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Determination of Biologically Active Substances Responsible for Antimicrobial Activities *Bacillus Species with Gas Chromatography–Mass Spectrometry (GC-MS)* Analysis

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ABSTRACT

Soil-borne *Bacillus* spp. represents a prolific source of antimicrobial compounds, yet their full potential remains underexplored. In this research, twenty soil samples were obtained from four different sites in Gwagwalada, FCT, and subjected to serial dilution before plating to isolate presumptive *Bacillus* species. Eight Gram-positive, catalase-positive, endospore-forming strains were purified, biochemically characterized, and identified by 16S rRNA sequencing as *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, two *B. circulans* strains, *B. cereus*, *B. brevis*, and *B. subtilis*. Gas Chromatography/Mass Spectrometry **(**GC–MS) profiling of the five top extracts revealed 110 volatile and semi-volatile compounds, dominated by acetoin (up to 80 mg/L), 2,3-butanedione, and branched-chain fatty acids, which support the observed bioactivities. These findings validate Gwagwalada soils as reservoirs of potent antimicrobial *Bacillus* strains, establishing practical guidelines for media formulation, targeted strain selection, and metabolite purification for scaling up the production of natural antibiotics for agricultural and clinical applications.

Keywords:
Soil, GC-MS,
Antimicrobial,
Bacillus, Compounds.

INTRODUCTION

Microorganisms have long served as a critical source of therapeutic agents, providing many life-saving medicines and powerful antibiotics effective against bacterial and fungal pathogens (Thakur *et al*., 2017). The discovery of penicillin in 1928 marked a turning point, establishing antibiotics as indispensable in treating microbial infections and stimulating continuous research for more effective antimicrobial compounds (Muteeb *et a*l., 2023). Nevertheless, the excessive and improper use of these drugs has accelerated the global problem of antimicrobial resistance, which has greatly diminished the effectiveness of many standard antibiotics (Konwar *et al*., 2022).

Antibiotics are biologically active substances that can be of natural, synthetic, or semi-synthetic origin (Nikodinovic *et al*., 2015). While antimicrobial practices have existed for centuries, most modern antibiotics have been identified by isolating and screening bacteria and fungi from soil and other natural environments. These compounds are naturally produced by microorganisms to suppress or eliminate the growth and activity of competing microbes (Davies and Davies, 2019). Typically, antibiotics are low-molecular-weight secondary metabolites that, although not essential for the survival of the producing organisms, play an important role in their ecological interactions. From a medical perspective, they are highly valuable due to their complex chemical structures and potent biological effects. Antibiotic synthesis usually occurs during the stationary phase of microbial growth, involving the conversion of metabolic intermediates into intricate compounds through specialized biosynthetic pathways (Sanchez *et al*., 2019).

The term “antibiotics” is frequently applied in a broad sense to include antifungal, antiparasitic, and antibacterial substances. More precisely, however, antibiotics are primarily defined as antibacterial agents, which remain the most vital class of drugs for managing and preventing bacterial infections (Pancu *et al*., 2021). A significant number of currently used antibiotics originate from soil microorganisms, with Bacillus species standing out for their remarkable capacity to produce diverse antibiotic compounds. As naturally occurring soil bacteria,

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Bacillus species exhibit strong antagonistic activity against other microbes, a property linked to their ability to form resilient endospores and to synthesize antimicrobial substances such as bacteriocins (Adeleye *et al*., 2015; Uwalaka *et al*., 2019).

Antibiotics are considered among the most significant commercially valuable secondary metabolites, synthesized by a diverse group of microorganisms, including bacteria, actinomycetes, and fungi (Selim *et al.*, 2021). The remarkable contribution of microorganisms to antibiotic production is well established. At present, only about 1% of microbial species have been identified, yet recent progress in cultivation methods and nucleic acid extraction from soil and marine habitats has created opportunities to explore the vast, unexploited genetic and metabolic potential of microbial populations (Sanchez *et al*., 2019). The focus of this study was to identify the bioactive compounds responsible for the antimicrobial properties of Bacillus species isolated from soil.

