyl-	C ₆ H ₁₄ O	102.174	5.315	1.29		Garrido <i>et al.</i> (2020)

p-aminobenzamide

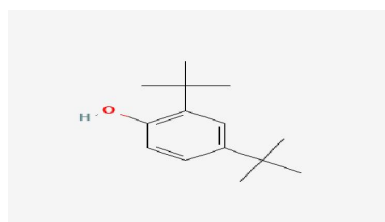
C₇H₈N₂O 136.15 5.904 1.90Bibens *et al.* (2023)

Benzonitrile,3,5-dimethoxy

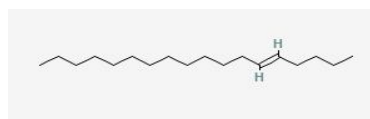
C₉H₉NO₂ 163.176 5.904 1.90

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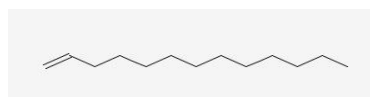
2,4-Di-tert-butylphenol

C₁₄H₂₂O 206.32 6.333 10.55Varsha *et al.* (2015)

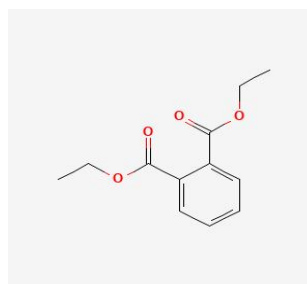
5-Octadecene

C₁₈H₃₆ 252.5 7.026 7.13Hamed *et al.* (2019)

1-Tridecene

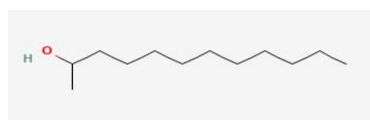
C₁₃H₂₆ 182.35 7.026 7.13Lammers *et al.* (2021)

Diethyl phthalate

C₁₂H₁₄O₄ 222.24 7.157 59.87

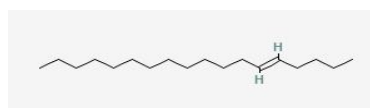
Premjanu and Jaynthy (2014)

2-Dodecanol

C₁₂H₂₆O 186.34 8.943 3.62

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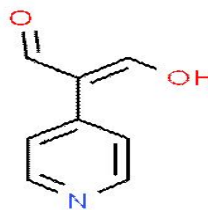
5-Octadecene

C₁₈H₃₆ 252.5 8.943 3.62Hamed *et al.* (2019)

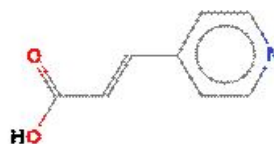
2-Heptadecanol

C₁₇H₃₆O 256.5 8.943 3.62Chatterjee *et al.* (2018)

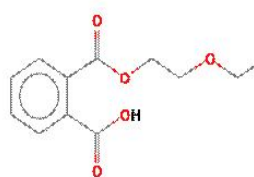
Propenal, 3-hydroxy-2-(4-pyridyl)-
C₈H₇NO₂ 149.147 9.761 1.74



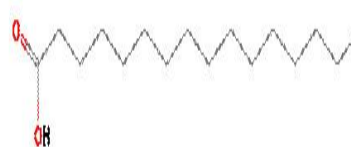
3-(4-Pyridyl)acrylic acid
C₈H₇NO 149.15 9.761 1.74



2-((2-Ethoxyethoxy)carbonyl)benzoic acid
C₁₂H₁₄O₅ 268.26 9.761 1.74



n-Hexadecanoic acid
C₁₆H₃₂O₂ 256.4 10.51 3.47



Kikukawa *et al.* (2023)

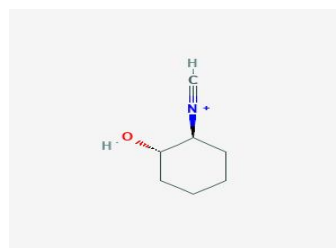
Dodecanoic acid
C₁₂H₂₄O₂ 200.3178 10.51 3.47



