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Production of Terpenoids, Terpene Alcohol, Fatty Acids and N_2 Compounds by Bacillus *amyloliquefaciens* S5i4 Isolated from Archaeological Egyptian Soil

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Abstract

The strain *Bacillus amyloliquefaciens* S_5I_4 (*B. amyloliquefaciens*) isolated from Idfou Temple, Egypt, was identified by 16S rRNA and has accession number AB813716. *B. amyloliquefaciens* S_5I_4 strain was tested for its antifungal and antibacterial activity against pathogenic fungi and bacteria. The antifungal activity of S_5I_4 strain examined by using *Aspergillus flavus* MD341 (*A. flavus*) producing aflatoxin B1 as an indicator. The antibacterial activity of S_5I_4 strain was examined by using *Staphylococcus aureus* KF 771028 (*S. aureus*) causing food poisoning as an indicator. This S_5I_4 strain used to produce the antimicrobial compounds then the extraction, purification and identification of the antimicrobial agents were carried by Gas liquid chromatographic mass spectrometry (GLC-MS). The extract was subjected to GLC-MS to afford 26 peaks corresponding to 26 compounds in ethyl acetate and 20 peaks corresponding to 20 compounds in methylene chloride extract. Most of these compounds are terpenoids (41.30%) terpene alcohols (15.22%), nitrogenous compounds (19.57%) and fatty acids (21.74%) with one miscellaneous group (2.17%) respectively. These compounds produced by S_5I_4 strain could be mechanism of biocontrol against some fungal diseases.

Keywords: Antifungal compounds; *B. amyloliquefaciens*; GLC-MS; Terpenoids; Terpene alcohols; Fatty acids

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Introduction

The pathogenic fungi and bacteria attack human, animals and plants. Since penicillin was discovered, the isolation and identification of new antimicrobial compounds produced by microorganisms hadn't been stopped. The fungal diseases affecting crops are a major threat to food production and food storage. Fungal growth on foods causes undesirable changes making them harmful for consumption and may cause mycosis [1]. *Aspergillus flavus is* producing aflatoxins which are potent agents causing liver cancer [2]. Chemical preservatives have been used carefully for the control of fungi because of their often toxicities to men and farm animals. These days, consumers prefer foods containing natural and safe preservatives [3]. Thus, biologically control of pathogenic fungi through the use of natural antagonistic microorganisms is alternative to chemical preservatives or chemical pesticides [4].

In this respect, bacilli particularly *Bacillus amyloliquefaciens* and *Bacillus subtilis* and their metabolites have great antimicrobial compounds and thus effectively compete with other microorganisms such as fungi and bacteria. *B. amyloliquefaciens* was reported effective for the biocontrol of multiple plant diseases and post harvested pathogen [5]. Members of Bacillus are called biopesticides. Bacillus-based product represents about half of commercially available bacterial biocontrol agents [6]. Bacilli can antagonize pathogens by producing fungal toxic compounds, competing for nutrients and stimulating the defense capacities of the host plant [7].

Bacillus amyloliquefaciens is important producer of antimicrobial molecules and secondary metabolites for biocontrol of pathogens [8,9]. *B. amyloliquefaciens* HNA3 strongly inhibited *Aspergillus niger* ATCC9642 [10]. Bioactive secondary metabolites are believed to play a key role in microbial interactions by mediating

antagonistic activity and intercellular communication [11]. Reda et al. [12] reported that *B. amyloliquefaciens* S_5I_4 produced antibacterial compound and was identified as butanedioic acid, octadecyl1(1carboxy1methylethyl) 4octyl ester. The commercially available strain of *Bacillus amyloliquefaciens* FZB24 is applied as bio-fertilizer, as it stimulates plant growth and suppress plant pathogenic organisms Secondary metabolites such as iturin and surfactin produced by *Bacillus amyloliquefaciens* BNM122 increase the antifungal activity [13].

In the present work *B. amyloliquefaciens* S_5I_4 inhibits *Aspergillus flavus* MD 341 and *S. aureus* KF 771028. Ethyl acetate extract of CFS of *B. amyloliquefaciens* S_5I_4 was subjected to GLC M.S to afford 26 peaks corresponding to 26 compounds and 20 compounds using ethyl acetate extract and methylene chloride solvents respectively. Most of these compounds are terpenoids, terpene alcohols, nitrogenous compounds and fatty acids. These compounds produced by S_5I_4 strain could be mechanism of biocontrol against some fungal diseases and Stapylococcal toxins.

Materials and Methods

Soil samples collection

Soil samples were collected from different archaeological regions of Egypt. These samples were obtained by removing and rejecting the first two inches and about 250 to 500 g for each site at a depth of 5-10 cm was taken into a clean sterilized plastic bag and transferred to the laboratory. Different samples were taken at randomly from each locality and were brought together into one composite soil sample.

Isolation and purification of bacterial isolates

Bacterial colonies are usually isolated and counted by using standard dilution plate procedure [14]. After incubation period for 24 h at 37°C, pure single colony transferred onto nutrient agar slants for more investigations [15].

Screening of *Bacillus amyloliquefaciens* S5I4 for its antifungal and antibacterial activities

Bacillus amyloliquefaciens S_5I_4 was screened for the antifungal using *Aspergillus flavus* MD 341 was obtained from the central Lab. Of Residues in Agric. Products, Agric, Pesticides Res. Centre, Dokki, Egypt producing aflatoxin B_1 and antibacterial using *S. aureus* KF 771028. The *B. amyloliquefaciens* S_5I_4 was grown on nutrient agar medium for 24 h at 37°C. Agar discs 7 mm in diameter were cut off by a cork borer and transferred to the surface of agar plates which freshly seeded with the *S. aureus* KF 771028. The tested fungal organisms were cultivated on Czapex' Dox agar media. The widths of inhibition zones produced by the producer organisms were measured 24 h and 5-7 days for bacteria and fungi, respectively.