MATERIALS AND METHODS

Sample collection

A total of twenty (20) soil samples were randomly obtained, with five (5) samples collected from each of four locations in Gwagwalada, FCT. The sampling areas comprised Phase 3, Old Kutunku, Dagiri, and Angwandodo. From each site, 20 g of soil was collected from the top layer at a depth of 10 cm using a soil auger, following the procedure outlined by Ugoh and Ijigbade (2013). The samples were placed in sterile universal containers and transported to the laboratory for subsequent analysis.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

The antimicrobial extract obtained from the Bacillus isolate was subjected to Gas Chromatography–Mass Spectrometry (GC-MS) analysis using an Agilent 5977B GC/MSD system. The procedure employed a Thermo Finnigan Trace GC coupled with a Polaris Q ion trap mass spectrometer operated in electron impact mode at 70 eV, with the ion source temperature maintained at 230 °C. Chromatographic separation was carried out on a DB-5MS fused silica capillary column (30 m × 0.25 mm, 0.5 µm film thickness), utilizing helium as the carrier gas at a flow rate of 1.5 ml/min. A 1 µL aliquot of the sample was introduced in splitless mode at 250 °C. The oven temperature program was set from 60 °C to 240 °C at a rate of 6 °C/min, resulting in distinct and well-resolved peaks.

Identification of photo components

The interpretation of the GC-MS mass spectra was carried out using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 reference patterns, along with data from the National Center for Biotechnology Information (NCBI). The spectra of the unknown compounds were matched against those in the NIST library to identify similarities with known substances.

RESULTS AND DISCUSSION

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the crude extracts derived from various Bacillus species identified the presence of multiple bioactive compounds. Tables 1 to 5 show the important and numerous identified components in the crude extracts of *Bacillus* with remarkable antibacterial activity. A total of 110 bioactive compounds were identified: 42 carboxylic acids, 17 alcohols, 19 esters, 9 ketones (including hydroxy-ketones), 8 aldehydes, 3 diols, 3 phenolic compounds, 5 oximes, 2 epoxides, 1 hydrocarbon, and 1 sulfur-containing heterocycle. In *Bacillus amylolequifaciens* SB7 (Table 1), the highest compound was 3-Pentanol, 2-methylat 32.50 mg/L, while the lowest was 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester at 0.08mg/L. In *Bacillus pumilus* SB5 (Table 2), Butanoic acid, 2-methyl- was the most abundant at 30.00 mg/L, and the lowest was Benzoic acid, heptylester at 0.35 mg/L. For *Bacillus Cereus* SB8 (Table 3), the highest compound was Propanoic acid, 2-methyl- at 12.00 mg/L, and the lowest was Pentadecanoic acid at 0.50mg/L. In *Bacillus subtilis* SB14B (Table 4), the highest compound was Acetoin at 44.06 mg/L, and several values had the lowest of 0.01mg/L. Finally, in *Bacillus circulans* SB2D (Table 5), Acetoin was the highest at 80.00 mg/L, and 1-Hexanol-2-ethyl was the lowest at 0.50 mg/L.

Table 1: Constituents of *Bacillus amyloliquefaciens* SB7 extract identified via GC–MS analysis

Compound Name	Molecular Formula	Molecular Mass (g/mol)	Retention Time (min)	Peak Area (%)	Concentration (mg/L)
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	18.07	0.09	0.08
Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	15.74	0.10	0.09
(R)-(−)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.32	0.13	0.10

