Identification of volatile organic compounds in *Aspergillus ostianus*

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Abstract
Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Separation and quantitative determination of a wide variety of organic and inorganic components in microorganisms are carried out by chromatographic methods. In this study, *Aspergillus ostianus* spores was grown in a liquid culture of potato dextrose broth (PDB) and the broth was filtered. The filtrate was extracted with EtOAc. The production of compounds were determined by TLC and GC-MS. The results showed that, *A.ostianus* produced different compound such as: Octanoic Acid, Metformin, Linoleic acid, Oleic acid, Palmitic acid, Myristic acid and Squalene.

Keywords: *Aspergillus ostianus*, GC-MS, Volatile compounds

1. Introduction

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Many bacteria and some fungi make one or more volatile organic compounds (VOCs). However, the composition of VOCs produced by microorganisms varies with the microbial species and its growth environment [7]. Fungi of the genus Aspergillus are soilborne ascomycetes that can be found all over the world. *Aspergillus spp*. are highly successful colonizers of their habitats, which is reflected both by their efficient utilization of the substrate at hand as well as their secretion capacity for antibiotic metabolites and enzymes. The separation and quantitative determination of a wide variety of organic and inorganic components in microorganisms are carried out by chromatographic methods. At present, analysis by gas chromatography–mass spectrometry (GC–MS) is essential for the identification of natural organic compounds obtained from cultures of *Aspergillus ostianus*. Usually , determination of volatile compounds, such as aromatic compounds, fatty acids, general hydrocarbons, and hydroxy or amino metabolites is achieved using GC–MS techniques. In this study, for detection of some volatile compounds from the cultures of *Aspergillus ostianus* performed by GC-MS [6].

2. Materials and methods

2.2. Cultivation conditions

In morphological studies, *Aspergillus ostianus* was identified based on standard identification keys. *A.ostianus* spores was grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25 ºC on a rotary shaker for 14 days at 130 rpm [6].

2.3. Extraction procedure

For extraction of volatile compounds from the mycelium, the cells were lysed by adding 10 ml Ethyle acetate to each flask and transferring the upper transparent phase to a new tub [5]. The suspension

was incubated at room temperature (25°C) for 15 - 20 min. The residue was resuspended in 500 µl of methanol and aseptically filtered using a 0.2 µm syringe filter. It was kept at 4°C before been used for TLC and GC-MS tests [9].

2.3. Thin layer chromatography

Twenty µl of fungal extract were applied to a silica gel TLC plate (Merck) and metabolites were separated in the developing solvent toluene:ethyl acetate:formic acid (TFA, 5:4:1). Photographs were taken following exposure to UV radiation at 302 nm [2].

2.4. GC-MS

Separation of volatile compounds was achived using a gas chromatography . An Hp-5MS fused silica capillary column (Hewlett- Packed, 30 m, 0.25 mm i.d., 0.25 µm film Thickness, cross-linked to 5% phenyl methyl siloxane stationary phase) was used . The entire system was controlled by MS ChemStation software (Hewlett- Packed, version A.01. 01). Electron impact mass spectra were recorded at 70 eV. Ultra-high purity helium (99.999%) was used as the carrier gas at flow rate of 1mL/min. The injection volume was 1µL and all the injections were performed in a splitless mode. Injector and detector temperatures were 270 and 280 ºC respectively. Column over temperature was initially set at 50 ºC for 5 min, then increased to 260 ºC (ramp, 5ºC/min), and held for 5 min.

3. Results and discussion

3.1. TLC analysis

Spores of *Aspergillus ostianus* were inoculated into PDB liquid media and incubated for 14 days at 25°C. The secondary metabolites were extracted with ethyl acetate and extracts were loaded on to UV-coated silica TLC plates. Metabolites were separated in developing solvent (toluene:ethyl acetate:formic acid 5:4:1). A TLC examination of Ethyle acetate extract of *A. ostianus* showed the production of several metabolites(Fig.1).