Molecular identification of *Bacillus amyloliquefaciens* S5I4

Identification of the most antagonistic activity isolate code S_5I_4 was confirmed by investigation of 16S rRNA gene sequence which submitted to Gene Bank with accession number AB813716. The identification of the selected isolate was carried out by the authorities of the Unit of Molecular Biology Sigma Laboratory, EL Mohandesein; Egypt.

Gas liquid chromatography analysis

Thin layer chromatography was performed in Silica Gel 60 F_{254} were precoated TLC aluminium sheets for Thin Layer Chromatography.

Size: 5 × 20 cm, Layer Thickness: 0.2 mm, Sheets Package: 100.

A Division of EM Industries, Inc. Associate of Merck KGa A, Dramastadt. Made in Germany.

Method of gas liquid chromatography-mass spectrometry (GLC/MS): Data of experiment were determined in Al-Azhar University, Faculty of Science; The Regional Center for Mycology and Biotechnology.

Two different samples with different solvents ethyl acetate and methylene chloride have been submitted for Chromatographic analysis in Gas Chromatography Unit.

Instrumentation and chromatographic conditions:

GC/MS system: SHIMADZU GC/MS-QP5050A

Software: CLASS 5000

Searched library: Wily Mass Spectral Data Base

Column: DB1, 30 m; 0.53 mm ID; 1.5 um film (J&W scientific)

Carrier gas: Helium

Ionization mode: Electric Ionization (EI)

Ionization voltage: 70 ev

Temperature program: 40°C (1 min) - 160°C (1 min) at 5°C/min-270°C (2 min) at 7.5°C/min

Detector temperature: 300°C

Injector temperature: 230°C

Results

Strain identification

The identification of *B. amyloliquefaciens* S_5I_4 was molecularly confirmed by investigation of 16S rRNA analysis. Sequence data were submitted to GenBank at NCBI web site (http://www.ncbi.nlm.nih.gov) with accession number AB813716. BLAST program (http://www.ncbi.nlm.gov/blast) for phylogenetic analysis was used to assess the similarities of obtained 16S rDNA gene sequence **(Figure 1)**.

The strain S_5I_4 revealed definitely antifungal properties against *A. flavus* MD 341 producing aflatoxin B1 aflatoxin B1as besides strong antibacterial activities against *S. aureus* KF 771028 producing food toxins as an indicator **(Figures 2A and 2B)**.

Gas liquid chromatographic mass spectrometry (GC-MS)

In the current study, it was found that after culturing 4 liters of

liquid nutrient broth media, the yield that obtained from *Bacillus amyloliquefaciens* S_5I_4 strain by using two different polarity solvents methylene chloride and ethyl acetate was very small yield (7 mg) so, we used GLC- MS in identification of the extracted compounds.

The results in **Table 1** and **Figures 3 and 4** revealed that ethyl acetate extract was subjected to GC-M.S to afford 26 peaks corresponding to 26 compounds and 20 peaks corresponding to 20 compounds in methylene chloride extract (46 compounds from both extracts). The results in **Table 2** and **Figures 5-8** represented the classification and identification of these compounds into five groups; nineteen terpenoids, seven terpene alcohols, nine nitrogenous compounds, ten fatty acids and one miscellaneous group.

The results implied that solvent extraction had different roles to recover different compounds, which had effective inhibition in various pathogenic microorganisms. In addition, the amount of crude extracts from each solvent is one of the factors used in determining the choice of solvent for extraction.

ACTCTGTCACTTCGGCGGCTGGCTCCATAAAGGTTACCTCACCGACTTCGGGTGTTACAAA
CTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCT
GATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACT
GAGAACAGATTTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATT
GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCC
TCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCA
AGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCA
TGCACCACCTGTCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATG
TCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTG
CGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCT
TAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTT
ACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGT
CAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCA
CCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCC
TCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACG
CCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTA
GCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCGCCCTATTTGAACGGCACTTGTTCTT
CCCTAACACAGAGCTTTACGATCGAAACTTCATCACTCACGCGGCGTGCTCGTCAGACTTT
CGTCATTGCGGAGATCCCTACTGCTGCCTCCGTAGAGTCTGGGCGTGTCTCAGTCCAGTGT
GACGATCACCCTCTCAGTCGCTACGCCATCGTCGCCTGGTGAGCGTACCTCACCAACCAGG
CTATGGCGGGTCACTGTAGGTAGCGGAAGCAACTTATGTTCGTGACACATGTCCGGTTAGT

Figure 1 Sequence of 16S rRNA gene of DNA the most potent antibacterial of *Bacillus amyloliquefaciens* S5I4.

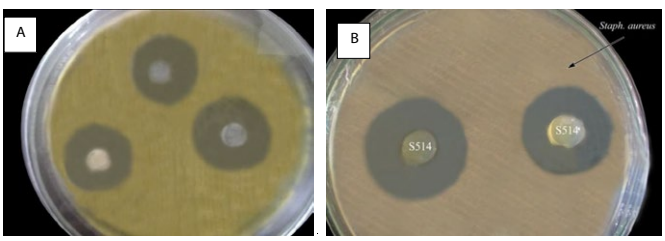


Figure 2 A) The antifungal activity of S5I4 strain examined by using *Aspergillus flavus* MD341 producing aflatoxin B1as an indicator. B) The antibacterial activity of S5I4 strain was examined by using *Staphylococcus aureus* KF 771028 causing food poisoning as an indicator.

