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Article

GC–MS Profiling of Volatile Metabolites Produced by Some *Bacillus* spp. and Evaluation of Their Antibacterial and Antibiotic Activities

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Abstract: *Bacillus* species produce different classes of antimicrobial and antioxidant substances: peptides or proteins with different structural composition and molecular mass and a broad range of volatile organic compounds (VOCs), some of which may serve as biomarkers for microorganism identification. The aim of this study is the identification of biologically active compounds synthesized by five *Bacillus* species using gas chromatography coupled to mass spectrometry (GC–MS). The current study profoundly enhances the knowledge of antibacterial and antioxidant metabolites ensuring the unambiguous identification of VOCs produced by some *Bacillus* species, which were isolated from vegetable samples of potato, carrot, and tomato. Phylogenetic and biochemical studies were used to identify the bacterial isolates after culturing. Phylogenetic analysis proved that five bacterial isolates BSS12, BSS13, BSS16, BSS21, and BSS25 showed 99% nucleotide sequence similarities with *Bacillus safensis* AS-08, *Bacillus cereus* WAB2133, and *Bacillus acidiproducens* NiuFun, *Bacillus toyonesis* FORT 102 and *Bacillus thuringiensis* F3, respectively. The crude extract was prepared from bacterial isolates to assess the antibiotic resistance potency and the antimicrobial potential against various targeted multidrug-resistant strains, including *Candida albicans, Candida krusei, Enterococcus hirae, Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus group B, Streptococcus mutans, Shigella sonnei, Salmonella enteritidis, Serratia marcescens, Pseudomonas aeruginosa*, and *Proteus vulgaris*. GC–MS analysis of bacterial strains found that VOCs from *Bacillus* species come in a variety of chemical forms, such as ketones, alcohols, terpenoids, alkenes, etc. Overall, 69 volatile organic compounds were identified from five *Bacillus* species, and all five were found to share different chemical classes of volatile organic components, which have a variety of pharmacological applications. However, 8 antibacterial compounds with different concentrations were commonly found in all five species: acetoin, acetic acid, butanoic acid, 2-methyl-, oxime-, methoxy-phenyl, phenol, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, nonanoic acid, and hexadecanoic acid, methyl. **The biology and physiology of *Bacillus* can be better understood using these results, which can also be used to create novel biotechnological procedures and applications. Moreover,** because of its exceptional ability to synthesize and produce a variety of different

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antibacterial compounds, *Bacillus* species can serve as natural and universal carriers for antibiotic compounds in the form of probiotic cultures and strains to fight different pathogens, including mycobacteria.

Keywords: *antimicrobial activity; Bacillus subtilis; Bacillus thuringiensis*; *Bacillus toyonensis; Bacillus acidiproducens; Bacillus cereus; Bacillus safensis*; GC–MC analysis, volatile organic compounds

1. Introduction

The emergence of bacterial strains that previously were susceptible to existing antibiotics but now cause serious infectious diseases makes it necessary to find and create novel treatments for these illnesses [1]. The most well-known and clinically significant example of this issue is the rise in multidrug-resistant strains of *Staphylococcus aureus* (MRSA), which is most pathogenic and leads to the formation of an abscess. Moreover, it can cause pneumonia, endocarditis, and osteomyelitis. According to some investigations, MRSA is resistant not only to some antibiotics such as methicillin, macrolides, tetracycline, aminoglycosides, and chloramphenicol but also to some disinfectants [2].

In order to create new antibiotic treatments or disinfectants, it is also necessary to look for and analyze compounds that have bactericidal or bacteriostatic capabilities against human and animal infections. Currently, analysis of the potential of natural compounds from various sources as antimicrobials has received significant attention in addition to the synthesis of new chemical substances. The observation of antagonism, or the interaction between microorganisms, is frequently the starting point for the development of antibiotics with activity against human infections. The creation and release of chemicals that impede or entirely stop the growth of other species serve as the physical manifestation of this hostility. Under natural circumstances, an agent released by a microbe that prevents the growth of another organism has an edge in the competition for environmental resources. The majority of antibiotics used in medicine are secreted by or derived from bacteria. Hence, it is a fact that the bacterial world has a vast repository of potentially antimicrobial chemicals that have not yet been identified or exploited. In this respect, members of the genus *Bacillus* are recognized as manufacturers of a wide variety of enzymes and antibacterial substances. For example, 23 peptide antibiotics are produced by *Bacillus brevis*, while *Bacillus subtilis* produces roughly 70 antimicrobials which are ribosomal peptides, non-ribosomal peptides, polyketides, hybrids, and volatile compounds [3,4]. Consequently, there is increased interest in taking these compounds into consideration as disjunctive antimicrobials for the healing of human infections [5–9].

Nowadays, a novel strategy for the management and prevention of numerous infectious illnesses is the use of bacterial probiotic strains and their metabolic products. Probiotics from the *Bacillus* genus have been shown to exhibit antibacterial effects in experiments on animals. As an affordable and infrequently resistant alternative to antibiotics, the application of bacteriocins and antimicrobial peptides synthesized by probiotic strains is recommended [13,14]. They hold promise for clinical usage since many of these molecules are efficient and affordable [15]. Due to their desirable medicinal qualities, such as their antibacterial, antiviral, anticancer, and contraceptive effects, a few natural peptides from bacterial isolates have demonstrated potential. Furthermore, when combined with traditional antibiotics, they have been demonstrated to offer protection against systemic and topical infections. Therefore, the justification for using probiotics in medicine is founded on the notion that giving oral or topically applied probiotics could restore the depleted state of the human microbiome [16].

Development, registration, and commercialization of biocontrol drugs based on microbial antagonists have advanced significantly during the past few decades. Although their use has side effects on both human and animal health. *Bacillus* species compete directly with fungal pathogens for resources and habitats and through a variety of processes such as the generation of siderophores. Hence, they also indirectly create systemic resistance or stimulate the growth of plants [17,18]. Moreover, they produce a vast array of volatile organic compounds with strong inhibitory potential against plant pathogens: alcohols, alkenes, benzenoids, terpenoids, ketones, sulfur-containing compounds, and others [19,20]. Non-volatile components of these metabolites have gotten a lot of scientific interest, whereas volatile components are examined less frequently. Numerous applications in biology, environmental sciences, health, the food industry, and national security include the study

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and detection of volatile organic compounds (VOCs) that come from or interact with creatures ranging from bacteria to people. Low-molecular-weight organic molecules with a lipophilic nature and a low boiling point are known as volatile organic compounds (VOCs) [21]. According to several studies, VOCs released by bacteria may help plants by fostering development, triggering defence mechanisms, and inhibiting or removing dangerous infections [22–26]. Furthermore, VOCs released by microorganisms are biodegradable as they are naturally occurring compounds. As a result, using VOCs produced by microorganisms is a sustainable method of crop protection and promotion. According to some recent studies, because they can stop certain pathogenic fungi's mycelial growth and spore germination, VOCs produced by *B. subtilis* have been suggested as an alternate control approach for postharvest fruit illnesses [27]. For instance, different VOCs produced by *B. subtilis* TB09 and TB72, such as nonan-2-one, β-benzeneethanamine, and 2-methyl-1,4-diazine effectively controlled the anthracnose pathogen on postharvest mangoes [28]. Likewise, some VOCs synthesized by *B. subtilis* PPCB001 helped to evaluate its antagonistic activity and it was found that *B. subtilis* PPCB001 reduces growth one of the imperfect fungi which is called *Penicillium crustosum* [29]. Additionally, the GC–MS analysis of three bacterial isolates of *Bacillus subtilis* Md1-42, *Bacillus subtilis* O-3, and *Bacillus subtilis* Khozestan2 samples proved the presence of phenol, benzoic acid, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl), methoxyphenyl-oxime, and benzaldehyde, which are known for their antimicrobial and other properties [30].

Overall, representatives of the genus *Bacillus* are found as producers of a wide range of antimicrobial compounds. The synergetic mechanism of antimicrobial compounds such as VOCs, polyketides, ribosomal peptides, and others explains why they have an increased industrial interest as therapeutic agents, food preservatives and biopesticides. Since *Bacillus* species have the unique ability to produce a variety of diverse antibacterial chemicals, they can serve as a natural carrier for antibiotics in the form of probiotic cultures and strains to fight different pathogens, including mycobacteria. Nevertheless, the dangerous effects of several antibacterials on humans and animals have prevented some of them from being used medically despite their promising in vitro antimycobacterial activity [31,32].

Another instance of a *Bacillus* species' employment is in the manufacture of food-grade amylase, glucoamylase, protease, pectinase, and cellulase in a variety of foods [33–36]. Additionally, many species of *Bacillus* have been employed to synthesize a number of dietary supplements for human use, including vitamins (such as riboflavin, cobalamin, and inositol) and carotenoids [36–39]. However, despite the foregoing advantages, these strains have not attracted much interest in the contemporary functional food market because of their relationships with a limited number of human diseases.

This study focused on isolating potential *Bacillus* species from vegetable samples (potato, carrot, and tomato) and preparing a crude extract from isolated bacterial strains to assess antimicrobial activities against the most common human pathogens. In addition, bacterial isolates were tested for antibiotic resistance using an inhibition zone diameter when determined via the disk diffusion method. GC–MC analysis was performed to determine bioactive compounds from the bacterial isolates. This study will facilitate the development of novel antibiotics against MDR bacterial strains and help to explore possible probiotics typical for the representatives of the genus *Bacillus*.

2. Results

2.1. Isolation and Identification

A total of n = 25 bacteria strains were isolated and identified using colony morphology, microscopy, biochemical properties, and sugar fermentation. Among all, Gram-strain-positive, rod-shaped, mycelial, and spore-forming bacterial strains were chosen for further verifying tests. The molecular analysis further validated the bacterial strains (BSS25, BSS21, BSS16, BSS13, and BSS12) as *Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08.

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2.2. Microbial Morphology and Colony Characteristics

The morphology of each colony by different bacterial isolates showed regular, irregular, slightly raised, flat, white, and cream-coloured colonies. By motility test, bacterial isolates were motile and possessed terminal and subterminal spores (Table 1).

Table 1. Colony morphology and microscopic presentation of isolated bacterial species.