(S)-(+)-5-Methyl-1-heptanol
C₈H₁₈O 130.23 10.84 1.42



Cyclohexanol, 2-isocyano-, trans-
C₇H₁₂NO+ 126.18 10.84 1.42



Cyclobut-1-enylmethanol
C₅H₈O 84.12 10.84 1.42

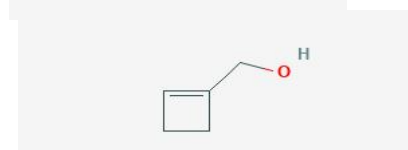
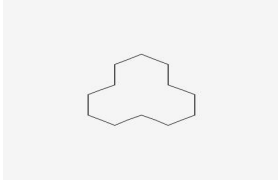
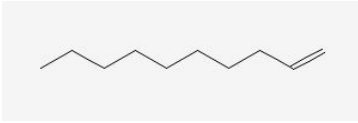
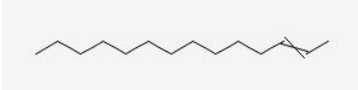
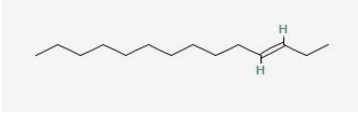
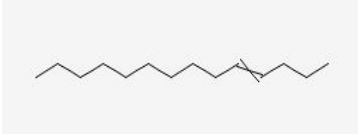
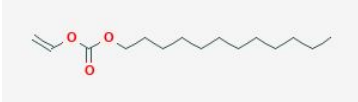
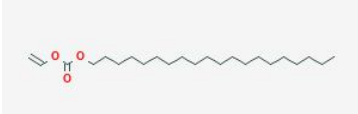
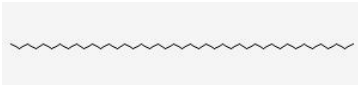
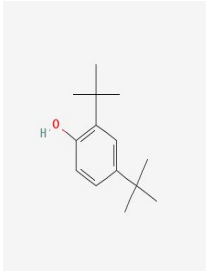
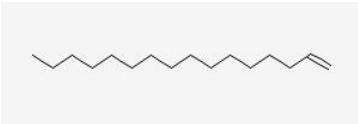
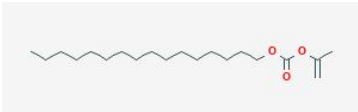
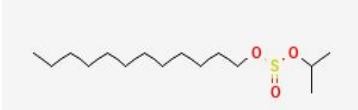
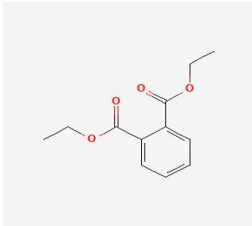
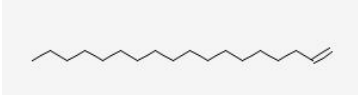
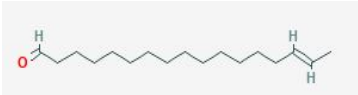
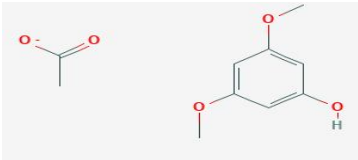
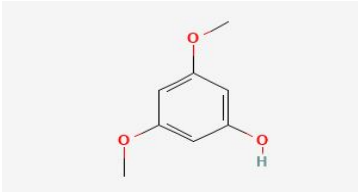
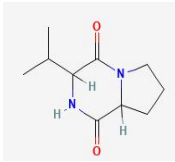
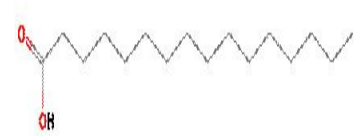
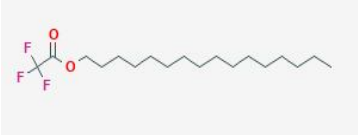


Table 4: Major volatile compound identified from the culture filtrate of *Achromobacter xylosoxidans* in solvent chloroform