Discussion

It is important to find safe and cheap antimicrobial agents to inhibit pathogenic fungi and bacteria. The strain S_5I_4 showed antifungal and antibacterial activities. This may due to secondary metabolites such as terpenoids, alkaloids and flavinoids. This is similar to previous work. Aflatoxin B_1 (AFB_1), produced by *A. flavus*, is secondary metabolite, highly toxic and carcinogenic. *S. aureus* produce toxins in food. Although *S. aureus* are easily killed by cooking the toxins are resistant to heat and couldn't be destroyed by cooking. Staphylococcal toxins are fast acting symptoms usually develop within 30 min to 6 h [16]. The data revealed that the strain *Bacillus amyloliquefaciens* S_5I_4, showed strong antibacterial and antifungal activities. Our results supported by reports that most *Bacillus* spp. produce many antibiotics such as bacillomycin, fengycin, mycosubtilin and zwittermicin, which are all effective at suppressing growth of target pathogens *in vitro* and/or *in situ* [17].

A microbial biological control agent may act against pathogens differently: by weakening or destroying the pathogen, competing for space and nutrients or producing antimicrobial compounds and enzymes that attack the cell components of the pathogens [18]. In order to investigate the potential biocontrol mechanisms of strain *Bacillus amyloliquefaciens* NJZJSB3, the nonvolatile antifungal compounds it produces were identified as iturin homologs using HPLC-ESI-MS. Antifungal volatile organic compounds were identified by gas chromatography-mass spectrometry. The detected volatiles toluene, phenol, and benzothiazole showed antifungal effects against *S. sclerotiorum*in chemical control experiments. Strain NJZJSB3 also produced biofilm, siderophores and cell-wall-degrading enzymes (protease and β-1,3-glucanase) [19].

Most of terpenoids, terpene alcohols, nitrogenous compounds and fatty acids are previously known from essential oils [20] and reported here from a microorganism. These results are in agreement with Yuan et al. [21] who characterized the volatile organic compounds (VOCs) produced by *B. amyloliquefaciens* strain NJN-6 by using solid-phase micro extraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) to extract and identify the VOCs and identified antagonistic VOCs as those that reduced the growth and inhibited the spore germination of *F. oxysporum.* Also, it was detected 36 compounds, including 12 benzenes, seven alkyls, three alcohols, seven ketones, two aldehydes, three naphthyls, one ester and one ether compound. In this connection, some of our identified compounds are very chemically similar in structure to; N-Isopropyl-beta-lactimimide and 17 beta, hydroxy-1alpha, 17alpha dimethyl-5 alpha androstan-3-one abundantly used as antimicrobial flagyl and fusidic acid .

Although many of the bacterial volatiles could not be identified due to no matches being found with mass-spectra of volatiles in the data base. Most of them were species-specific [22].

Table 1 The structure, molecular weight and molecular formula of 46 compounds from ethyl acetate and methylene chloride extracts when subjected to GLC M.S (gas liquid chromatographic mass spectrometry); 26 compounds at ethyl acetate extract and 20 compounds at methylene chloride extract.

26 compounds at ethyl acetate extract				20 compounds at methylene chloride			
No.	Compound structure	M.W.	Molecular form	No.	Compound structure	M.W.	Molecular form
1	Cyclo4-methyl or 4-methyl cyclohexene or 4-methyl-1-cyclohexene	96.	C_7H_{12}	1	1,2-Cyclopentanedione,3,3,5,5-tetramethyl or 3,3,5,5-tetramethyl,1,2-cyclopentane	154	$C_9H_{14}O_2$
2	N-Isopropyl-beta-lactimimide	112	$C_6H_{12}N_2$	2	Dodecanoic acid	200	$C_{12}H_{24}O_2$
3	2-methyl-1-dodecene or 1-dodecene, 2-methyl	182	$C_{13}H_{26}$	3	(3-t-butyl-1-methyl-3-piperidinyl)propan-2-one	211	$C_{13}H_{25}NO$
4	-4-hexene-3-one, 5-methyl	112	C_7H_2O	4	3, 3-Dimethyl-2-phenylbutyl phenyl sulphide	270	$C_{18}H_{22}S$
5	3-Ethyl-5-Methyl-1-Propyl-Cyclohexane	168	$C_{12}H_{24}$	5	11-(aminomethyl)-1,4-dioxaspiro(4.7)dodec-7-ene	197	$C_{11}H_{19}NO_2$
6	Bis-(3,5,5trimethylhexyl) ether	270	$C_{18}H_{38}O$	6	N-Formyl-alpha-aminocrotonic acid methyl ester	143	$C_6H_9NO_3$
7	2-Hexene-1-ol, 2-ethyl	128.	$C_8H_{16}O$	7	BORNEOL	154	$C_{10}H_{18}O$
8	Tridecanaldehyde Or Tridecyl aldehyde	198.	$C_{13}H_{26}O$	8	Camphor	152	$C_{10}H_{16}O$
9	N-Hexyl Tiglate	184	$C_{11}H_{20}O_2$	9	1-carboxymethyl-4-hydroxy-6-methyl-2-pyridone	183	$C_8H_9NO_4$
10	-Cyclo hexane,1,5-diethyl-2,3-dimethyl	168.	$C_{12}H_{24}$	10	Decanoic Acid or Capric Acid	172	$C_{10}H_{20}O_2$
11	Hexadecanoic acid or Palmitic acid or n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	11	Menthyl-beta-D-glucopyranoside	318	$C_{16}H_{30}O_6$
12	Dodecanoic acid Or Lauric acid Or Neo-Fat 12 Or Vulvic acid	200	$C_{12}H_{24}O_2$	12	Octanoic,2-methylcyclohexyl ester trans	240	$C_{15}H_{28}O_2$
13	2-cyclododecylethanol	212	$C_{14}H_{28}O_2$	13	3-pyrrolidino-3-oxo-propylsuccinimide	224	$C_{11}H_{16}N_2$
14	2,2-Dimethyl-3-cyclohexene-1-ol Or Cyclohexene-1-ol,2,2dimethyl	126	$C_8H_{14}O$	14	Phenyl Alanin-Proline Diketopiprazine	244	$C_{14}H_{16}N_2O_2$
15	2-Methylcyclohexanol	114	$C_7H_{14}O$	15	1,3-Cyclopentanedione, 4-hydroxy-5-(3-methyl1-butenyl)	182	$C_{10}H_{14}O_3$
16	Citronellyl propionate or 6-Octene-1-ol,3,7-dimethyl-propionate	212	$C_{13}H_{24}O_2$	16	-Cis-8-Endo-Ethoxybicyclo(4,3,0)-3-Nonene-7-exo-carboxaldehyde	194	$C_{12}H_{18}O_2$
17	2,6,6-Trimethylcyclohex-2-ene-1,4-Dione	152	$C_9H_{12}O_2$	17	Phthalic acid, Didecyl ester Or 1,2-benzenedicarboxylic acid, Didecyl ester	446	$C_{28}H_{46}O_4$
18	3-(trans-2-hydroxy cyclohexyl) propanol	158	$C_9H_{18}O_2$	18	Decanoic acid or capric acid	172	$C_{10}H_{20}O_2$
19	Octanoic acid, 2-methylcyclohexyl ester, trans	240	$C_{15}H_{28}O_2$	19	Bicyclo(2,2,2)octan-1-amine	125	$C_8H_{15}N$
20	Heptadecanoic acid Or Margaric acid Or Hexadecane carbonic acid Or Margarinic acid	270	$C_{17}H_{34}O_2$	20	Dodecanol Molecular weight: Molecular form:	186	$C_{12}H_{26}O$
21	Beta Tocopherol Or 2H-1-Benzopyran-6-ol,3,4-dihydro-2,5,8-trimethyl-2-(4,8,12-trimethyl)	416	$C_{28}H_{48}O_2$				
22	Oleic acid, Propyl ester	324	$C_{21}H_{40}O_2$				
23	2-propenyl-3-cyclohexylpropenoate	196	$C_{12}H_{20}O_2$				
24	Androstan-3-one,17-hydroxy-1,17-dimethyl-(1 alpha, 5 alpha, 17 beta) Or 17 beta,hydroxy-1alpha,17alpha dimethyl-5 alpha androstan-3-one	318	$C_{21}H_{34}O_2$				
25	3-beta, 6 alpha, 20 beta trihydroxy-5 alpha-pregnane	336	$C_{21}H_{36}O_3$				
26	Nonacosanol	424	$C_{29}H_{60}O$				