Bacterial Species	Media	Colony Color and Texture	Microscopic Presentation
Bacillus thuringiensis F3 (BSS25)	*Bacillus* Medium.	White, irregular, flat.	Gram-strain-positive, spore-forming, rod.
Bacillus toyonensis FORT 102 (BSS21)	*Bacillus* Medium.	White, irregular, flat.	Gram-strain-positive, spore-forming, rod.
Bacillus acidiproducens NiuFun (BSS16)	*Bacillus* Medium.	White, irregular, flat.	Gram-strain-positive, spore-forming, rod.
Bacillus cereus WAB2133 (BSS13)	*Bacillus* Medium.	White, irregular, flat.	Gram-strain-positive, spore-forming, rod.
Bacillus safensis AS-08 (BSS12)	*Bacillus* Medium.	White, irregular, flat.	Gram-strain-positive, spore-forming, rod.

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2.3. Antimicrobial Potency Evaluation

Five bacterial cultures of BSS25, BSS21, BSS16, BSS13 and BSS12 were tested for their antagonistic activity against 15 pathogens such as *Salmonella enterica* ATCC 35664, *Klebsiella aerogenes* ATCC 13048, *Serratia marcescens* ATCC 13880, *Klebsiella pneumoniae* ATCC 13883, *Streptococcus group B, Escherichia coli* ATCC 25922, *Candida krusei* ATCC 14243, *Shigella sonnei* ATCC 25931, *Streptococcus mutans* ATCC 25175, *Enterococcus hirae* ATCC 10541, *Proteus Vulgaris* ATCC 6380, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 2091 (Figure 1).

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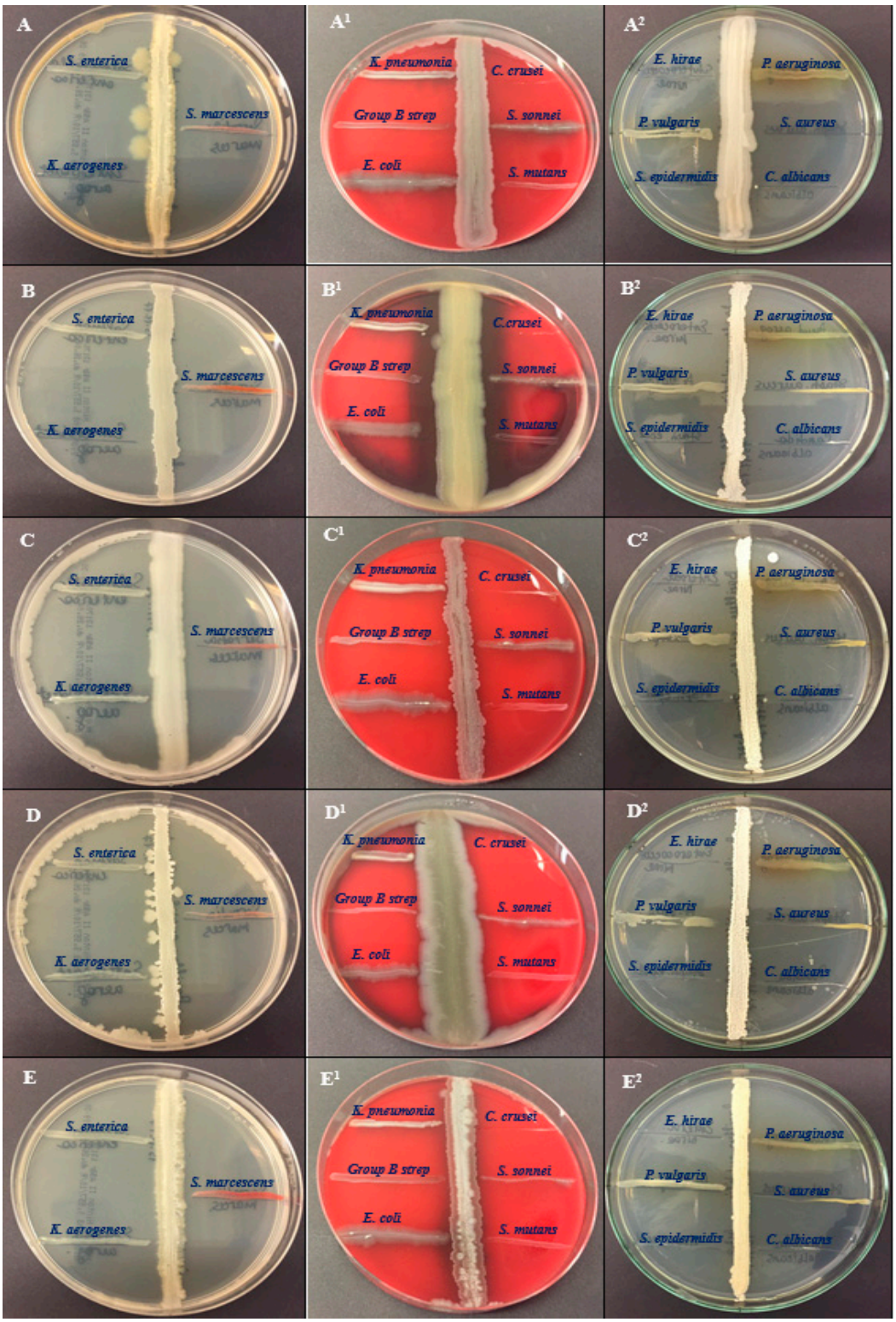

Figure 1. Antagonistic activity of the bacteria of the genus *Bacillus* against pathogens. The antagonistic efficacy of all five isolates was examined against pathogenic bacteria, such as *Salmonella enterica* ATCC 35664, *Serratia marcescens* ATCC 13880, *Klebsiella aerogenes* ATCC 13048, *Candida krusei* ATCC 14243, *Shigella sonnei* ATCC 25931, *Streptococcus mutans* ATCC 25175, *Klebseiella pneumoniae* ATCC 13883, *Group B Streptococcus*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 2091, *Enterococcus hirae* ATCC 10541, *Proteus vulgaris* ATCC 6380, and *Staphylococcus epidermidis* ATCC 12228. (**A**–**A2**)—BSS25, (**B**–**B2**)—BSS21, (**C**–**C2**)—BSS16, (**D**–**D2**)—BSS13, and (**E**–**E2**)—BSS12.

The five extracts showed antibacterial activity against all the bacterial pathogens except *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus hirae* ATCC 10541 (Table 2). The strain of

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Bacillus thuringiensis F3 (BSS25), showed a better zone of inhibition for *Staphylococcus aureus* ATCC 29213 (35±1,27 mm),*Staphylococcus epidermidis* ATCC 12228 (37±1,47 mm),*Candida albicans* ATCC 2091 (36±1,43 mm),*Candida krusei* ATCC 14243 (37±1,41 mm), *Klebsiella aerogenes* ATCC 13048 (37±1,27 mm), and *Enterococcus hirae* ATCC 10541 (37±1,25 mm). Additionally, the strain of *Bacillus toyonensis FORT 102* (BSS21) was effective against the pathogens *Staphylococcus epidermidis* ATCC 12228 (36±1,27), *Candida albicans* ATCC 2091 (38±1,21 mm), *Candida krusei* ATCC 14243 (36±1,28 mm), *Klebsiella aerogenes* ATCC 13048 (36±1,37 mm), and *Enterococcus hirae* ATCC 10541 (38±1,27 mm).

Table 2. Antibacterial activity of the bacterial culture extracts against pathogenic strains.

Species of Microorganism	BSS25	BSS21	BSS16	BSS13	BSS12	Control (Streptomycin)
Staphylococcus aureus ATCC 29213	35±1,27	9±1,53*	23±0,50*	20±2,50*	20±1,54	24±0,33***
Staphylococcus epidermidis ATCC 12228	37±1,47	36±1,27	38±1,27	37±1,07	35±1,47	22±0.33***
Streptococcus group B	18±1,56*	19±1,31*	19±1,23*	18±1,56*	17±1,39*	17±0.33***
Streptococcus mutans ATCC 25175	20±1.47*	19±1,27*	23±1,33	20±1,33	20±1,37*	19±0.33***
Candida albicans ATCC 2091	36±1,43	38±1,21	38±1,27	35±1,26	34±1,22	31±0.33***
Candida krusei ATCC 14243	37±1,41	36±1,28	8±1,38*	36±1,27	13±1,27*	30±0.33***
Pseudomonas aeruginosa ATCC 9027	18±0,53*	17±1,27*	17±0,33*	17±1,10*	16±1,33*	15±0.33***
Shigella sonnei ATCC 25931	20±1,27*	33±1,37*	21±1,57*	21±1,37*	21±1,06*	19±0.33***
Klebsiella pneumonia ATCC 13883	9±1,53*	9±1,27*	8±1,27*	8±1,37*	8±1,37*	12±0.33***
Salmonella enterica ATCC 35664	17±1,36*	15±1,25*	18±1,27*	15±1,27*	17±1,27*	19±0.33***
Klebsiella aerogenes ATCC 13048	37±1,27	36±1,37	32±1,33*	35±0,63*	33±1,33*	23±0.33***
Enterococcus hirae ATCC 10541	37±1,25	38±1,27	38±1,27	39±1,27	38±1,27	22±0.33***
Escherichia coli ATCC 25922	17±1,37*	16±1,07*	19±1,44*	18±1,33*	20±1,08*	16±0.33***
Serratia marcescens ATCC 13880	27±0,56*	29±1,36*	25±1,32*	28±0,53*	27±1,53*	22±0.33***
Proteus Vulgaris ATCC 6380	20±0,31*	19±1,31*	21±1,33*	20±1,23*	21±0,33*	22±0.33***

* Data are represented as the means ± SE (n = 3). Values with the same superscript symbols are not statistically different. Significance level * < ***.

2.4. Antibiotic Susceptibility Profile of the Isolates

With the exception of bacitromycin (B, 10), polymyxin (PB, 300), and cloxacillin (CX, 5), none of the five *Bacillus* species examined were resistant to any antibiotics, according to the analysis of the antibiogram (Table 3). All five strains, which are BSS25, BSS21, BSS16, BSS13 and BSS13 strains showed the highest vulnerability to gentamicin (CN, 120) with 39 ± 0,37 mm, 38 ± 0,43 mm, 40± 0,12 mm, 41 ± 0,23 and 39 ± 0,31 sensitivity diameters, respectively

Table 3. Colony morphology and microscopic presentation of isolated bacterial species.