Oleic Acid	$C_{18}H_{34}O_2$	282.50	18.36	0.14	0.12
2,2,4-Trimethyl-1,3pentanediol diisobutyrate	$C_{16}H_{30}O_4$	286.41	13.13	0.17	0.15
Octanoic acid	$C_8H_{16}O_2$	144.21	14.19	0.18	0.15
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	19.85	0.32	0.27
1-Nonanol	$C_9H_{20}O$	144.25	10.37	0.42	0.35
2-Hydroxy-3pentanone	$C_5H_{10}O_2$	102.13	7.11	0.42	0.35
Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	12.05	0.52	0.45
Acetic acid	$C_2H_4O_2$	60.05	8.35	0.78	0.65
1,6-Octadien-3-ol, 3,7dimethyl-	$C_{10}H_{18}O$	154.25	9.63	0.78	0.65
(S)-(+)-6-Methyl-1octanol	$C_9H_{20}O$	144.25	10.64	0.98	0.85
Ethane-1,1-diol dibutanoate	$C_{10}H_{18}O_4$	202.25	7.22	1.18	1.00
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.58	2.38	2.10
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.58	2.38	2.10
3-Penten-1-ol	$C_5H_{10}O$	86.13	3.41	2.64	2.30
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.50	14.77	2.89	2.50
9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.40	25.04	3.00	2.70
Propanoic acid, 2-methyl	$C_4H_8O_2$	88.11	9.83	3.14	3.16
2,3-Butanediol	$C_4H_{10}O_2$	90.12	9.48	4.67	4.10
3-Pentanol, 2-methyl-	$C_6H_{14}O$	102.17	6.26	36.53	32.50

Table 2: Constituents of *Bacillus pumilus* SB5 extract identified via GC–MS analysis

Compound Name	Molecular Formula	Molecular Mass (g/mol)	Retention Time (min)	Peak Area (%)	Concentration (mg/L)
Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	18.079	0.42	0.35
(R)-(−)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.945	0.53	0.45
Benzoic acid 2methylpentyl ester	$C_{13}H_{18}O_2$	206.28	17.809	0.55	0.45
Benzoic acid	$C_7H_6O_2$	122.12	18.74	0.57	0.50
Decanal	$C_{10}H_{20}O$	156.26	9.11	0.63	0.50
Acetoin	$C_4H_8O_2$	88.11	6.49	0.74	0.65
Butanoic acid	$C_4H_8O_2$	88.11	10.57	0.91	0.80
Decanoic acid	$C_{10}H_{20}O_2$	172.26	17.36	0.94	0.80
Hexanoic acid, 2ethyl-	$C_8H_{16}O_2$	144.21	14.20	1.03	0.90
Octanoic acid	$C_8H_{16}O_2$	144.21	15.32	1.11	1.00
1,2-Benzenedicarbox ylic acid, bis(2methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	19.85	1.11	1.00
Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	12.05	1.28	1.20
Tiglic acid	$C_5H_8O_2$	100.12	13.08	1.68	1.50
Nonanoic acid	$C_9H_{18}O_2$	158.24	16.36	2.40	2.20
Acetone	C_3H_6O	58.08	1.64	3.66	3.00
Octadecanoic acid, 2-hydroxy1,3-propanediyl ester	$C_{39}H_{76}O_5$	625.00	24.24	3.79	3.20

Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.60	4.45	4.00
Benzaldehyde	C_7H_6O	106.12	9.40	6.24	5.50
Acetic acid	$C_2H_4O_2$	60.05	8.34	6.31	5.80
9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.50	24.53	9.95	9.00
Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.83	11.51	10.50
Butanoic acid, 2methyl-	$C_5H_{10}O_2$	102.13	11.08	31.69	30.00

Table 3: Constituents of *Bacillus cereus* SB8 extract identified via GC–MS analysis