Compound name	Molecular Formula	Mol. weight	RT	Area %	Structure	Bioactivity
Cyclododecane	C ₁₂ H ₂₄	168.319	3.816	2.77		Mohan and Anjali (2005)
1-Decene	C ₁₀ H ₂₀	140.266	3.816	2.77		-
2-Tetradecene	C ₁₄ H ₂₈	196.37	5.269	8.15		Mahdavi <i>et al.</i> (2017)
3-Tetradecene	C ₁₄ H ₂₈	196.37	5.269	8.15		Sharma and Thakur (2020)
4-Tetradecene	C ₁₄ H ₂₈	196.37	5.269	8.15		Ara <i>et al.</i> (2013)
Carbonic acid, dodecyl vinyl ester	C ₁₅ H ₂₈ O ₃	256.38	5.326	0.63		-
Carbonic acid, eicosyl vinyl ester	C ₂₃ H ₄₄ O ₃	368.6	5.326	0.63		-
Tritetracontane	C ₄₃ H ₈₈	605.2	5.326	0.63		Rhetso <i>et al.</i> (2020)
2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32	6.339	24.25		Seenivasan <i>et al.</i> (2022)
Cetene	C ₁₆ H ₃₂	224.425	7.032	12.76		Femi-Adepoju <i>et al.</i> (2018)

Carbonic acid, hexadecyl prop-1-en-2-yl ester	$C_{20}H_{38}O_3$	326.5	7.095	0.49		-
Sulfurous acid, dodecyl 2-propyl ester	$C_{15}H_{32}O_3S$	292.5	7.095	0.49		Mary Mawumenyo et al. (2023)
Diethyl phthalate	$C_{12}H_{14}O_4$	222.24	7.163	17.13		Premjanu and Jaynthy (2014)
1-Octadecene	$C_{18}H_{36}$	252.5	8.943	13.39		Kayode et al. (2018)
E-15-Heptadecenal	$C_{17}H_{32}O$	252.4	8.943	13.39		Teoh et al. (2023)
3,5-Dimethoxyphenol acetate	$C_{10}H_{13}O_5$	213.21	9.538	0.64		-
3,5-Dimethoxyphenol	$C_8H_{10}O_3$	154.16	9.538	0.64		Reddy et al. (2015)
Pyrrolo(1,2-a)pyrazine-1,4-dione, hexahydro-3-(1-methylethyl)-	$C_{10}H_{16}N_2O_2$	196.25	10.459	0.57		-
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	10.459	0.57		Nabi et al. (2022)
Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.4	10.843	10.63		-

110 GC-MS analysis of antifungal metabolites secreted by two endophytes [J.Mycopathol.Res :

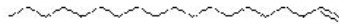
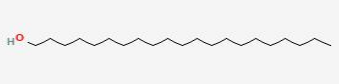