Antibiotic (AB, charge in µg) used	*Bacillus* strains									
	BSS25		BSS21		BSS16		BSS13		BSS12	
	Diameter (mm)	S/R	Diameter (mm)	S/R	Diameter (mm)	S/R	Diameter (mm)	S/R	Diameter (mm)	S/R
Penicillins:										
Penicillin G (PEN, 10)	$37\pm0,19^{a}$	S	$20\pm0,35^{ab}$	S	$34\pm0,48^{abc}$	S	$22\pm1,43^{a}$	S	$22\pm1,43^{a}$	S
Ampicillin (AMP, 10)	$30\pm0,21^{ab}$	S	$20\pm0,35^{ab}$	S	$36\pm0,36^{ab}$	S	$32\pm0,98^{ab}$	S	$32\pm0,98^{ab}$	S
Amoxycillin (AMOX, 30)	$40\pm0,21^{a}$	S	$20\pm0,35^{ab}$	S	$38\pm0,41^{abc}$	S	$35\pm1,81^{abc}$	S	$35\pm1,81^{abc}$	S
Amoxycillin-clavulanic acid (AMC, 30)	$35\pm0,98^{c}$	S	$20\pm0,35^{abc}$	S	$36\pm0,98^{abc}$	S	$30\pm1,45^{ab}$	S	$30\pm1,45^{b}$	S
Carbenicillin (CAR, 100)	$35\pm0,32^{abs}$	S	$20\pm0,35^{ab}$	S	$34\pm0,56^{abc}$	S	$35\pm1,43^{abc}$	S	$35\pm1,43^{abc}$	S
Cloxacillin (CX, 5)	$14\pm0,23^{a}$	R	$14\pm0,23^{a}$	R	$14\pm0,23^{ab}$	R	$14\pm0,23^{abc}$	R	$14\pm0,23^{a}$	R
Macrolides:										
Erythromycin (ERO, 15)	$30\pm0,21^{a}$	S	$20\pm0,11^{abc}$	S	$40\pm0,39^{ab}$	S	$40\pm0,28^{a}$	S	$30\pm0,21^{a}$	S

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Azithromycin (AZM, 15)	30 ± 0,22^{a}	S	20 ± 0,35^{a}	S	35 ± 0,59^{ab}	S	37 ± 1,52^{a}	S	30 ± 0,22^{a}	S
Cephalosporins:										
Cefepime (FEP, 30)	30 ± 0,35^{bcd}	S	30 ± 0,35^{abc}	S	30 ± 0,36^{ab}	S	20 ± 1,43^{ab}	S	20 ± 1,43^{ab}	S
Cefepime/clavulanic acid FEC-40	30 ± 0,35^{a}	S	30 ± 0,35^{ab}	S	33 ± 0,28^{a}	S	26 ± 0,23^{a}	S	26 ± 0,23^{a}	S
Cephalatin (KF, 30)	34 ± 0,21^{ab}	S	30 ± 0,35^{a}	S	30 ± 0,54^{a}	S	25 ± 0,98^{ab}	S	25 ± 0,98^{abs}	S
Cefotaxime (CTX, 30)	27 ± 0,35^{a}	S	28 ± 0,11^{a}	S	25 ± 0,28^{a}	S	35 ± 1,29^{ab}	S	35 ± 1,29^{a}	S
Aminoglycosides:										
Gentamicin (CN, 120)	39 ± 0,37^{ab}	S	38 ± 0,43^{ab}	S	40± 0,12^{ab}	S	41 ± 0,23^{ab}	S	39 ± 0,31^{ab}	S
Streptomycin (STR, 10)	23 ± 0,36^{ab}	S	25 ± 0,31^{ab}	S	28 ± 1,41^{ab}	S	23 ± 1,41^{ab}	S	25 ± 0,31^{ab}	S
Tobramycin (TOB, 10)	32 ± 0,32^{a}	S	25 ± 0,31^{a}	S	34± 1,18^{a}	S	35 ± 0,98^{ab}	S	35 ± 1,29^{a}	S
Tetracyclines:										
Tetracycline (TET, 30)	30 ± 0,52^{a}	S	26 ± 0,15^{a}	S	36 ± 1,43^{a}	S	30 ± 0,23^{a}	S	30 ± 0,52^{ab}	S
Polypeptides:										
Polymyxin (PB, 300)	7 ± 0,28^{b}	R	0 ± 0,00^{b}	R	8 ± 1,49^{b}	R	10± 0,23^{b}	R	0 ± 0,00^{b}	R
Bacitromycin (B, 10)	0 ± 0,00^{b}	R	0 ± 0,00^{b}	R	0 ± 0,00^{b}	R	0 ± 0,00^{b}	R	0 ± 0,00^{b}	R

* The Newman–Keuls test was used to compare means in a data set, and the results suggest that there are statistically significant differences among some of the groups. Here, "±" is a standard error; legend: D and S/R are dimension and sensible/resistant, respectively.

2.5. GC–MS Analysis

According to the results of the GC–MS analysis, crude extracts from different *Bacillus* bacterium species contained a variety of compounds. Tables 4–8 explain the most important and plentiful components identified in the crude extracts that were subjected to the GC–MS analysis, as well as information about where the chemicals found in this study had previously been identified. These substances exhibited similarities to natural products of a variety of organisms, such as bacterial, plant and fungi origin**.** In a study, the majority of the compounds detected were derived from volatile substances. Different *Bacillus* species have been found to produce volatile compounds belonging to various classes, such as alcohols, ketones, fatty acids, and aromatic compounds, in addition to esters and ethers. *In strain BSS25, ethyl acetate extraction showed the presence of 33 compounds (Table 4) compared to 37 compounds arising out of the same extraction strain BSS21 (Table 5). In the* ethyl acetate extract of the BSS16 bacterial strain were identified 23 compounds (Table 6). Acetoin, benzaldehyde, 3(2H)-thiophenone, dihydro-2-methyl-, propanoic acid, 2-methyl-, and oleic acid were identified in the BSS16 extract with important concentrations of 8.440992%, 4.751143%, 6.072668%, 13.97511%, and 9.677555%, respectively. In the BSS25 bacterial isolate, the major compounds were butanoic acid, 2-methyl- at 29.39467% and 9,12-octadecadienoic acid (Z, Z)- at 11.09688% and other three compounds such as 3(2H)-thiophenone, dihydro-2-methyl-, benzoic acid, tridecyl ester and pentadecanoic acid were identified only in this bacterial extract. In bacterial strain BSS13, some compounds such as acetone, acetic acid, benzaldehyde, hexadecanoic acid, octadecanoic acid, 2-hydroxy-1,3-*propanediyl ester, 9-Octadecenoic acid, (E)-, and 9,12-Octadecadienoic acid (Z,Z)- were found with high concentrations 3.660169%, 6.313543%, 6.242524%, 4.45127%, 3.793011%, 9.952046%, and 5.86713%, respectively (Table 7). The solvent with metabolites for isolate BSS12 was ethyl acetate, which also contained 38 chemicals (Table 8), whereas the same solvent with metabolites for isolate BSS13 was found to contain 33 compounds (Table 7). GC–MS analysis for five bacterial (BSS25, BSS21, BSS16, BSS13 and BSS12) analyses also confirmed the presence of the same volatile organic compounds, while some components were found only in some bacterial isolates.*

Table 4. The main constituents of bacterial extract BSS25 were identified through a GC–MS analysis.

***Bacillus thuringiensis* F3 (BSS25)**							
No.	**Name**	**Molecular formula**	**Molecular Mass, g/mol**	**Retention Time (min)**	**PubChem Compound CID**	**Similarities**	**Area, %**
1	Acetone	C_3H_6O	58.08	1.642	180	87	0.170794
2	2,3-Butanedione	C_4H_6O	86.09	2.64	650	93	15.84782
3	Hexanal	$C_6H_{12}O$	100.16	3.617	6184	65	0.343629
4	Acetoin	$C_4H_8O_2$	88.111	6.239	179	78	44.063
5	3-Pentanol, 2-methyl-	$C_6H_{14}O$	102.17	7.012	11264	80	1.373128

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6	Oxirane, (methoxymethyl)-	$C_4H_8O_2$	88.11	7.219	13589	79	1.360574
7	Acetic acid	$C_2H_4O_2$	60.05	8.368	176	86	0.887442
8	Decanal	$C_{10}H_{20}O$	156.26	8.504	8175	73	0.3926
9	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130,229	8.896	7720	87	0.244481
10	Benzaldehyde	C_7H_6O	106.12	9.411	240	94	2.222227
11	2,3-Butanediol, [S-(R*,R*)]-	$C_4H_{10}O$	90.12	9.491	439888	82	4.077882
12	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154.25	9.64	6549	85	0.781074
13	1-Hepten-4-ol	$C_7H_{14}O$	114.19	9.847	19040	70	3.418443
14	1-Nonanol	$C_9H_{20}O$	144.25	10.47	8914	78	0.731353
15	(S)-(+)-6-Methyl-1-octanol	$C_9H_{20}O$	144.25	10.639	13548104	85	1.248967
16	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.091	8314	81	2.237883
17	Oxime-, methoxy-phenyl	$C_8H_9NO_2$	151.16	12.063	9602988	73	1.136385
18	1-Decanol	$C_{10}H_{22}O$	158.28	12.21	8174	60	0.516912
19	Hexanoic acid	$C_6H_{12}O_2$	116.16	13.085	8892	91	1.862169
20	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	$C_{13}H_{22}O$	194.31	13.329	1549778	60	0.661766
21	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O$	216.32	13.462	551387	65	0.99794
22	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	286.41	13.63	23284	81	0.906841
23	(R)-(-)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.965	12600623	70	0.615997
24	Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$	144.21	14.226	8697	89	2.90418
25	Cetene	$C_{16}H_{32}$	224.42	14.477	12395	81	0.750762
26	Phenol	C_6H_6O	94.11	14.825	996	89	0.81691
27	Neodecanoic acid	$C_{10}H_{20}O_2$	172.26	15.305	62838	61	0.428921
28	Octanoic acid	$C_8H_{16}O_2$	144.21	15.386	379	91	2.465807
29	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	16.151	6782	77	0.437683
30	Nonanoic acid	$C_9H_{18}O_2$	158.24	16.478	8158	90	2.797785
31	Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$	234.33	17.083	94310	61	0.172171

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32	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	17.161	8181	91	1.221765
33	2-Octyl benzoate	$C_{15}H_{22}O_2$	234.33	17.531	243800	69	0.908645

Table 5. The main constituents of bacterial extract BSS21 were identified through a GC–MS analysis.