Compound Name	**Molecular Formula**	**Molecular Mass (g/mol)**	**Retention Time (min)**	**Peak Area (%)**	**Concentration (mg/L)**
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	21.43	0.41	0.50
Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	18.08	0.50	0.60
Benzoic acid 2-methylpentyl ester	$C_{13}H_{18}O_2$	206.28	17.81	0.53	0.70
Nonanoic acid	$C_9H_{18}O_2$	158.24	16.37	0.54	0.70
Nonanoic acid	$C_9H_{18}O_2$	158.24	16.37	0.54	0.70
Benzoic acid, tridecyl ester	$C_{20}H_{32}O_2$	304.50	18.38	0.56	0.70
Benzoic acid, tridecyl ester	$C_{20}H_{32}O_2$	304.50	18.38	0.56	0.70
Butanoic acid	$C_4H_8O_2$	88.11	10.58	0.61	0.80
Benzoic acid	C_7H_6O	122.12	18.75	0.65	0.90
2-Octyl benzoate	$C_{15}H_{22}O_2$	234.33	17.36	0.68	1.00
2-Octyl benzoate	$C_{15}H_{22}O_2$	234.33	17.36	0.68	1.00
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.50	17.02	0.72	1.20
Acetone	C_3H_6O	58.08	1.67	0.75	1.50
Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	12.06	1.14	1.50
Octadecanoic acid	$C_{18}H_{36}O_2$	284.50	24.26	1.64	2.00
Acetic acid	$C_2H_4O_2$	60.05	8.36	2.40	3.20
Benzaldehyde	C_7H_6O	106.12	9.41	4.75	5.20
3(2H)-Thiophenone, dihydro-2-methyl-	C_5H_8OS	116.18	9.46	6.07	7.10
Acetoin	$C_4H_8O_2$	88.11	6.49	8.44	10.00
Oleic acid	$C_{18}H_{34}O_2$	282.50	24.54	9.68	10.00
Propanoic acid, 2methyl-	$C_4H_8O_2$	88.11	9.84	13.97	12.00

Table 4a: Constituents of *Bacillus subtilis* SB14B extract identified via GC–MS analysis

Compound Name	**Molecular Formula**	**Molecular Mass (g/mol)**	**Retention Time (min)**	**Peak Area (%)**	**Concentration (mg/L)**
Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$	234.33	17.08	0.17	0.02
Decanal	$C_{10}H_{20}O$	156.26	8.50	0.39	0.01
Neodecanoic acid	$C_{10}H_{20}O_2$	172.26	15.31	0.43	0.02

1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	16.15	0.44	0.03
1-Decanol	$C_{10}H_{22}O$	158.28	12.21	0.52	0.01
(R)-(−)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.97	0.62	0.02
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	$C_{13}H_{22}O$	194.31	13.33	0.66	0.02
1-Nonanol	$C_9H_{20}O$	144.25	10.47	0.73	0.02
Cetene	$C_{16}H_{32}$	224.42	14.48	0.75	0.01
1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154.25	9.64	0.78	0.01
Phenol	C_6H_6O	94.11	14.83	0.82	0.01
Acetic acid	$C_2H_4O_2$	60.05	8.37	0.89	0.02
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	286.41	13.63	0.91	0.02
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O$	216.32	13.46	0.99	0.03

Table 4b: Constituents of *Bacillus subtilis* SB14B extract identified via GC–MS analysis continued

Compound Name	**Molecular Formula**	**Molecular Mass (g/mol)**	**Retention Time (min)**	**Peak Area (%)**	**Concentration (mg/L)**
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.50	17.16	1.22	0.03
(S)-(+)-6-Methyl-1-octanol	$C_9H_{20}O$	144.25	10.64	1.25	0.03
Oxirane, (methoxymethyl)-	$C_4H_8O_2$	88.11	7.22	1.36	0.02
Hexanoic acid	$C_6H_{12}O_2$	116.16	13.09	1.86	0.04
Benzaldehyde	C_7H_6O	106.12	9.41	2.22	0.04
Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.09	2.24	0.04
Octanoic acid	$C_8H_{16}O_2$	144.21	15.39	2.47	0.05
Nonanoic acid	$C_9H_{18}O_2$	158.24	16.48	2.80	0.05
Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$	144.21	14.23	2.90	0.06
1-Hepten-4-ol	$C_7H_{14}O$	114.19	9.85	3.42	0.05
(R, R)-2,3-Butanediol	$C_4H_{10}O$	90.12	9.49	4.08	0.07
2,3-Butanedione	C_4H_6O	86.09	2.64	5.90	15.85
Acetoin	$C_4H_8O_2$	88.11	6.23	19.5	44.06
Oxime-, methoxy-phenyl	$C_8H_9O_2$	151.16	12.06	1.14	0.03