No: Number of compound; M.W: Molecular Weight

Bacillus species are good secretors of proteins and metabolites. Also *Bacillus* strains produce one of the most potent lipopeptide biosurfactants, surfactin which shows high surface activity and therapeutic potential [23,24].

Production of antimicrobial substance(s) by members of *Bacillus amyloliquefaciens* was reported by many investigators. In this connection, Arguelles-Ariaset et al. [8] reported that *Bacillus amyloliquefaciens* GA1 as a good candidate for the development of biocontrol agents. The genome of the plant-associated *B. amyloliquefaciens* GA1 contained three gene clusters directing

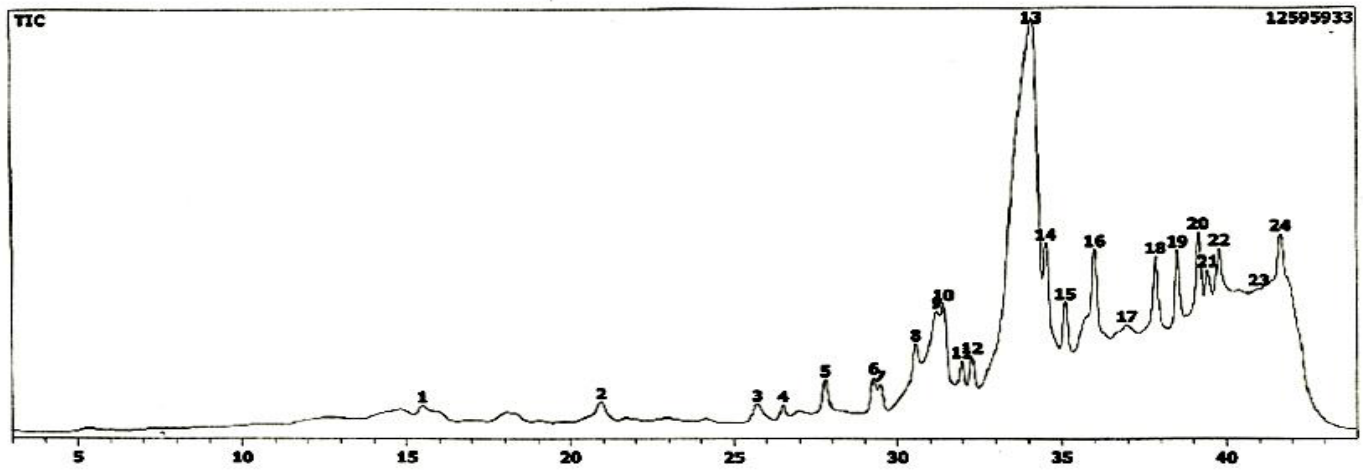


Figure 3 Gas chromatography (G.C) of ethyl acetate extract afforded 26 compounds.