Bacillus toyonensis FORT 102 (BSS21)							
No.	**Name**	**Molecular formula**	**Molecular Mass, g/mol**	**Retention Time (min)**	**PubChem Compound CID**	**Similarities**	**Area, %**
1	Acetone	C_3H_6O	58.08	1.661	180	79	0,551252
2	2,3-Butanedione	$C_4H_6O_2$	86.09	2.652	650	92	16,97044
3	2,3-Pentanedione	$C_5H_8O_2$	100.12	3.404	11747	68	2,035411
4	Acetoin	$C_4H_8O_2$	88.11	6.246	179	84	38.25336
5	3-Pentanol, 2-methyl-	$C_6H_{14}O$	102.17	7.008	11264	81	2.916132
6	Oxirane, (methoxymethyl)-	$C_4H_8O_2$	88.11	7.216	13589	80	2.409396
7	Nonanal	$C_9H_{18}O$	142.24	7.73	31289	85	0.746341
8	Acetic acid	$C_2H_4O_2$	60.05	8.343	176	97	2.456759
9	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.229	8.889	7720	88	0.154366
10	E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	226.4	9.048	5363322	65	0.135906
11	2,3-Butanediol, [S-(R*,R*)]-	$C_4H_{10}O_2$	90.12	9.486	439888	88	7.796162
12	Formic acid, octyl ester	$C_9H_{18}O_2$	158.24	9.751	8176	69	0.371511
13	Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.832	6590	76	1.406486
14	2-Octanol	$C_8H_{18}O$	130.229	9.928	20083	74	0.263263
15	(S)-(+)-6-Methyl-1-octanol	$C_9H_{20}O$	144.25	10.631	13548104	82	0.282974
16	1-Nonanol	$C_9H_{20}O$	144.25	11.003	8914	78	0.129921
17	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.073	8314	87	3.572379
18	Dodecanal	$C_{12}H_{24}O$	184.32	11.714	8194	92	0.683743
19	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	151.16	12.052	9602988	73	0.348646
20	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152.23	12.851	5283349	77	0.282056
21	Pentanoic acid	$C_5H_{10}O_2$	102.13	13.071	7991	79	0.233567
22	3-Buten-2-one, 4-(1-cyclopenten-1-yl)-, (E)-	$C_9H_{12}O$	136.19	13.461	5370075	76	0.401939
23	Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$	144.21	14.199	8697	80	0.315248
24	1-Dodecanol	$C_{12}H_{26}O$	186.33	14.446	8193	81	0.150351
25	Phenol	C_6H_6O	94.11	14.774	996	90	0.191971
26	Octanoic acid	$C_8H_{16}O_2$	144.21	15.314	379	79	0.398877
27	Nonanoic acid	$C_9H_{18}O_2$	158.24	16.359	8158	88	0.730917
28	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	17.01	8181	88	0.315637
29	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222.32	17.298	75550	63	0.17709
30	Decanoic acid	$C_{10}H_{20}O_2$	172.26	17.356	2969	64	0.307782
31	Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	18.369	81591	73	0.161383
32	Benzoic acid	$C_7H_6O_2$	122.12	18.739	243	85	0.221304

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33	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	19.85	6782	81	0.333842
34	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	21.099	3026	67	0.325750
35	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.611	985	76	4.002248
36	Oleic Acid	$C_{18}H_{34}O_2$	282.5	24.54	445639	86	5.853397
37	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	25.063	5280450	87	3.796889

Table 6. The main constituents of bacterial extract BSS16 were identified through a GC–MS analysis.

Bacillus acidiproducens NiuFun (BSS16)							
No.	**Name**	**Molecular formula**	**Molecular Mass, g/mol**	**Retention Time (min)**	**PubChem Compound CID**	**Similarities**	**Area, %**
1	Acetone	C_3H_6O	58.08	1.67	180	93	0.745862
2	Acetoin	$C_4H_8O_2$	88.11	6.49	179	81	8.440992
3	Acetic acid	$C_2H_4O_2$	60.05	8.363	176	95	2.398269
4	Benzaldehyde	C_7H_6O	106.12	9.411	240	94	4.751143
5	3(2H)-Thiophenone, dihydro-2-methyl-	C_5H_8OS	116.18	9.463	61664	84	6.072668
6	Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.839	6590	92	13.97511
7	Butanoic acid	$C_4H_8O_2$	88.11	10.581	264	83	0.612913
8	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.084	8314	83	29.39467
9	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	151.16	12.063	9602988	76	1.137091
10	Phenol	C_6H_6O	94.11	14.788	996	90	0.318667
11	Nonanoic acid	$C_9H_{18}O_2$	158.24	16.372	8158	85	0.544397
12	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	17.016	8181	91	0.720873
13	2-Octyl benzoate	$C_{15}H_{22}O_2$	234.33	17.363	243800	68	0.683898
14	Benzoic acid 2-methylpentyl ester	$C_{13}H_{18}O_2$	206.28	17.813	570433	66	0.529643
15	Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	18.084	81591	80	0.501309
16	Benzoic acid, tridecyl ester	$C_{20}H_{32}O_2$	304.5	18.375	9814973	75	0.560423
17	Benzoic acid	$C_7H_6O_2$	122.12	18.752	243	79	0.652983
18	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	19.859	6782	91	0.940027
19	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	21.427	13849	63	0.410235
20	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.604	985	88	3.605440
21	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	24.256	5281	72	1.638403
22	Oleic acid	$C_{18}H_{34}O_2$	282.5	24.542	445639	90	9.677555
23	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	25.067	5280450	91	11.09688

Table 7. The main constituents of bacterial extract BSS13 were identified through a GC–MS analysis.

Bacillus cereus WAB2133 (BSS13)

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No.	Name	Molecular formula	Molecular Mass, g/mol	Retention Time (min)	PubChem Compound CID	Similarities	Area, %
1	Acetone	C_3H_6O	58.08	1.644	180	94	3.660169
2	Acetoin	$C_4H_8O_2$	88.11	6.49	179	81	0.745862
3	Acetic acid	$C_2H_4O_2$	60.05	8.335	176	97	6.313543
4	Decanal	$C_{10}H_{20}O$	156.26	9.114	8175	70	0.631996
5	Benzaldehyde	C_7H_6O	106.12	9.402	240	95	6.242524
6	Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.828	6590	92	11,51283
7	Butanoic acid	$C_4H_8O_2$	88.11	10.569	264	86	0.911113
8	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.081	8314	83	31.6913
9	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	151.16	12.054	9602988	67	1.284529
10	Tiglic acid	$C_5H_8O_2$	100.12	13.078	125468	81	1.676206
11	(R)-(-)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.945	12600623	84	0.532951
12	Hexanoic acid, 2-ethyl-	$C_8H_{16}O_2$	144.21	14.197	8697	88	1.032308
13	Phenol	C_6H_6O	94.11	14.777	996	87	0.439273
14	Octanoic acid	$C_8H_{16}O_2$	144.21	15.317	379	90	1.111877
15	Nonanoic acid	$C_9H_{18}O_2$	158.24	16.362	8158	90	2.401952
16	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	17.016	8181	80	1.091995
17	Decanoic acid	$C_{10}H_{20}O_2$	172.26	17.359	2969	66	0.942792
18	Benzoic acid 2-methylpentyl ester	$C_{13}H_{18}O_2$	206.28	17.809	570433	64	0.556621
19	Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	18.079	81591	78	0.427025
20	Benzoic acid	$C_7H_6O_2$	122.12	18.742	243	87	0.568841
21	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	19.852	6782	93	1.113468
22	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	21.116	3026	69	0.936291
23	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.596	985	84	4.45127
24	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	625	24.244	101269	67	3.793011
25	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5	24.53	637517	86	9.952046
26	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	25.052	5280450	83	5.86713

Table 8. The main constituents of bacterial extract BSS12 were identified through a GC–MS analysis.

Bacillus safensis AS-08 (BSS12)							
No.	Name	Molecular formula	Molecular Mass, g/mol	Retention Time (min)	PubChem Compound CID	Similarities	Area, %
1	(2-Aziridinylethyl)amine	$C_4H_{10}N_2$	86.14	1.162	97697	96	0.389922
2	1-Propen-2-ol, acetate	$C_5H_8O_2$	100.12	1.667	7916	65	0.784972
3	2,3-Butanedione	$C_4H_6O_2$	86.09	2.647	650	93	21.42702
4	3-Penten-1-ol	$C_5H_{10}O$	86.13	3.411	510370	69	2.637362