3.2. Identification and Biological activity of compounds

Aspergillus ostianus grown on PDB media produced different volatile compounds. Metabolites obtained by GC-MS analysis are collected inTable 1. The different groups in the compounds separated by GC–MS, including some of alkenes, alcohols, ketones, aldehyes ethers, esters, terpenes, fatty acids and other volatile compounds. Some of the isolated compounds were Ocatadecan, Palmitic (Hexadecanoic) acid, Caprolactam, Tridecane, Heptadecane, Eicosane [6], Myristic (Tetradecanoic) acid, Linoleic (9,12-Octadecadienoic) acid, Oleic (9-Octadecenoic) acid [1,3], Squalene[5] Octanoic (Caprylic) acid [8] and Metform. The Linoleic acid (Polyunsaturated), Oleic acid (Unsatuted) Palmitic acid and Myristic acid (Saturated) fatty acids have antifungal activity against several plant pathogenic fungi [4]. Chemical structure and chromatograms of some compounds are shown in figure 2 and 3 Respectively.

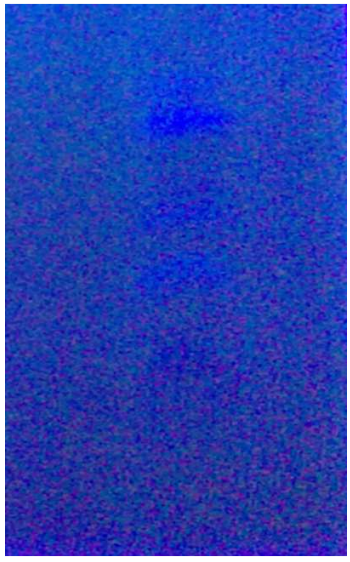
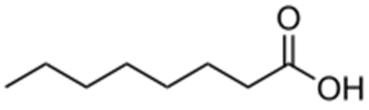


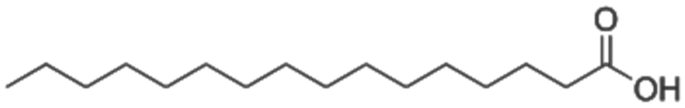
Fig. 1 TLC analysis of production of secondary metabolites *A. ostianus* in PDB. Metabolites were separated in developing solvent (toluene:ethyl acetate:formic acid,5:4:1) and photos were taken following exposure to 302 nm UV radiation.

Table 1. GC-MS analysis of the VOCs produced by *Aspergillus ostianus* after 14 days incubation in a PDB medium at 25 ºC .

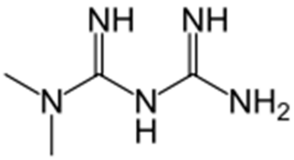
Compounds	Molecular formula	Retention time (min)	Abundance(%)
Ocatadecan	$C_{17}H_{36}$	33.685	74
Palmitic acid	$C_{16}H_{32}O_2$	36.026	99
Myristic acid	$C_{14}H_{28}O_2$	32.896	99
Eicosane	$C_{20}H_{42}$	38.601	99
Linoleic (9,12-Octadecadienoic) acid	$C_{18}H_{32}O_2$	41.685	99
Oleic (9-Octadecenoic) acid	$C_{18}H_{34}O_2$	41.811	99
Squalene	$C_{30}H_{50}$	55.103	91
Metformin	$C_4H_{11}N_5$	13.727	27
Octanoic (Caprylic) acid	$C_8H_{16}O_2$	15.375	59
Caprolactam	$C_6H_{11}NO$	16.067	43
Heptadecane	$C_{17}H_{36}$	43.087	96
Tridecane	$C_{13}H_{28}$	51.046	89