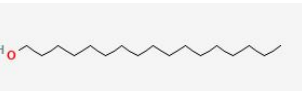
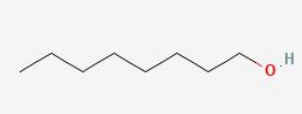
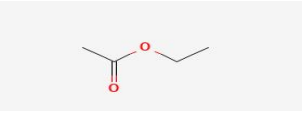
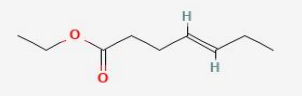
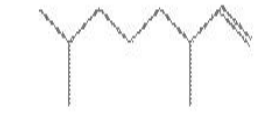
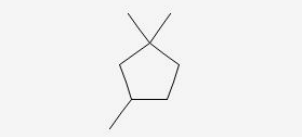
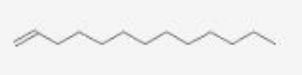
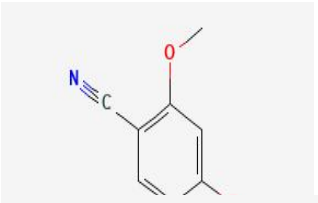
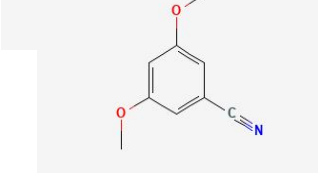
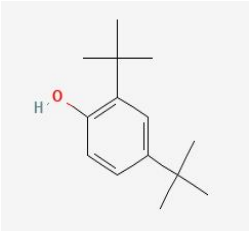
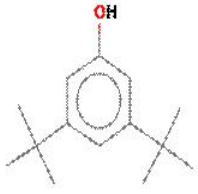
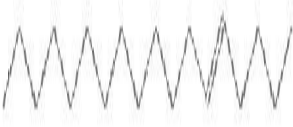
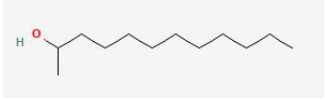
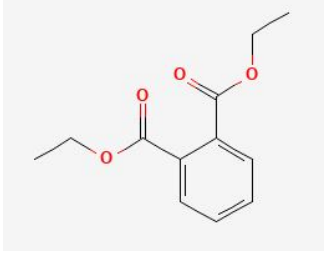
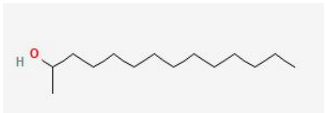
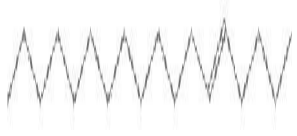
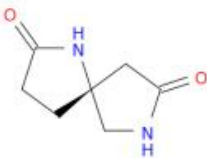
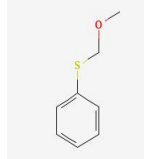
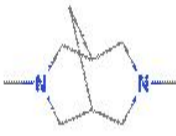
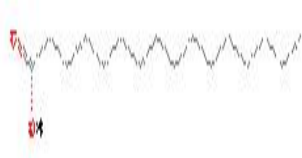
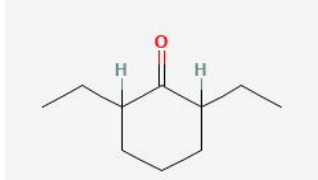


	$C_{19}H_{38}$	266.5	10.843	10.63		Ghavam <i>et al.</i> 2021
1-Nonadecene						
1-Heneicosanol	$C_{21}H_{44}O$	312.6	10.843	10.63		Rhetso <i>et al.</i> (2020)
Behenic alcohol	$C_{22}H_{46}O$	326.6000	12.645	4.11		-
Heptacosyl acetate	$C_{29}H_{58}O_2$	438.7696	14.327	0.93		-

Table 5: Major volatile compound identified from the culture filtrate of *Achromobacter xylosoxidans* in solvent ethyl acetate

Compound name	Molecular Formula	Mol. weight	RT	Area %	Structure	Bioactivity
1-Heptadecanol	$C_{17}H_{36}O$	256.5	3.787	4.35		Chatterjee <i>et al.</i> (2018)
1-Octanol	$C_8H_{18}O$	130.23	3.787	4.35		Neelakandan <i>et al.</i> (2021)
Ethyl Acetate	$C_4H_8O_2$	88.11	3.936	2.92		Satria <i>et al.</i> (2023)
4-Heptenoic Acid, Ethyl Ester, (E)-	$C_9H_{16}O_2$	156.2221	3.936	2.92		-
1-Octene, 3,7-Dimethyl	$C_{10}H_{20}$	140.27	5.258	7.41		-
Cyclopentane, 1,1,3-Trimethyl-	C_8H_{16}	112.21	5.258	7.41		-
1-Tridecene	$C_{13}H_{26}$	182.35	5.258	7.41		Arogbodo <i>et al.</i> (2022)

Benzonitrile, 2,4-Dimethoxy-	$C_9H_9NO_2$	163.17	5.904	1.57		-
3,5-Dimethoxybenzonitrile	$C_9H_9NO_2$	163.18	5.904	1.57		-
2,4-Di-Tert-Butylphenol	$C_{14}H_{22}O$	206.32	6.333	8.80		Seenivasan <i>et al.</i> (2022)
Phenol, 3,5-Bis(1,1-Dimethylethyl)	$C_{14}H_{22}O$	206.33	6.333	8.80		Rizvi <i>et al.</i> (2014)
5-Octadecene, (E)	$C_{18}H_{36}$	252.5	7.026	6.29		Hamed <i>et al.</i> (2019)
2-Dodecanol	$C_{12}H_{26}O$	186.33	7.026	6.29		Togashi <i>et al.</i> (2007)
Diethyl phthalate	$C_{12}H_{14}O_4$	222.24	7.152	58.09		Premjanu and Jayanthi (2014)
2-Tetradecanol	$C_{14}H_{30}O$	214.39	8.943	3.62		Schwob <i>et al.</i> (2006)