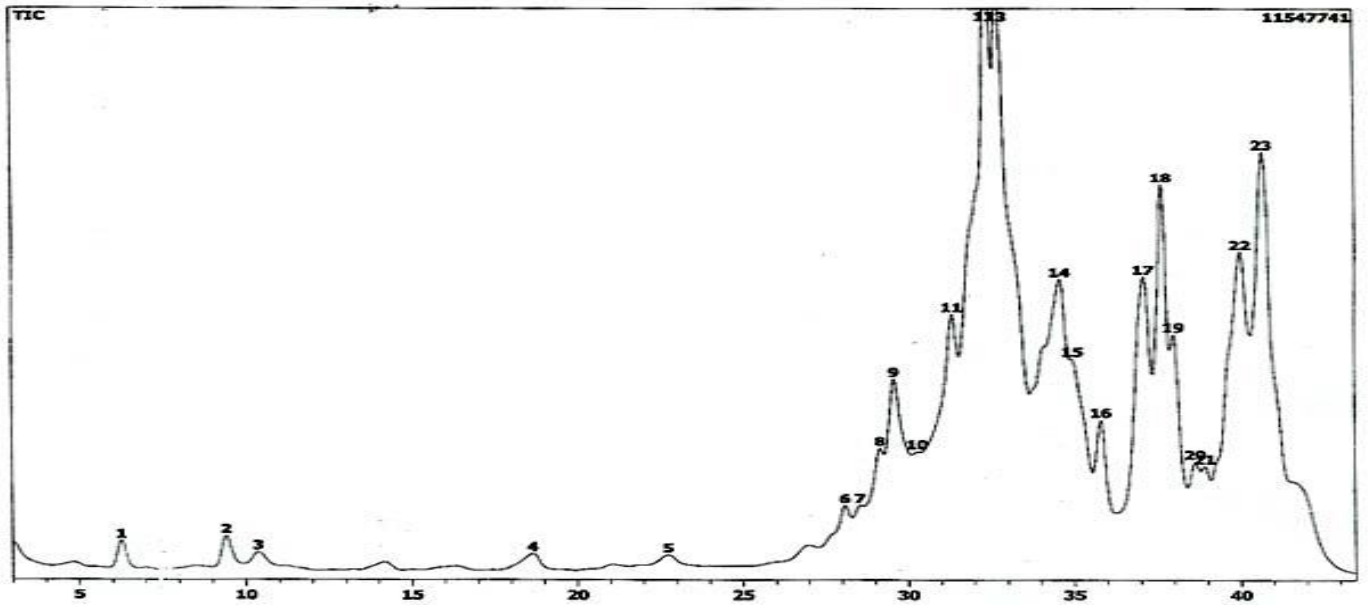


Figure 4 Gas chromatography (GC) of methylene chloride solvent afforded 20 compounds.

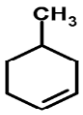
the synthesis of the antibacterial polyketides macrolactin, bacillaene and difficidin. Secondary metabolites produced by endophytic bacteria *Bacillus pumilus* MAIIIM4A showed a strong inhibitory activity against the fungi *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii* and LC-MS/MS was used to identify the active fraction assigned as punilacidin [25].

About 50.000 terpenoid metabolites have been isolated from terrestrial and marine plants, liverworts and fungi. However, rarely terpenoids metabolites have been identified in prokaryotes. The first study of bacterial terepenes reported in 1891 by Beithelot and Andre. Also, Gerber and Lechevalier [26] were carried out studies on bacterial terpenes production. GC-MS analysis showed that *Streptomyces avermitilis* ATCC 31267 produce terpenoid metabolites. The volatile compounds produced by *Bacillus atrophaeus* (CAB-1) include a range of amines and alkanamides, alcohols, phenols, hexadecane and O-anisaldehyde which inhibited fungal pathogen *Botrytis cinerea* [27].

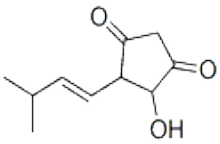
Our results showed that *B. amyloliquefaciens* S_5I_4 and/or its bioactive compounds may be used in the control of toxogenic fungi *A. flavus* MD 341. *Bacillus subtilis* and *Bacillus amyloliquefaciens* are well known for their biocontrol of fungal and bacterial diseases. The main mechanisms known to be involved in biocontrol include antibiosis, competition, growth promotion and induction of systemically acquired resistance [28]. Antibiotics are powerful weapons used by biocontrol strains to compete with other microorganisms [29].

Table 2 Classification and identification of extracted antimicrobial compounds produced by *B. amyloliquefaciens* S5I4 strain using gas liquid chromatographic mass spectrometry (GC-MS).

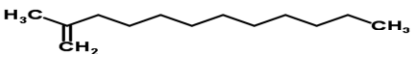
Type of Compound Number of Compound	Terpenoids	Terpene Alcohols	Nitrogenous compounds	Fatty acids
1	Cyclo4-Methyl Or 4-Methyl Cyclohexene	2-Hexene-1-Ol, 2-Ethyl	N-Isopropyl-Beta-Lactimimide	N-Hexyl Tiglate
2	2-Methyl-1-Dodecene	2-CyclododecylEthanol	(3-T-Butyl-1-Methyl-3-Piperidinyl)Propan-2-One	Hexadecanoic Acid Or Palmitic Acid
3	4-Hexene-3-One, 5-Methyl	2,2Dimethyl-3-Cyclohexene-1-Ol	3, 3-Dimethyl-2-Phenylbutyl Phenyl Sulphide	Dodecanoic Acid Or Lauric Acid
4	3-Ethyl-5-Methyl-1-Propyl-Cyclohexane	Cyclohexanol, 2-Methyl	11-(Aminomethyl)-1,4-Dioxaspiro(4.7)Dodec-7-Ene	Heptadecanoic Acid Or Margaric Acid
5	Bis-(3,5,5-Trimethylhexyl) Ether	3-(Trans-2-Hydroxy Cyclohexyl) Propanol	N-Formyl-Alpha-Aminocrotonic Acid Methyl Ester	Beta Tocopherol
6	Tridecanaldehyde	Nonacosanol	1-Carboxymethyl-4-Hydroxy-6-Methyl-2-Pyridone	Androstan-3-One,17-Hydroxy-1,17-Dimethyl-(1 Alpha.,5 Alpha.,17 Beta) (Vitamen C)
7	Cyclohexane,1,5-Diethyl-2, 3-Dimethyl	Dodecanol	3-Pyrrolidino-3-Oxo-Propylsuccinimide	3-Beta, 6 Alpha, 20 Beta Trihydroxy-5 Alpha-Pregnane (Hormone)
8	Citronellyl Propionate		Phenyl Alanin-Proline Diketopiprazine	Decanoic Acid Or Capric Acid
9	2,6,6-Trimethylcyclohex-2-Ene-1,4-Dione		Bicyclo(2,2,2)Octan-1-Amine	Phthalic Acid ,Didecyl Ester
10	Octanoic Acid, 2-Methylcyclohexyl Ester,Trans			Decanoic Acid Or Capric Acid
11	Oleic Acid, Propyl Ester			
12	2-Propenyl 3-Cyclohexylpropenoate			
13	3,3,5,5-Tetramethyl,1,2-Cyclopentane			
14	Dodecanoic Acid			
15	Borneol			
16	Camphor			
17	Octanoic, 2-Methylcyclohexyl Ester Trans			
18	1,3-Cyclopentanedione, 4-Hydroxy-5-(3-Methyl1-Butenyl)			
19	Cis-8-Endo-Ethoxybicyclo (4,3,0)-3-Nonene-7-Exo-Carboxaldehyde			
	19	7	9	10+Miscellaneous=Menthyl-beta-D-glucopyranoside
	41.30	15.22	19.57	21.74+2.17