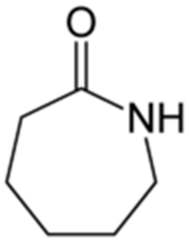
Octanoic Acid



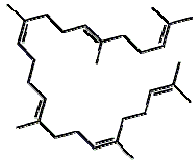
Palmitic acid



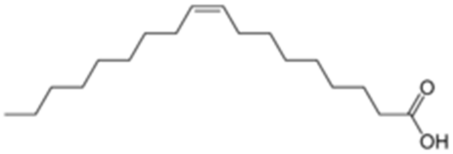
Metformin



Caprolactam



Squalene



Oleic acid

Fig. 2. Chemical structure some of compounds extracted from *A.ostianus*

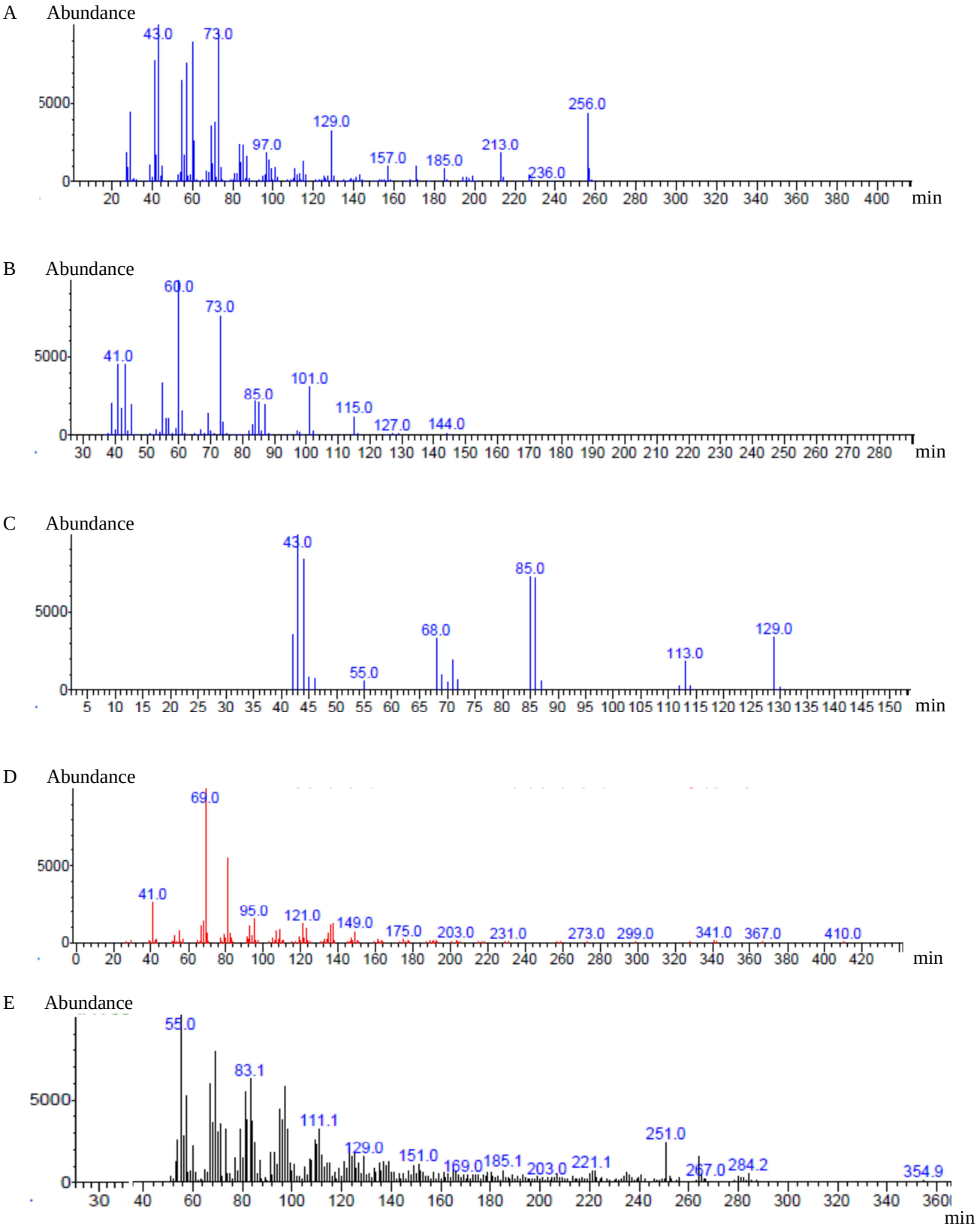


Fig. 3. The parts (A, B, D and E) of gas chromatography and mass spectrometry (GC–MS) of volatile compounds identified from the cultured *A. ostianus:* Palmitic acid(A), Octanoic Acid (b), Metformin (c), Squalene (d), Oleic acid (E).

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