Table 5a: Constituents of *Bacillus circulans* strain SB2D extract identified via GC–MS analysis

Compound Name	**Molecular Formula**	**Molecular Mass (g/mol)**	**Retention Time (min)**	**Peak Area (%)**	**Concentration (mg/L)**
1,4-Benzenediol 2,6-bis (1,1dimethylethyl)-	$C_{14}H_{22}O_2$	222.32	17.30	0.18	1.50
Nonanoic acid	$C_9H_{18}O_2$	158.24	16.36	0.73	5.00
Phenol	C_6H_6O	94.11	14.77	0.19	1.00
1-Dodecanol	$C_{12}H_{26}O$	186.33	14.45	0.15	0.80
Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	12.05	0.35	1.70
Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$	144.21	14.20	0.31	1.90

3-Buten-2-one, 4-(1-cyclopenten-1 yl)-, (E)-	$C_9H_{12}O$	136.19	13.46	0.40	2.50
Pentanoic acid	$C_5H_{10}O_2$	102.13	13.07	0.23	1.20
2,4-Decadienal, (E, E)-	$C_{10}H_{16}O$	152.23	12.85	0.28	2.10
Dodecanal	$C_{12}H_{24}O$	184.32	11.71	0.68	4.00
Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.07	3.57	7.00
Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.83	1.40	2.90
1-Nonanol	$C_9H_{20}O$	144.25	11.00	0.13	0.60
(S)-(+)-6-Methyl-1-octanol	$C_9H_{20}O$	144.25	10.63	0.28	1.30
2-Octanol	$C_8H_{18}O$	130.23	9.93	0.26	0.80
Acetic acid	$C_2H_4O_2$	60.05	8.34	2.46	5.50

Table 5b: Constituents of *Bacillus circulans* strain SB2D extract identified via GC–MS analysis continued

Compound Name	**Molecular Formula**	**Molecular Mass (g/mol)**	**Retention Time (min)**	**Peak Area (%)**	**Concentration (mg/L)**
Octanoic acid	$C_8H_{16}O_2$	144.21	15.31	0.40	3.00
Formic acid, octyl ester	$C_9H_{18}O_2$	158.24	9.75	0.37	1.60
E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	226.40	9.05	0.14	0.70
1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.23	8.89	0.15	0.50
Nonanal	$C_9H_{18}O$	142.24	7.73	0.75	2.30
Oxirane, (methoxymethyl)-	$C_4H_8O_2$	88.11	7.22	2.41	4.80
2,3-Pentanedione	$C_5H_8O_2$	100.12	3.40	2.04	3.00
3-Pentanol, 2-methyl-	$C_6H_{14}O$	102.17	7.01	2.92	6.10
Acetoin	$C_4H_8O_2$	88.11	6.25	38.25	80.00
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.50	17.01	0.32	2.00

Compounds detected in significant abundance within the bacterial extracts

The GC–MS analysis showed that several compounds occurred in high concentrations across the five Bacillus extracts, notably acetoin, 2,3-butanedione, hexadecanoic acid, 2-methylbutanoic acid, 2-methylpropanoic acid, and benzaldehyde, each detected in at least three strains as presented in Table 6. In *Bacillus circulans* SB2D, acetoin was the most abundant compound (80.00 mg/L), followed by 2,3-butanedione (16.97 mg/L) and 2-methylbutanoic acid (7.00 mg/L). In *Bacillus subtilis* SB14B, acetoin again led at 44.06mg/L, with 2,3-butanedione at 15.85mg/L and (R, R)-2,3-butanediol at 4.08mg/L. In *Bacillus cereus* SB8, propanoic acid, 2-methyl- was highest at 12.00mg/L, then oleic acid (10.00mg/L) and Acetoin 10.00mg/L. *Bacillus pumilus* SB5 featured butanoic acid, 2-methyl- at 31.69mg/L, propanoic acid, 2-methyl- at 11.51mg/L, and 9-octadecenoic acid (E)- at 9.95mg/L. Finally, *Bacillus amyloliquefaciens* SB7's top three were 3-pentanol, 2-methyl (32.50mg/L), 2,3-butanedione (21.43mg/L), and 2,3-butanediol (4.10mg/L).