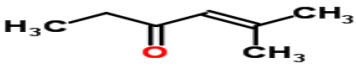
4-methyl-1-cyclohexene



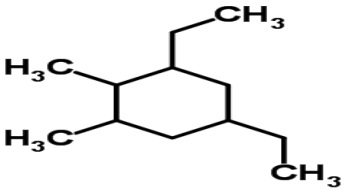
1,3-Cyclopentanedione, 4-hydroxy-5-(3-methyl1-butenyl)



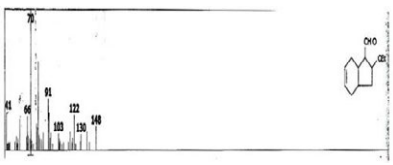
2-methyl-1-dodecene



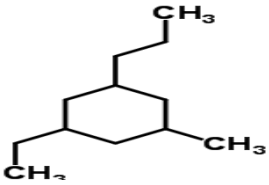
4-hexene-3-one, 5-methyl



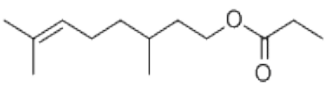
Cyclo hexane, 1, 5-diethyl-2, 3-dimethyl



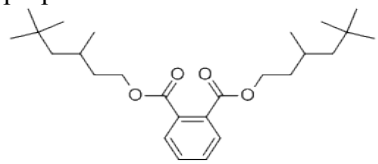
Cis -8-Endo-Ethoxybicyclo (4, 3, 0)-3-Nonene-7-exo-carboxaldehyde



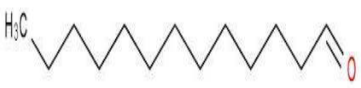
3-ethyl-5-methyl-1-propyl-cyclohexane



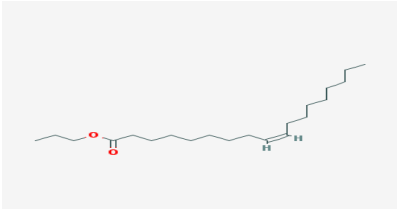
Citronellyl propionate or 6-Octene-1-ol, 3, 7-dimethyl-propionate



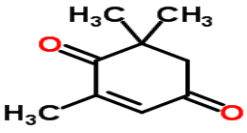
Bis-(3, 5, 5 trimethylhexyl) ether



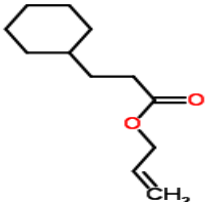
Tridecanaldehyde



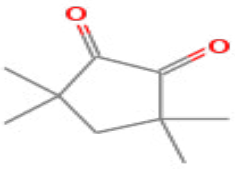
Oleic acid,propyl ester



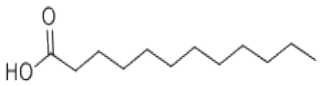
2, 6, 6-Trimethylcyclohex-2-ene-1, 4-Dione



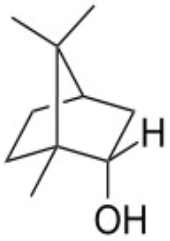
2-propenyl 3-cyclohexylpropenoate



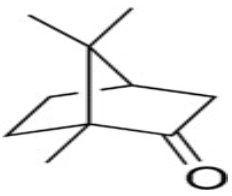
1,2-Cyclopentanedione,.3,3,5,5-tetramethyl