5-Octadecene, (E)	$C_{18}H_{36}$	252.5	8.943	3.62		Hamed <i>et al.</i> (2019)
2,6-Diazaspiro(4,4)Nonane-3,7-Dione	$C_7H_{10}N_2O_2$	154.1665	10.453	1.36		-
Methoxymethyl Phenyl Sulphide	$C_8H_{10}OS$	154.23	10.453	1.36		-
3,7-Diazabicyclo[3.3.1]Nonane, 3,7-Dimethyl	$C_9H_{18}N_2$	154.2526	10.453	1.36		Lenin <i>et al.</i> (2017)
n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.4241	10.510	4.22		Krishnan <i>et al.</i> (2016)
Cyclohexanone, 2,6-Diethyl-	$C_{10}H_{18}O$	126.20	10.510	4.22		-
2-Hexenal, (E)	$C_8H_{16}O$	98.1430	10.842	1.39		Wang <i>et al.</i> (2022)
3-Hexene, 2-Methyl-, (E)	C_7H_{14}	98.1861	10.842	1.39		-

5-Octadecene (E)- , 3,7-Diazabicyclo [3.3.1] Nonane, 3,7-Dimethyl-, n-Hexadecanoic Acid, 2-Hexenal (E)-. (Tables 4 and 5).

CONCLUSION

The present study depicts about the roles of culture filtrates of endophytic bacteria as potent antagonistic activities in controlling the growth of fungal pathogens. GCMS analysis of antifungal metabolites, secreted by two potent endophytes

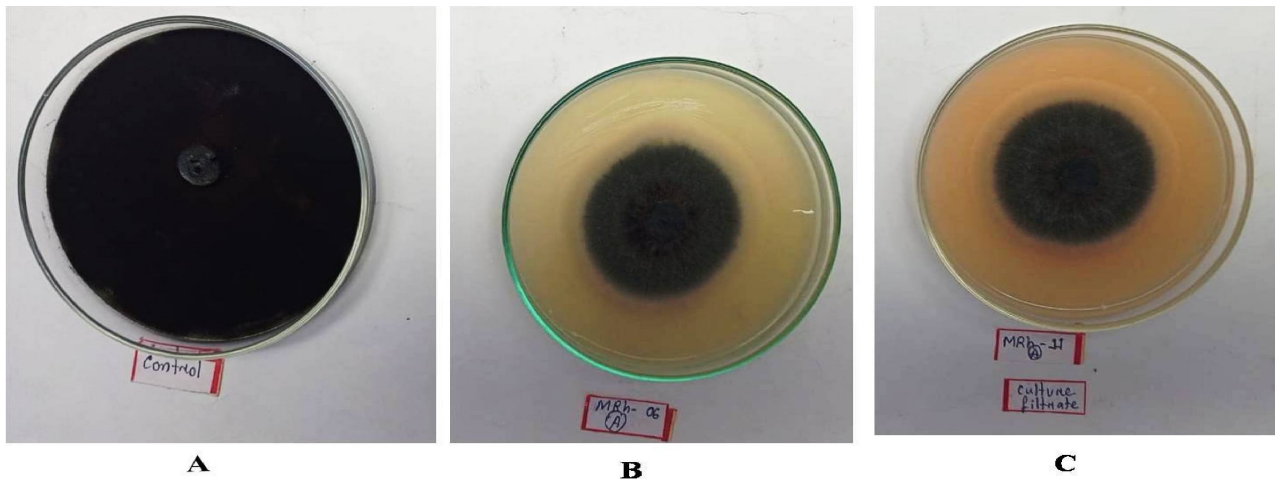


Fig 1: (A) *Curvularia lunata* grown with out adding any bacterial culture filtrate. (B) *Curvularia lunata* grown with culture filtrate of *Enterobacter cloacae*. (C) *Curvularia lunata* grown with culture filtrate of *Achromobacter xylosoxidans*