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5	Acetoin	$C_4H_8O_2$	88.11	5.703	179	72	0.263788
6	3-Pentanol, 2-methyl-	$C_6H_{14}O$	102.17	6.259	11264	72	36.53327
7	2-Nonen-1-ol	$C_9H_{18}O$	142.24	7.011	61896	82	1.230591
8	2-Hydroxy-3-pentanone	$C_5H_{10}O_2$	102.13	7.109	521790	73	0.422042
9	Ethane-1,1-diol dibutanoate	$C_{10}H_{18}O_4$	202.25	7.215	551339	83	1.183593
10	Acetic acid	$C_2H_4O_2$	60.05	8.354	176	90	0.783519
11	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	130.229	8.888	7720	93	0.440399
12	Benzaldehyde	C_7H_6O	106.12	9.397	240	96	2.170694
13	2,3-Butanediol	$C_4H_{10}O_2$	90.12	9.484	262	89	4.672893
14	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154.25	9.632	6549	87	0.782704
15	Propanoic acid, 2-methyl-	$C_4H_8O_2$	88.11	9.832	6590	67	3.156132
16	2,3-Butanediol, [R-(R*,R*)]-	$C_4H_{10}O_2$	90.12	9.925	225936	74	0.530592
17	1-Nonanol	$C_9H_{20}O$	144.25	10.366	8914	82	0.418021
18	(S)-(+)-6-Methyl-1-octanol	$C_9H_{20}O$	144.25	10.635	13548104	89	0.984823
19	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102.13	11.082	8314	82	1.894643
20	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	12.053	9602988	67	0.518031
21	2,4-Decadienal	$C_{10}H_{16}O$	152.23	12.853	5283349	79	0.322369
22	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	286.41	13.131	23284	82	0.168580
23	(R)-(-)-4-Methylhexanoic acid	$C_7H_{14}O_2$	130.18	13.315	12600623	62	0.133451
24	Phenol	C_6H_6O	94.11	13.711	996	75	0.205061
25	Octanoic acid	$C_8H_{16}O_2$	144.21	14.194	379	76	0.185012
26	Nonanoic acid	$C_9H_{18}O_2$	158.24	14.587	8158	76	0.138322
27	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	14.766	8181	96	2.885995
28	2-Octyl benzoate	$C_{15}H_{22}O_2$	234.33	15.31	243800	70	0.17637
29	Benzoic acid, heptyl ester	$C_{14}H_{20}O_2$	220.31	15.742	81591	75	0.10189
30	Benzoic acid, undecyl ester	$C_{18}H_{28}O_2$	276.4	16.355	229159	88	0.402725
31	Benzoic acid	$C_7H_6O_2$	122.12	17.008	243	89	0.281133
32	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	18.074	6782	69	0.097665
33	Oleic Acid	$C_{18}H_{34}O_2$	282.5	18.364	445639	76	0.140625
34	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	19.845	3026	89	0.325177
35	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	22.582	985	82	2.377302
36	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	24.235	5281	69	2.671248
37	Oleic Acid	$C_{18}H_{34}O_2$	282.5	24.517	445639	87	4.585822

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38	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	25.039	5280450	83	3.576243

The GC–MS based metabolite profiling of the ethyl acetate extracts of BSS25, BSS21, BSS16, BSS13, and BSS12 bacterial isolates revealed a total of 69 volatile organic substances. Based on the analysis of bacterial isolates, all five isolates were found to share a similar composition of volatile organic components, such as acetoin, acetic acid, butanoic acid, 2-methyl-, oxime-, methoxy-phenyl, phenol, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, nonanoic acid, and hexadecanoic acid, methyl ester. Their chemical structure details are available, as illustrated in Figure 2.

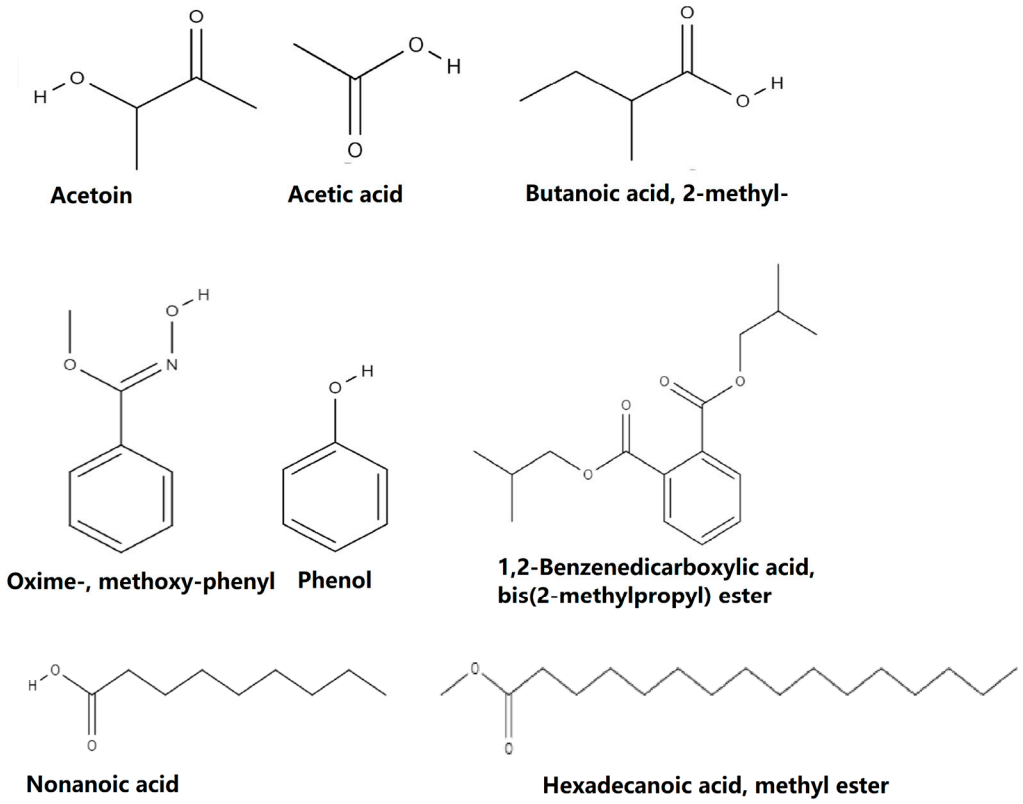

Figure 2. Structure of common components identified from *Bacillus* spp. isolates.

Table 9. List of various classes of compounds identified from five *Bacillus* spp. and their pharmacological activities.

No.	Name	Chemical classes	Known pharmacological activities
1	3-Pentanol, 2-methyl-	alcohols	–
2	2,3-Butanediol, [S-(R*,R*)]-	alcohols	–
3	1,6-Octadien-3-ol, 3,7-dimethyl-	monoterpene alcohols	anti-inflammatory, anticancer, anti-hyperlipidemic, antimicrobial, antinociceptive, analgesic, anxiolytic, anti-depressive and neuroprotective [40]
4	1-Hepten-4-ol	alcohols	–
5	1-Nonanol	alcohols	antifungal [41] and antibacterial[42]
6	(S)-(+)-6-Methyl-1-octanol	alcohols	–

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7	1-Decanol	alcohols	antibacterial[42], antioxidant and neuroprotective [43]
8	E-3-Pentadecen-2-ol	alcohols	–
9	2-Octanol	alcohols	–
10	3-Penten-1-ol	alcohols	–
11	2-Nonen-1-ol	alcohols	–
12	2,3-Butanediol	alcohols	CNS depressant [44], antimicrobial and antagonistic [45]
13	1-Hexanol, 2-ethyl-	alcohols	–
14	1-Dodecanol	alcohols	antibacterial [42]
15	Hexanal	aldehydes	antimicrobial [46]
16	Nonanal	aldehydes	anti-fungal [47]
17	Decanal	aldehydes	anti-fungal [48]
18	Dodecanal	aldehydes	–
19	2,4-Decadienal, (E,E)-	aldehydes	flavoring agent, fragrance agent, toxic [49]
20	Acetic acid	aromatic aldehydes	antibacterial and antifungal, anticancer [50]
21	Benzaldehyde	carboxylic acids (simple acids)	denaturant and a flavoring agent [51]
22	Butanoic acid, 2-methyl-	carboxylic acids (simple acids)	laxative [52]
23	Hexanoic acid	carboxylic acids (fatty acids)	–
24	(R)-(-)-4-Methylhexanoic acid	carboxylic acids (fatty acids)	–
25	Hexanoic acid, 2-ethyl-	carboxylic acids (fatty acids)	–
26	Neodecanoic acid	carboxylic acids (fatty acids)	–
27	Octanoic acid	carboxylic acids (fatty acids)	anticancer [53], antibacterial [54], antimicrobial [55]
28	Nonanoic acid	carboxylic acids (fatty acids)	skin-conditioning agent [56], anti-fungal [57]
29	Propanoic acid, 2-methyl-	carboxylic acids (fatty acids)	–
30	Butanoic acid	carboxylic acids (fatty acids)	the main energetic substrate of the colonocyte [58]
31	Pentadecanoic acid	carboxylic acids (fatty acids)	a JAK2/STAT3 signaling inhibitor in breast cancer cells [59], anti-biofilm agent [60]
32	Oleic Acid	carboxylic acids (fatty acids)	anticancer, anti-inflammatory, wound healing [61]

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33	Hexadecanoic acid	carboxylic acids (fatty acids)	anti-inflammatory [62], antibacterial [63],
34	Octadecanoic acid	carboxylic acids (fatty acids)	anticancer [64]
35	9,12-Octadecadienoic acid (Z,Z)-	carboxylic acids (fatty acids)	used for the treatment or prevention of cardiac arrhythmias [65]
36	Tiglic acid	carboxylic acids (fatty acids)	–
37	Decanoic acid	carboxylic acids (fatty acids)	enhances antibacterial effect [66], anti-inflammatory [67]
38	9-Octadecenoic acid, (E)-	carboxylic acids (fatty acids)	–
39	Pentanoic acid	carboxylic acids (fatty acids)	neuroprotective agent and suppresses oxidative stress [68]
40	Acetone	ketones	–
41	2,3-Butanedione	ketones	–
42	Acetoin	ketones	CNS depressant [44]
43	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	ketones (sesquiterpenoid)	–
44	2,3-Pentanedione	ketones	–
45	3-Buten-2-one, 4-(1-cyclopenten-1-yl)-, (E)-	ketones (cyclic)	–
46	2-Hydroxy-3-pentanone	ketones (acyloins)	–
47	Oxime-, methoxy-phenyl	Esters	–
48	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	Esters	–
49	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	fatty acid esters	–
50	Hexadecanoic acid, methyl ester	fatty acid esters	shows cardioprotective effect against the ischemia/reperfusion (I/R) injury [70], antibacterial [71], counteracts cyclophosphamide cardiotoxicity [72]
51	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	fatty acid esters	–
52	Ethane-1,1-diol dibutanoate	fatty acid esters	–
53	Benzoic acid, 2-ethylhexyl ester	benzoic acid esters	–
54	2-Octyl benzoate	benzoic acid esters	–
55	Benzoic acid 2-methylpentyl ester	benzoic acid esters	–
56	Benzoic acid, heptyl ester	benzoic acid esters	–
57	Benzoic acid, tridecyl ester	benzoic acid esters	–
58	Benzoic acid, undecyl ester	benzoic acid esters	–

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59	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	phthalate esters	–
60	Diibutyl phthalate	phthalate esters	–
61	Formic acid, octyl ester	fatty alcohol esters	–
62	1-Propen-2-ol, acetate	fatty alcohol esters	–
63	Oxirane, (methoxymethyl)-	heterocyclic ethers	–
64	(2-Aziridinylethyl)amine	amines	–
65	Cetene	alkenes	–
66	Benzoic acid	benzenoids	antibacterial and antifungal [73]
67	Phenol	phenols	desinfectant [74]
68	3(2H)-Thiophenone, dihydro-2-methyl-	tetrahydrothiophenes	–
69	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	quinones	–

2.5. GC–MS Analysis

Five (n=5) bacterial isolates with increased antibacterial activity were isolated from distinct samples. Phylogenetic analysis of the 16S rRNA gene sequences indicated that all the five candidate bacterial isolates, BSS25, BSS21, BSS16, BSS13 and BSS12, belong to five different *Bacillus spp.*, respectively (Figure 3) as they are related to the aforementioned bacterial species in the phylogenetic tree.