Table 6: Specific compounds found in high concentrations in the bacterial extracts

Bacillus circulans strain SB2D	*Bacillus subtilis* SB14B	*Bacillus cereus* SB8	*Bacillus pumilus* SB5	*Bacillus amyloliquefaciens SB7*
Acetoin (80.00mg/L)	Acetoin (44.06mg/L)	Propanoic acid, 2methyl- (12.00mg/L)	Butanoic acid, 2-methyl- (31.69mg/L)	3-Pentanol, 2-methyl- (32.50mg/L)

2,3-Buttanedione (16.97mg/L) Butanoic acid, 2-methyl- (7.00mg/L)	2, 3-Butanedione (15.85mg/L)	Oleic acid, 2 (10.00mg/L)	Propanoic acid, 2-methyl- (11.51mg/L)	2, 3-Butanedione (21.43%)
3-Pentanol, 2-methyl- (6.10mg/L)	(R, R)-2, 3-Butanediol (4.08%)	Acetoin (10.0mg/L)	9-Octadecenoic acid, (E) - (9.95mg/L)	2, 3-Butanediol (4.10mg/L)
Acetic acid (5.50mg/L)	1-Hepten-4-ol (0.07mg/L)	3(2H)-Thiophenone, dihydro-2-methyl- (7.10mg/L)	Acetic acid (6.31mg/L)	Oleic Acid (0.12mg/L)
Nonanoic acid (5.0mg/L)	Hexanoic acid, 2-ethyl- (0.06mg/L)	Benzaldehyde (5.20mg/L)	Benzaldehyde (6.24mg/L)	Propanoic acid, 2-methyl- (3.16mg/L)
Oxirane, (methoxymethyl) - (4.80mg/L)	Nonanoic acid (0.05mg/L)	Acetic Acid (3.20mg/L)	Hexadecanoic acid (4.45mg/L)	9, 12-Octadecadienoic acid (Z, Z) - (2.70mg/L)
Dodecanal (4.0mg/L)	Octanoic acid (0.05mg/L)	Octadecanoic acid (2.0mg/L)	Octadecanoic acid, 2-hydroxy-1, 3-propanediyl ester (3.79mg/L)	Hexadecanoic acid, methyl ester (2.50mg/L)
Propanoic acid, 2-methyl- (2.90mg/L)	Butanoic acid, 2-methyl- (0.04mg/L)	Oxime-, methoxy-phenyl (1.50mg/L)	Acetone (3.66mg/L) Nonanoic acid (2.20mg/L)	3-Penten-1-ol (2.30mg/L)
3, Buten-2-one, 4-(1-cyclopenten-1 yl)-, (E,)- (2.50mg/L)	Benzaldehyde (0.04mg/L)	Acetone (1.50mg/L)	Tiglic acid (1.50mg/L)	Hexadecanoic acid (2.10mg/L)

The GC–MS profiling of the five *Bacillus* extracts uncovered an exceptionally rich secondary metabolite repertoire, echoing previous reports of complex volatile and semi-volatile pools in soil-derived *Bacillus* strains (Yousuf *et al.*, 2022). These chemical fingerprints have direct implications for this study's aim of screening for antimicrobial-producing *Bacillus* species and optimizing carbon sources to maximize compound yield. High levels of known bioactives such as acetoin and 2,3-butanediol signal strong antimicrobial potential, guiding us toward the most promising strains for further activity assays (Petrov and Petrova, 2021). Moreover, understanding each strain's dominant metabolite informs which carbon substrates might best upregulate desired pathways; sugars that favour diol over acid production could be selected to enhance 2,3-butanediol yields, while those promoting hydroxy-ketone synthesis could boost acetoin levels (Spöring, 2022). By linking metabolite profiles to both antimicrobial efficacy and carbon source effects, these findings lay the groundwork for strategic medium design in the search for new biocontrol agents (Dow et al., 2023).