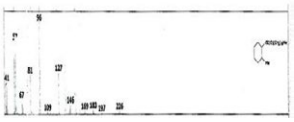
Dodecanoic acid



Borneol

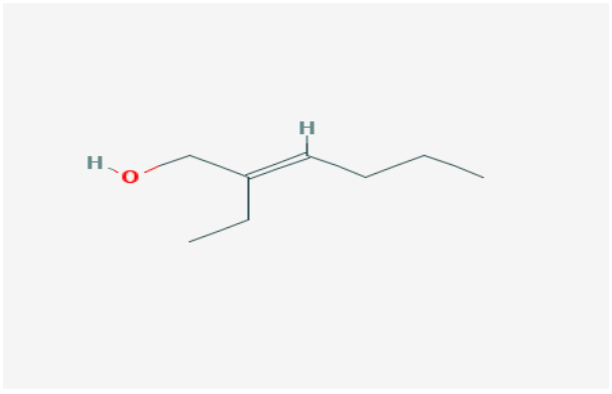


Camphor

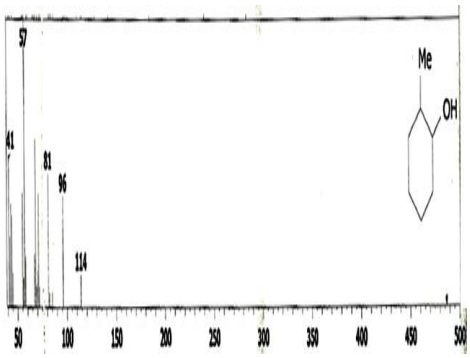


Octanoic,2-methylcyclohexyl ester trans

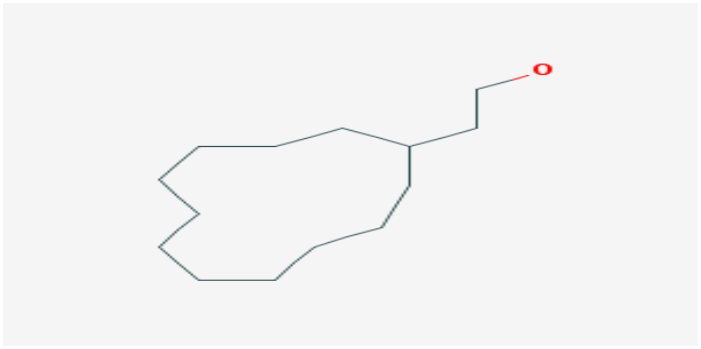
Figure 5 Terpenoids produced by *Bacillus amyloliquefaciens* S_5I_4 that interfere with *Aspergillus flavus* and *Staphylococcus aureus*.



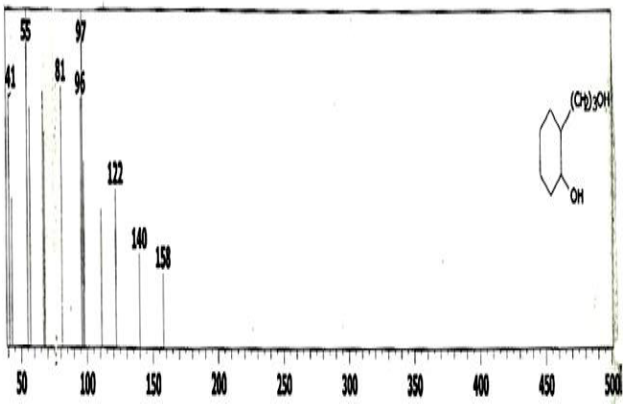
2-Hexene-1-Ol, 2-Ethyl



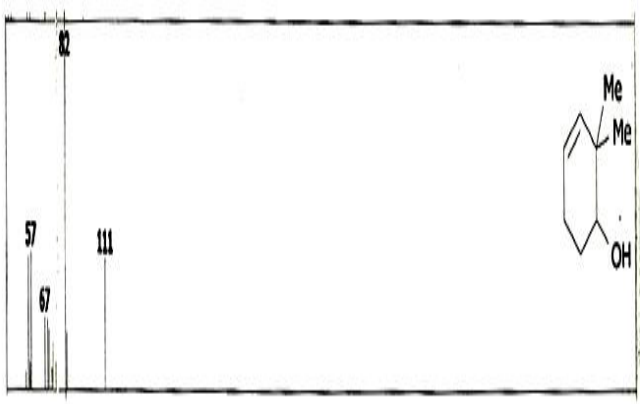
CYCLOHEXANOL, 2-METHYL Or 2-methylcyclohexanol



2-Cyclododecylethanol



3-(trans-2-hydroxy cyclohexyl) propanol



2,2Dimethyl-3-cyclohexene-1-ol or cyclohexene-1-ol,2,2dimethy

Nonacosanol

Dodecanol

Figure 6 Terpene alcohols produced by *Bacillus amyloliquefaciens* S_5I_4 that interfere with *Aspergillus flavus* and *Staphylococcus aureus.*