Bacillus species, i.e., *Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08 were identified as having the highest hit sequence similarity for these bacterial isolates (Table 10). High bootstrap values were obtained following a phylogenetic analysis and tree topology both served to confirm the presumably described taxonomy.

Table 10. Colony morphology and microscopic presentation of isolated bacterial species.

No.	Isolates	16S rRNA Amplified Region Length	Bacterial Species	NCBI Accession No
1	BSS25	1420 bp	99% with *Bacillus thuringiensis* F3	MF135173
2	BSS21	1492 bp	99% with *Bacillus toyonensis* FORT 102	MG561363
3	BSS16	1452 bp	99% with *Bacillus acidiproducens* NiuFun	MF446886
4	BSS13	1474 bp	98% with *Bacillus cereus* WAB2133	MH169322
5	BSS12	1449 bp	99% with *Bacillus safensis* AS-08	JX849661

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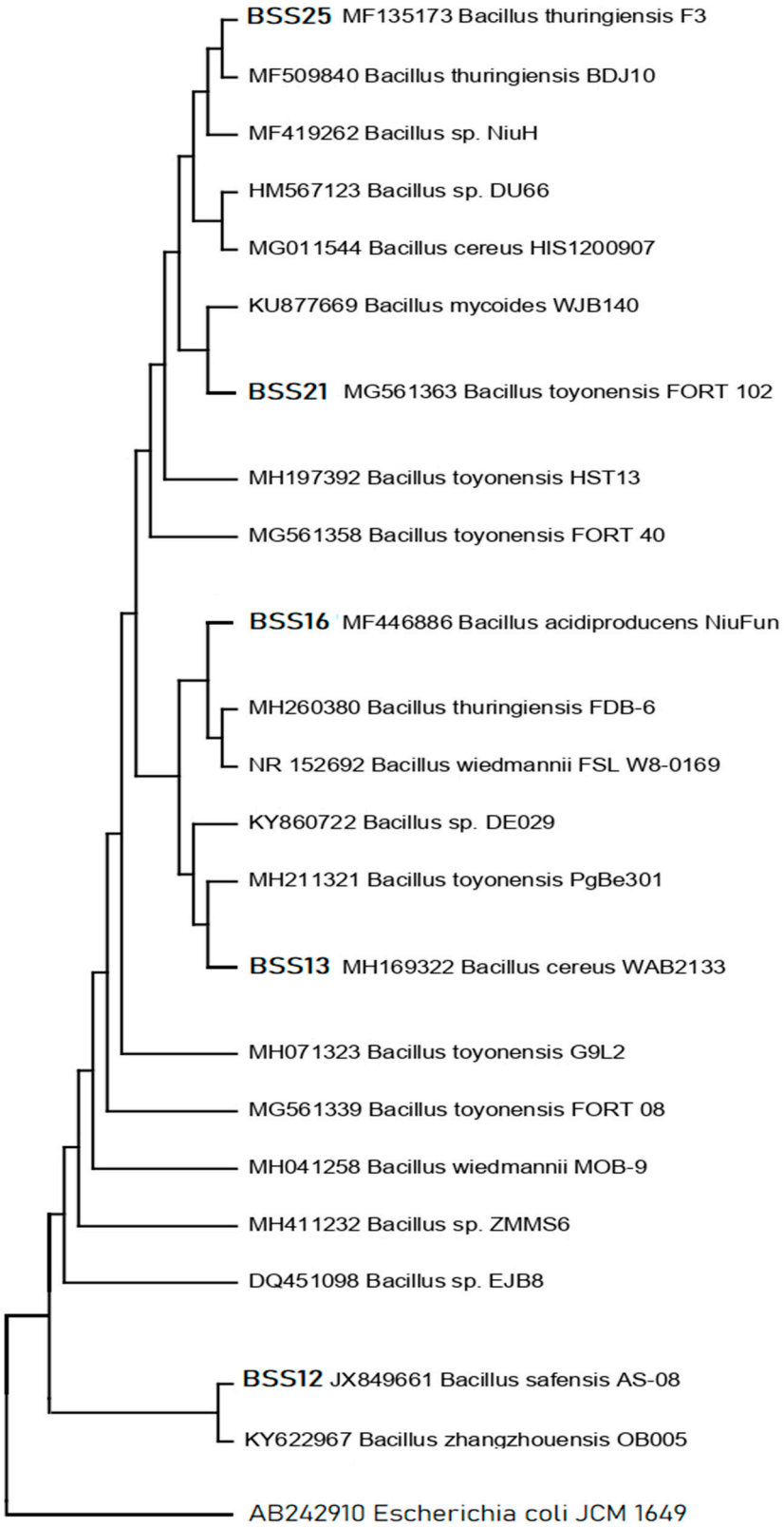

Figure 3. The phylogenetic tree using the Neighbour-joining model was constructed according to 16S rRNA gene sequences representing different *Bacillus* species, i.e., *Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08, respectively. *E. coli JCM 1649 (AB242910)* was used as an outgroup in a phylogenetic tree.

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3. Discussion

Microorganisms, including bacteria, archaea, fungi, and even viruses, inhabit diverse environments and contribute to the cycling of nutrients and the production of a wide array of metabolites. Especially extreme microbial diversity, abundance, and structure can have significant implications for the production of various metabolites with diverse functions, including anti-parasitic, antimicrobial, anti-pesticidal anti-cancerous functions. Consequently, these metabolites can have important applications in various fields. The goal of the present study was to investigate the possibility of particular vegetable microbial communities displaying antibacterial properties and to establish the possible relationship between isolated compounds through GC–MS and the antagonistic activity of studied bacterial strains. As a result of various phases of isolation, the identification of diverse general objectives with the selection of bacterial growth conditions, and biochemical tests, nineteen (n=19) different bacterial isolates were detected. In recent years, the ongoing exploration of microbial diversity, along with advancements in culturing techniques, genomics, and metagenomics, has rejuvenated the search for new antibiotics. This is a promising development in the fight against infectious diseases and antibiotic resistance, as it offers the potential for a new generation of antimicrobial agents to tackle previously untreatable infections [75,76]. However, both, mobile genetic elements and inherent characteristics (natural phenotypic traits) causes contribute to the development and spread of antibiotic resistance, making it a complex and evolving problem in healthcare and public health. The inappropriate use of antibiotics, both in clinical settings and in agriculture, accelerates the selection and dissemination of antibiotic-resistant bacteria by providing a selective advantage to those carrying resistance genes. Addressing antibiotic resistance requires a multifaceted approach that includes responsible antibiotic use, surveillance, development of new antibiotics, and strategies to prevent the spread of resistant bacteria [77]. Nearly all antibiotics, with the exception of bacitracin, polymyxin, and cloxacillin, were found to be effective against *Bacillus species*, i.e., *Bacillus thuringiensis* F3 (BSS25), *Bacillus toyonensis* FORT 102 (BSS21), *Bacillus acidiproducens* NiuFun (BSS16), *Bacillus cereus* WAB2133 (BSS13), and *Bacillus safensis* AS-08 (BSS12) (Table 3). Similar findings on the susceptibility of various antibiotic-susceptible *Bacillus* species were observed in some recent studies [30,78–81]. Here, resistance in specific *Bacillus* strains to particular antibiotics can result from both inherent (natural) mechanisms and acquired resistance due to the presence of resistance genes associated with the production of resistance enzymes [82]. The probability of passing on resistance genes to other bacteria, particularly dangerous pathogens, may be lower when resistance is due to inherent (natural) resistance mechanisms rather than acquired resistance through the acquisition of resistance genes. This distinction is important in the context of antibiotic resistance transmission and the potential for the development of multidrug-resistant or extensively drug-resistant bacteria. Since antibiotic resistance has indeed become a serious global concern, and the spread of resistant bacteria through the food chain is one of the pathways contributing to this problem [83]. Although, Isolated *Bacillus* strains may not necessarily harbor antibiotic resistance genes that can be horizontally transferred to dangerous pathogens, but they can still exhibit natural resistance or insensitivity to a wide variety of antibiotics due to their inherent characteristics. Indeed, further research into *Bacillus* strains, especially those with unique characteristics or inherent resistance to antibiotics, can be valuable for various applications, including the development of probiotic starter cultures and the production of high-quality, medicinal, and health-promoting substances.

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique commonly used to detect and identify various compounds in biological samples, including microbial cells and their metabolites helped in this study to detect markers in biological material, such as components of microbial cells and metabolites like fatty acids, aldehydes, and phenolic compounds. Additionally, using GC–MS without the need for the preliminary isolation of pure cultures of microorganisms offers several advantages in the case of both endogenous and exogenous microflora, which is especially important when considering the difficulties in cultivating anaerobes. The method's unique benefits were quick analytical times and the capacity to quantify marker content. According to the GC–MS analysis, the *Bacillus* species produce a variety of chemicals (Table 9), which possess different pharmacological activities such as antiviral, antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, hyperlipidemic, antimicrobial, antinociceptive, analgesic, anxiolytic, anti-depressive, neuroprotective and so on. Overall, 69 compounds were determined by the GC–

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MS analysis of their crude metabolites from five *Bacillus* species. 8 biologically active compounds such as acetoin, acetic acid, butanoic acid, 2-methyl-, oxime-, methoxy-phenyl, phenol, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, nonanoic acid, and hexadecanoic acid, methyl ester were found as common for all five strains (Table 4, Table 5, Table 6, Table 7, and Table 8 and Figure 2).