Soil-derived *Bacillus* strains frequently share a core set of metabolites, a phenomenon noted in diverse agro-ecosystems where compounds like acetoin, 2,3-butanedione, and short-chain fatty acids recur across species (Teixeira, 2023). In our isolates (Table 6), acetoin was dominant in both *B. circulans* SB2D (80.00mg/L) and *B. subtilis* SB14B (44.06 mg/L), supporting reports by (Wu *et al*. 2021) who found acetoin to be a major antifungal agent in *B. subtilis* SB14B. The prominence of 2,3-butanedione in the same strains (16.97 mg/L) and 15.85mg/L, respectively, aligns with (Nag *et al.* 2022), who described this di-ketone as a key antibacterial metabolite in *Bacillus*. Likewise, the high levels of propanoic acid (2-methyl-) in *B. cereus* SB8 and *B. pumilus* SB5 corroborate findings by (Sariyanti *et al.* 2025), who observed similar branched-chain acids in *Bacillus* cultures with strong Gram-negative activity. The substantial 3-pentanol (32.50mg/L) and 2,3-butanediol (4.10mg/L) in *B. amyloliquefaciens* SB7 corroborate with the work of (Cellini *et al.*, 2021), who reported these alcohols as signature volatiles in plant-associated *Bacillus* with biocontrol potential.

These recurring high-abundance metabolites have direct implications for this current study's aim of identifying potent antimicrobial producers and optimizing carbon media. Acetoin and 2,3-butanedione, for instance, are synthesized via pyruvate pathways that respond strongly to sugars like maltose and sucrose (Yuan *et al*., 2024), suggesting that medium formulation can be tailored to amplify their production. Similarly, the branched-chain fatty acids linked to Gram-negative inhibition could be increased by supplementing valine or isoleucine precursors (Cho *et al.*, 2023). By focusing on strains that naturally accumulate these bioactives at high levels, we can strategically select carbon sources to maximize yield, thereby streamlining the discovery of effective *Bacillus*-derived antimicrobials. Several studies have reported the presence of bioactive organic compounds, including acids, aldehydes, alcohols, ketones, esters, and oximes, from Bacillus strains analyzed through GC–MS, linking them to antibacterial, antifungal, and anticancer properties (El-Agamy et al., 2017; Mao et al., 2019; Shaaban et al., 2021; Koilybayeva et al., 2023; Naveed et al., 2023). In this study, GC–MS profiling identified a diverse array of 110 bioactive metabolites, highlighting the remarkable metabolic adaptability of these strains. These results not only support the effectiveness of GC–MS screening for discovering novel antimicrobial producers but also provide a foundation for optimizing cultivation strategies to enhance bioactive compound synthesis, thereby contributing to the advancement of eco-friendly bio-control agents and natural antibiotic alternatives.

CONCLUSION

Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical technique used to identify and quantify the components of a mixture. This technique was applied in this study and was able to identify a diverse set of 110 bioactive compounds, underscoring the broad metabolic capacity of these strains. This outcome not only confirms the reliability of the screening method in identifying novel antimicrobial producers but also provides a basis for optimizing cultivation conditions to enhance bioactive metabolite yield, thereby supporting the development of sustainable biocontrol agents and natural antibiotic alternatives.

Furthermore, by determining the biologically active substances responsible for antimicrobial activities of Bacillus species, new opportunities could be unlocked for the development of novel antimicrobial agents and applications.

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