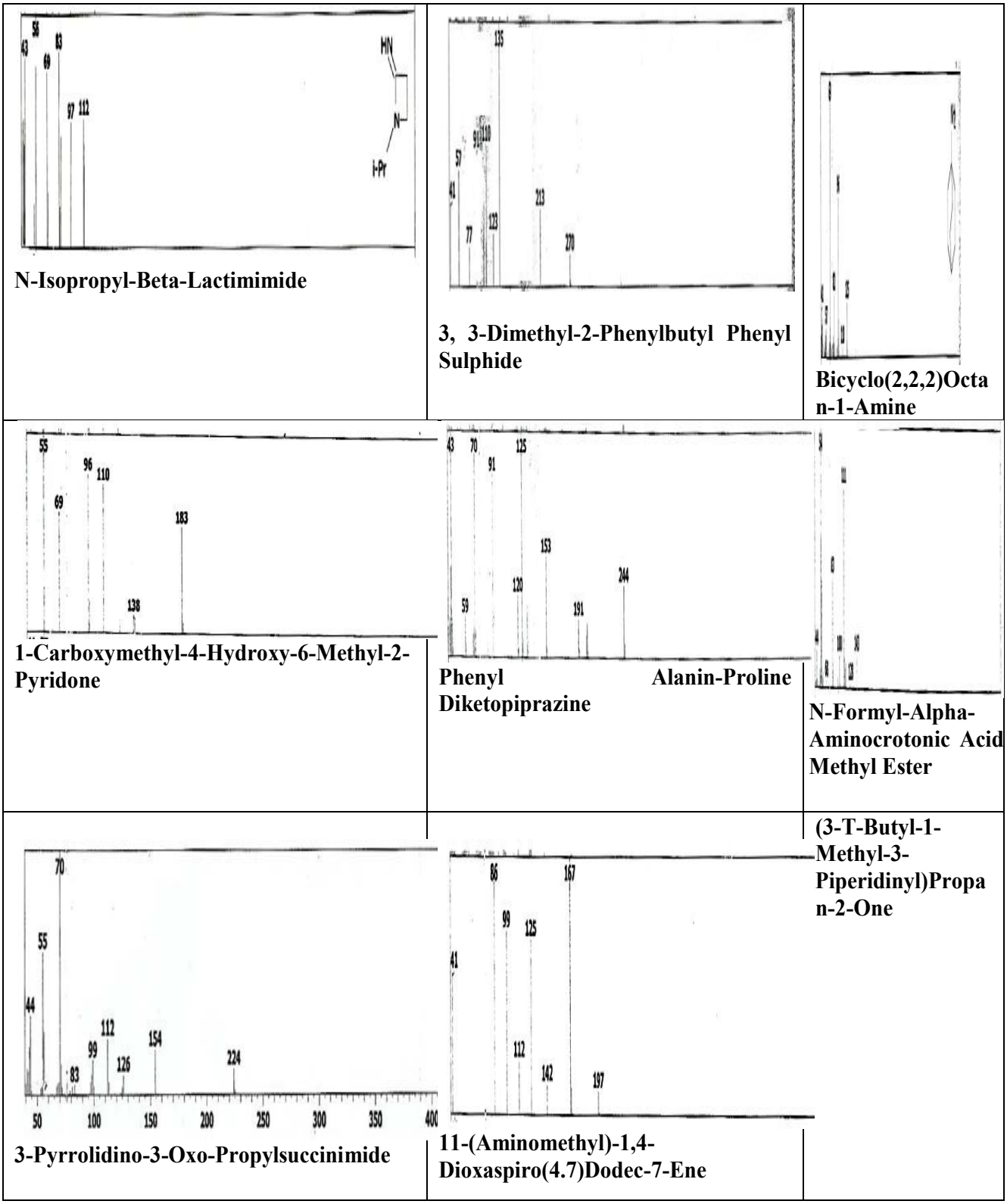
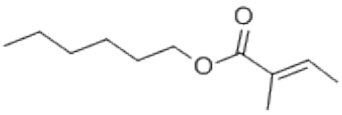
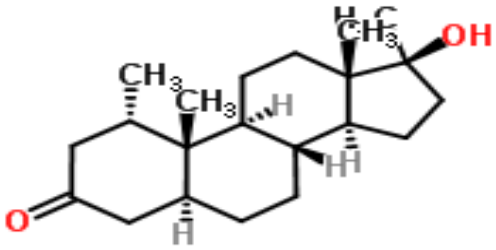


Figure 7 Nitrogenous compounds produced by *Bacillus amyloliquefaciens* S_5I_4 that interfere with *Aspergillus flavus* and *Staphylococcus aureus*.



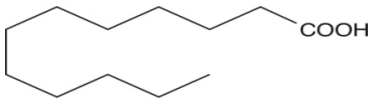
N-Hexyl Tiglate



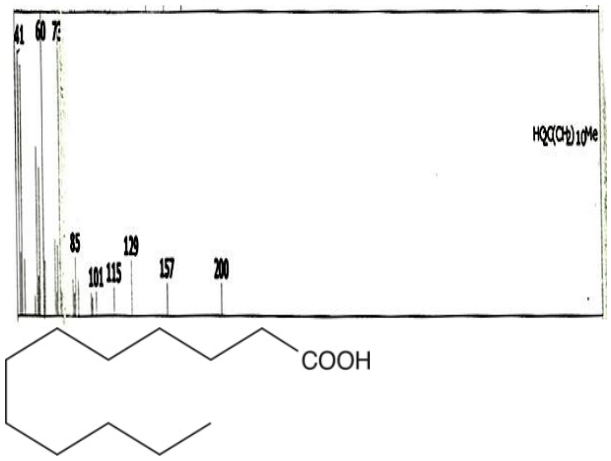
Androstan-3-One,17-Hydroxy-1,17-Dimethyl-(1 Alpha.,5 Alpha.,17 Beta) (Vitamen C)

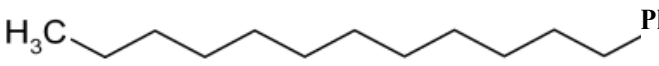
Hexadecanoic Acid Or Palmitic Acid

Dodecanoic acid or Lauric acid or Neo-Fat 12 or vulvic acid
Molecular weight: 200. Molecular form: $C_{12}H_{24}O_2$



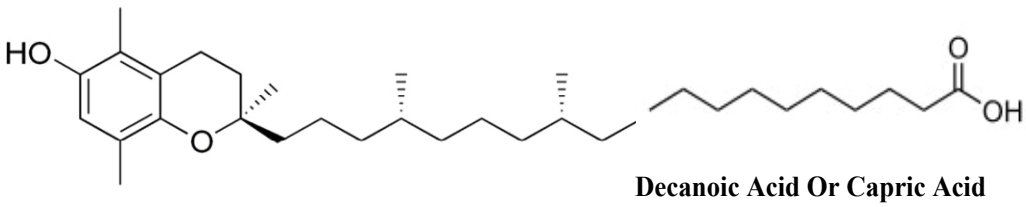
Dodecanoic Acid Or Lauric Acid





Phthalic Acid ,Didecyl Ester

Heptadecanoic Acid Or Margaric Acid



Decanoic Acid Or Capric Acid

Beta Tocopherol

Figure 8 Fatty acids produced by *Bacillus amyloliquefaciens* S_5I_4 that interfere with *Aspergillus flavus* and *Staphylococcus aureus.*

Conclusion

The identification of new bioactive compound with broad activity is very important to inhibit or kill the antibiotic resistance bacteria. The extraction and identification of the pure effective bioactive compounds from *B. amyloliquefaciens* strain as; terpenoids and alcohol terpenes were reported here in which previously known from essential oils. These compounds could be utilized both to make best use of their antibacterial and antifungal activities and to reduce the growth of pathogen required to achieve a particular antibacterial effect for food safety and health purposes. Also, required to achieve inhibition to pathogenic fungi, e.g. *A. flavus*.a

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