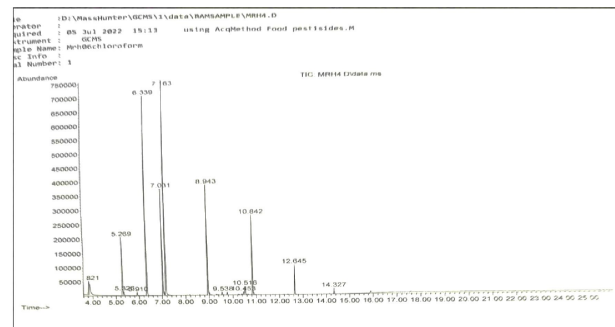


Fig. 2 : GC-MS analysis of *Enterobacter cloacae* by using solvent-chloroform and Chromatogram of GC-MS analysis where each separated substance is represented by a peak and retention time

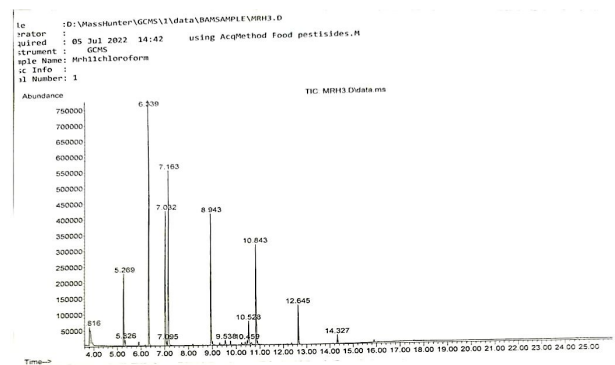


Fig. 4: GC-MS analysis of *Achromobacter xylosoxidans* by using the solvent chloroform and chromatogram of GC-MS analysis where each separated substance is represented by a peak and retention time

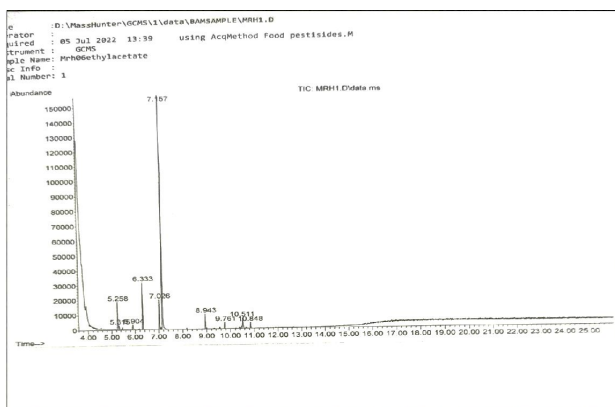


Fig. 3: GCMS analysis of *Enterobacter cloacae* by using the solvent ethyl acetate and Chromatogram of GC-MS analysis where each separated substance is represented by a peak and retention time

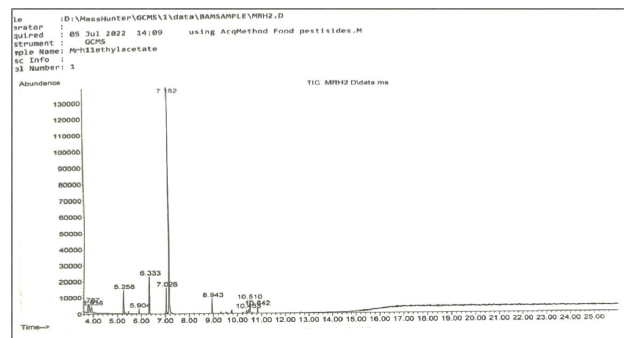


Fig. 5: GCMS analysis of *Achromobacter xylosoxidans* by using the solvent ethyl acetate and chromatogram of GC-MS analysis where each separated substance is represented by a paeak and retention time

Enterobacter cloacae and *Achromobacter xylosoxidans* proved their biocontrol potentials against banana leaf spot disease causing pathogen *Curvularia lunata*.

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Bengal, India are thankfully acknowledged for the molecular identification of endophytic bacterial strain and fungus isolate.

DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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