Previously, we found that acetic acid is present in bacterial isolates such as *Bacillus subtilis* O-3, *Bacillus subtilis* Md1-42, and *Bacillus subtilis* Khozestan2 [30]. According to the present study acetic acid seems a common organic compound almost for all *Bacillus* species as its presence is confirmed in other five *Bacillus* species i.e. *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08. BSS13 has the highest concentration of acetic acid at 6.313543%, while the other four strains were found to share similar low concentrations. Acetic acid is a common organic acid and a component of the volatile organic compounds (VOCs) produced by some bacterial species, including certain strains of Bacillus. Acetic acid is a byproduct of microbial metabolism, particularly in bacteria that undergo fermentation processes or produce acetic acid as part of their metabolic pathways. It has been known for antibacterial and antifungal, anticancer activities [50]. The second common compound for all five strains was acetoin, and its concentration for BSS25, BSS21, BSS16, BSS13, and BSS12 were different as follows 44.063%, 38.25336%, 8.440992%, 0.745862%, and 0.263788%, respectively. Acetoin is a common compound produced by various bacteria, and its concentration can vary among different strains. It is not typically used as a central nervous system (CNS) depressant in medical practice or for recreational purposes. However, in one recent study, it was proven that acetoin has a potent CNS depressant effect [44]. It was found that the third component that all bacteria share is butanoic acid, 2-methyl-, which has an application as a laxative. BSS16 and BSS13 have the highest concentrations of butanoic acid, 2-methyl- at 29.3946% and 31.6913%, while BSS16, BSS13, and BSS12 share very low concentrations at *2.237883%, 3.572379, and 1.894643, respectively.* Other 4 compounds common for all five strains, which are oxime-, methoxy-phenyl, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, nonanoic acid, and hexadecanoic acid, methyl ester, were found at low concentrations similarly to each other.

The GC-MS analysis of *Bacillus thuringiensis* F3 has revealed the presence of several chemical compounds of 2,3-butanedione, 2,3-butanediol, [S-(R*,R*)]-, 1-hepten-4-ol, hexanoic acid, hexanoic acid, 2-ethyl-, octanoic acid, benzaldehyde, (S)-(+)-6-methyl-1-octanol in relatively high concentrations at 15.84782%, 4.077882%, 3.418443%, 1.862169%, 2.90418%, 2.465807%, 2.222227%, and 1.248967%, respectively. The compositional study of *Bacillus toyonensis* FORT 102 established that it differs from other bacterial strains by composing of 2,3-butanedione (16,97044%), propanoic acid, 2-methyl- (1.406486%), hexadecanoic acid (4.002248%), oleic acid (5.853397%), 9,12-octadecadienoic acid (Z,Z)- (3.796889%), 2,3-pentanedione (2,035411%), 3-pentanol, 2-methyl- (2.916132%), oxirane, (methoxymethyl)- (2.409396%), 2,3-butanediol, [S-(R*,R*)]- (7.796162%), hexadecanoic acid (4.002248%), 9,12-octadecadienoic acid (Z,Z)- (3.796889%). The presence of some valuable compounds in *Bacillus acidiproducens* NiuFun also was confirmed, which are benzaldehyde (4.751143%); 3(2H)-thiophenone, dihydro-2-methyl- (6.072668%), propanoic acid, 2-methyl- (13.97511%), hexadecanoic acid (3.60544%), octadecanoic acid (1.638403%), oleic acid (9.677555%), and 9,12-octadecadienoic acid (Z,Z)- (11.09688%). The presence of some valuable compounds in *Bacillus acidiproducens* NiuFun also was confirmed, which are benzaldehyde (4.751143%); 3(2H)-thiophenone, dihydro-2-methyl- (6.072668%), propanoic acid, 2-methyl- (13.97511%), hexadecanoic acid (3.60544%), octadecanoic acid (1.638403%), oleic acid (9.677555%), and 9,12-octadecadienoic acid (Z,Z)- (11.09688%) and the presence of these chemical constituents indicates a diverse metabolic capacity of this bacterium. Finally, the specific VOCs with high concentrations produced by *Bacillus safensis* AS-08 were as 2,3-butanedione (21.42702%), 3-pentanol, 2-methyl- (36.53327%), benzaldehyde (2.170694%), 2,3-butanediol (4.672893%), propanoic acid, 2-methyl- (3.156132%), oleic acid (4.585822%), and 9,12-octadecadienoic acid (Z,Z)- (3.576243).

Additionally, the GC–MS analysis showed that all five bacterial isolates contained various fatty acids along with other volatile organic compounds. It is well-known that fatty acids and their derivatives can exhibit powerful antibacterial and antifungal activities [52–68]. Indeed, fatty acids are known for their biodegradability, low toxicity, and resistance to extremes in pH, salinity, and temperature, which make them environmentally friendly compounds. These properties have led to their acceptance and use as food additives in various applications. Antifungal fatty acids, particularly

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those found in natural sources, may have a lower likelihood of inducing resistance in pathogenic fungi compared to some synthetic antifungal drugs [84]. The identification of volatile compounds, including esters, alkaloids, ethers, and phenolics, in five different *Bacillus* species, is noteworthy and suggests a diverse array of secondary metabolites produced by these bacteria. The presence of volatile organic compounds (VOCs) as major constituents of a bacterial strain with properties against phytopathogens is significant and highlights the potential of these bacteria for various agricultural and environmental applications. The presence of common volatile compounds among different *Bacillus* species suggests the existence of conserved metabolic pathways or biochemical processes within the genus *Bacillus.* These shared compounds may be indicative of fundamental metabolic activities that are essential for the survival and growth of these bacteria. Our findings support prior research on the chemical composition of bacterial strains using GC–MS and show that *Bacillus* spp. share similar volatile chemicals [30,85–88].

The antibacterial characteristic of certain microorganisms, including *Bacillus* strains, plays a crucial role in various therapeutic activities. The perpendicular streak method is a common laboratory technique used to assess the antibacterial properties of bacterial isolates against selected human bacterial pathogens. In the present study, this method was used to analyze the antibacterial potency of the bacterial isolates labeled as BSS25, BSS21, BSS16, BSS13 and BSS12 against the selected human bacterial pathogens. It can be explained with the help of bioactive compounds produced by bacteria, which can have various effects on other organisms, including antimicrobial. The perpendicular streak method is recognized as a first-pass qualitative screening method for the detection of microbial activity, particularly when assessing the antibacterial properties of bacterial isolates. The research demonstrated strong antagonistic action against human pathogens such as *Enterococcus hirae* and *Staphylococcus epidermidis* by all bacterial isolates. It is obvious that the synergistic contribution of antibacterial potency refers to the combined effect of two or more antimicrobial agents (such as antibiotics or other antimicrobial com-pounds) that work together to produce a stronger inhibitory or bactericidal effect against bacteria than the individual agents would achieve on their own. According GC–MS analysis, many compounds out 69 were identified with antibacterial potency (Table 9) and their synergetic contribution explains how all bacterial strains have shown strong antagonistic action against *Enterococcus hirae* and *Staphylococcus epidermidis.* Moreover, the current research provided indicates that bacterial isolates BSS25, BSS21, and BSS16 exhibit strong antagonistic activity against *Candida albicans* while BSS25, BSS21, and BSS13 suppress the growth or activity of *Candida krusei*. Additionally, only BSS25 and BSS21 were found to show being studied exhibited strong inhibitory effects against *Klebsiella aerogenes*. This may be due to the high concentrations of acetoin in BSS25 and BSS21 and butanoic acid, 2-methyl- in BSS16 and BSS13. For sure acetoin and butanoic acid, 2-methyl- enhances the antibacterial potency of bacterial strains along with other organic volatile compounds, peptides, etc.

These are positive results, suggesting that these bacterial isolates may have potential applications in controlling or preventing infections caused by these human pathogens. Our results showed that *Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08 are effective at inhibiting the growth of some multidrug-resistant bacterial strains and this is similar to findings of our previous study [30]. Previuos researches found that the inhibitory impact of *Bacillus* species on other microorganisms, including pathogens, can indeed be attributed to various factors, including the pH of the growing medium and the generation of volatile chemicals. *Bacillus* species are known to produce a variety of polypeptide antibiotic substances, including bacitracin, polymyxin, gramicidin S, and tyrothricin. These antibiotic substances have shown effectiveness against a wide range of bacteria, including both Gram-positive and Gram-negative bacteria [88].

Bacterial extract investigation is a versatile and interdisciplinary field with far-reaching implications for science, medicine, agriculture, and industry. It involves the discovery and characterization of compounds and biological activities that can address various challenges and opportunities in these domains. Bacterial extracts are screened to discover novel antibiotic compounds, and antimicrobial peptides (AMPs) with potential therapeutic applications, as bacteria produce a wide variety of substances. Moreover, bacterial extracts can be a source of potential drug candidates for the treatment of various diseases, including infectious diseases and cancer. According to recent studies, bacterial extracts are also used in bioassays to evaluate the biological activity of

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compounds, including screening for enzyme inhibitors or activators while more of the studies proved the value of bacterial extracts' use in agriculture to manage plant diseases and pests. For example, *Bacillus* species are known for their significant roles in agriculture and biotechnology, primarily due to their ability to produce various bioactive compounds that can benefit plant health and promote agricultural sustainability [89,90]. Moreover, bacterial extracts are analyzed to identify probiotic strains with potential benefits for gut health.

Molecular investigations can provide valuable insights into the taxonomy and genetic relatedness of different bacterial isolates and our results revealed the taxonomy of five different isolated species of *Bacillus*, which are *Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08. It was determined that the five most viable candidates of bacterial isolates BSS25, BSS21, BSS16, BSS13 and BSS12 belong to *Bacillus thuringiensis* F3 (99%), *Bacillus toyonensis* FORT 102 (99%), *Bacillus acidiproducens* NiuFun (99%), *Bacillus cereus* WAB2133 (99%), and *Bacillus safensis* AS-08 (99%), respectively, based on top hit sequence similarity results and phylogenetic analysis.

The identification of the five separate bacterial strains (*Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, and *Bacillus safensis* AS-08) and their antibacterial activity can significantly facilitate microbial screening and the isolation of active metabolites, especially against multidrug-resistant strains. Consequently, knowing the specific strains that exhibit antibacterial activity allows for targeted screening of these strains against multidrug-resistant bacterial strains. This targeted approach saves time and resources compared to screening a wide range of microorganisms. As the antibacterial activity is confirmed, the isolation and purification of bioactive metabolites from these strains can be prioritized. This is crucial for identifying the specific compounds responsible for the antibacterial effects. Moreover, the isolated bioactive compounds may have potential as antibiotic adjuvants, especially against multidrug-resistant strains. This can be a valuable contribution to the fight against antibiotic resistance. Additionally, these compounds may serve as lead compounds for drug discovery efforts, where further modifications or structural optimization can be performed to enhance their efficacy and reduce potential side effects. Overall, the identification of metabolites from bacterial strains and evaluation of their antibacterial and antibiotic activity provides a strong foundation for focused research and applications in various fields.

4. Materials and Methods

4.1. Isolation of Potential Strains of the Genus Bacillus spp.

Five *Bacillus* strains (BSS25, BSS21, BSS16, BSS13 and BSS12) have been isolated from different vegetable samples such as tomato, potato, and carrot. A vegetable sample with a mass equal to 15 g was homogenized in a solvent with a volume of 100 ml of NaCl by shaking at 150 rpm for 20 min. After, the sample was steadily diluted and incubated for 10 min at 90 ◦C. After the incubation the sample has been cooled to room temperature. The sample with a volume of 0.1 mL was loaded onto nutrient agar/meat peptone agar (NA/MPA) plates, which serves as a fertile medium for the growth of undemanding microorganisms. NA/MPA plates were consisted of bacteriological agar (BA, 15 g/L), gelatin peptone (GP, 5 g/L), and meat extract (ME, 3 g/L). The plates were incubated at 37°C for 48 hours. The isolated pure strains were refrigerated at −20 ◦C in nutrient broth (NB) media supplemented with 20% (v/v) glycerin. Pure strains were stored at −20 ◦C in nutrient broth (NB), which contains 20% (v/v) glycerin as a supplement. Then, morphological identification was performed on the newly created culture. *Slightly raised, flat, white, and cream-colored colonies were chosen for further study.* Strain isolates were helpful in further studies, particularly in the preparation of ethyl acetate extract for subjecting GC–MS analysis.

4.2. Antagonistic Activity Study

On Mueller-Hinton agar (MHA) plates, a preliminary antibacterial investigation of the isolates was carried out using the perpendicular streak method against potent human pathogens. Overall, n=15 bacterial pathogens were used in antagonistic activity study: *Salmonella enterica* ATCC 35664, *Klebsiella aerogenes* ATCC 13048, *Serratia marcescens* ATCC 13880, *Klebsiella pneumoniae* ATCC 13883, *Streptococcus group B, Escherichia coli* ATCC 25922, *Candida krusei* ATCC 14243, *Shigella sonnei* ATCC

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25931, *Streptococcus mutans* ATCC 25175, *Enterococcus hirae* ATCC 10541, *Proteus Vulgaris* ATCC 6380, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 2091. On the basis of the perpendicular streak method, an exponential culture of the studied pathogens was streaked on the surface of an agar medium and incubated at 30 ± 4 ◦C for 24 h [91]. Then, an exponential culture of the test strain was inoculated perpendicularly from the edge of the cup to the stroke of the grown culture of the antagonist with a stroke by slightly touching the stroke of the antagonist strain. The plate was once more incubated in a setting that encouraged test culture growth. The samples were then processed using the first technique.

The second method an agar well-diffusion method with a few minor modifications was used to measure antimicrobial activity [92]. On the plate, bacterial suspensions were applied with turbidity that was calibrated to the McFarland 0.5 standard (about 108 colony forming units, or CFU per milliliter). Using the back end of a sterile 1-mL pipette tip, a 7-mm diameter well was punched aseptically onto Mueller-Hinton agar (Oxoid, Basingstoke, UK). The positive control was streptomycin (1 g/mL). Each well received 100 L of test agent in total. The diameter of the clear zone was measured during an incubation period of 16 to 24 hours at 37 °C.

4.3. Antibiotic Susceptibility of the Bacillus Isolates

Using the disk diffusion method, the antibiotic susceptibility test of all five *Bacillus* strains (BSS25, BSS21, BSS16, BSS13 and BSS12) was done in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019). Using an aliquot of 1 mL for each strain and a concentration of 106 CFU/mL (0.5 McFarland, Hi-media, India), *Bacillus* strains were spread-plated on Mueller-Hinton (MH) agar using sterile beads. The plates were subjected to drying for an hour. Consequently, antibiotic disks were inserted into the agar plates containing an inoculated strain. After incubation period (24 h) at 37 °C, the widths of the inhibition zones surrounding the antibiotic disks were measured using an electronic digital vernier caliper micrometer measuring instrument caliber digital ruler (ZHHRHC LCD, Hardened, China). This helped to identify following parameters such as the strain's antibiotic susceptibility (S), intermediate resistance (I), or resistance (R) according to the CLSI guidelines (2012) [93,94]. Antibiotic disks (n=18) contained a sample each of ampicillin (AMP, 10), amoxycillin (AMOX, 30), amoxycillin-clavulanic acid (AMC, 30), azithromycin (AZM, 15), bacitromycin (B, 10), carbenicillin (CAR, 100), cefepime (FEP, 30), cefepime/clavulanic acid FEC-40, cephalatin (KF, 30), cefotaxime (CTX, 30), cloxacillin (CX, 5), erythromycin (ERO, 15), gentamicin (CN, 120), polymyxin (PB, 300), penicillin G (PEN, 10), streptomycin (STR, 10), tobramycin (TOB, 10), tetracycline (TET, 30).

4.4. Gas Chromatography–Mass Spectrum Analysis

The volatile substances extraction for each bacterial isolate (*Bacillus thuringiensis* F3, *Bacillus toyonensis* FORT 102, *Bacillus acidiproducens* NiuFun, *Bacillus cereus* WAB2133, *Bacillus safensis* AS-08) was carried out separately two times from the culture broth with 25 mL ethyl acetate (Sigma-Aldrich, Germany) for 20 min and two extracts were combined. After that, 1.5 mL of the extract was transferred into plastic vials with a capacity of 2 mL, which were then set on the autosampler tray for GC-MS analysis. Bacterial secondary metabolites were examined through a GC-MS analysis on a Thermo Scientific GC Focus Series DSQ. As the carrier gas, helium gas was used at a constant flow rate of 1 mL per minute and the injection volume of sample was equal to 1 mL. The injector and hot oven were kept at 250 ◦C and 110 ◦C, respectively, with the temperature increasing by 10 ◦C per minute up to 200 ◦C, 5 ◦C per minute up to 280 ◦C, and shutting down after 9 min at a temperature of 280 ◦C [30]. The retention durations of several chemical peaks that were eluted from the GC column were recorded. After matching the data with the mass spectra of the compounds, the database was searched for compounds with comparable molecular masses and retention times. The current investigation discovered a parallel pattern in the bioactivities of previously studied natural compounds.

4.5. Molecular Characterization of the Bacterial Isolates

Isolated bacterial strains were molecularly characterized using universal bacterial primers and 16S rRNA conserved gene sequences. Using the conventional PCR method, the targeted gene

sequence was amplified. The final result was then processed via 1% gel electrophoresis to determine the size of the amplified fragments. The amplified materials were delivered for sequencing along with the pertinent sequencing fragments. The nucleotide sequences were phylogenetically analyzed using MEGA software (MEGA-11). The bacterial isolates were subsequently validated and categorized at the species level using GenBank NCBI's BLAST search (National Center for Biotechnology Information). The accession numbers (MF135173, MG561363, MF446886, MH169322, and JX849661) correspond to the 16S rRNA gene sequences of the probiotic strains and were used to retrieve and reference the sequences in the GenBank database. (www.ncbi.nlm.nih.gov/projects/genome/clone/, accessed on 9 July 2023).

4.6. Statistical Analysis

The XLSAT software version 2016.02.27444 was used to perform the one-factor analysis of variance at the significance level ($\alpha = 0.05$). The Newman-Keuls test was used to rank the means when there were substantial differences between the parameters under study.

5. Conclusions

In a current study, vegetable bacterial isolates obtained from five different species of Bacillus demonstrated the ability to inhibit the growth of multidrug-resistant bacterial strains. As a result of a screening process, three potent isolates named BSS25, BSS21, BSS16, BSS13 and BSS12 were identified. GC–MS was used to identify and quantify the chemical compounds present in bacterial species, despite the fact that they are all members of the same *Bacillus* subspecies. The observation was that volatile organic compounds (VOCs) differ among members of the same *Bacillus* subspecies despite their taxonomic similarity highlighting the chemical diversity that can exist within closely related bacterial strains. This study confirmed a variety of volatile inhibitory substances, including esters, phenolics, and ethers, which are believed to play a role in antimicrobial activity. The volatile compounds differ in chemical composition among the tested samples suggesting that these variations may have an impact on antimicrobial activity and antibiotic potency. Strains of the Bacillus subtilis group are known to have the capability of producing a wide range of secondary metabolites that contribute to their antimicrobial characteristics and this was also confirmed by our findings. In addition to volatile organic compounds, strains within the *Bacillu*s subtilis group are known to produce a variety of other bioactive compounds, including bacteriocins, polyketides, peptides, and more. Hence, it can be concluded that the discovered organic volatile substances i.e. acetoin and butanoic acid, 2-methyl- enhance the antimicrobial properties of *Bacillus* spp. together with the above substances. The remarkable metabolic capacity and adaptive biochemistry of *Bacillus species*, i.e., *Bacillus thuringiensis* F3 (BSS25), *Bacillus toyonensis* FORT 102 (BSS21), *Bacillus acidiproducens* NiuFun (BSS16), *Bacillus cereus* WAB2133 (BSS13), and *Bacillus safensis* AS-08 (BSS12) make these strains valuable for various commercial and biotechnological applications, as they have the potential to generate a wide range of bioactive chemical substances. Bacterial extracts, which contain bioactive chemicals produced by these *Bacillus* strains, have the potential to be used as antimicrobial agents. The anticipation of conducting a thorough investigation, similar to the one described, holds great promise in uncovering new microbiological possibilities and discovering previously unknown substances or metabolites with strong antibacterial potential. Such research endeavors are essential for addressing the burden and danger posed by bacterial strains that have developed resistance to multiple drugs.

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