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# MICROBIAL BIOTRANSFORMATION OF BIOACTIVE NATURAL PRODUCTS FOR DRUG DISCOVERY

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**Abstract:** Microbial biotransformation is used instead of chemical synthesis or mammalian models to study drug safety where many fungi possess cytochrome p450 as in mammalian. Biotransformation is a process used to convert one molecule to other by whole-cell or isolated enzymes. About 40 fungi were isolated from different soil and plant residues. All these isolates were identified morphologically and used to study their potential to transform alkaloid piperine. Among these fungi, four fungi namely Aspergillus herbariourm, Aspergillus ochraceous, Cladosporium sp, and Stachybotrius sp showed the ability to metabolize piperine. Among the four fungi, only Aspergillus herbariourm was used in the preparative scale fermentation procedure to give five metabolites identified by GC/MS analyses technique. These metabolites were identified as hexyl(methylidyne)ammonium, dimethyl phenyl ethyltethylidyl pyrrolidine, (4E)-hepta-1.2.4.6-tetraeane methyl laurate, 6,7diazabicyclo[3,2,2] and non a-3,6-diene,2-methylene.

**keywords**: Natural products, Fungi, Biotransformation, Piperine.

## 1.Introduction

It's taken for granted that, green chemistry has become a boundary area for producing more eco-friendly medicinal molecules. It has led to the transformation of compounds, through which hazardous chemical reactors can be avoided to arrive at a new product. Various methods are used to manufacture substances as; super-critical fluids microwave synthesis, catalysis of phase transfer, and, microbial biotransformation[1]

Biotransformation is a term used to explain enzyme-catalyzed reactions, including the utilization of cell extracts or isolated enzymes when chemicals are modified to another [2]

From both active and inactive natural products, biotransformation produces fine chemicals, agrochemical and, pharmaceutical compounds. Biotransformation also is appealing ways in chemical libraries that produce structural diversity to synthesize molecules difficult to achieve by conventional chemistry procedures [3].

The preservation of the original carbon skeleton after acquiring the products is one of the most noticeable aspects of biotransformation processes. The carbon atoms are transferred to other molecules with distinct chemical activities throughout metabolism[4]

Syntheses of drugs via biotransformation processes undergo three different principal approaches with a variety of complexity; (1) A purely chemical technique is applied. (2) A combination of biocatalysts and chemicals by using a chemo-enzymic pathway. (3) Use the complete biological synthesis via fermentation or biotransformation in many steps [5]

It is possible to identify two different kinds of biotransformation: I) xenobiotic; in which the substrate is entirely not identified to the organism II) biosynthetic: The substrate is associated with a natural intermediate of the organism's metabolic pathway [6]

The important aspects of using catalysts in biotransformation are regio-stereoselectivity reaction is high, it has a high degree of

enantioselectivity[7]. Limitation of the release of environmentally hazardous substances as heavy metal or chlorine [8]

This study aims to produce an alternative system to study drug metabolism (piperine) by the microbial system instead of mammalian models, also to produce large quantities of active metabolites.

## 2. Materials and methods

### Collection of soil and peanut samples

Soil samples were collected from different locations in Dakahlia, Talkha, Manzala and, Belkas at 10 cm depth. The sample was put in sterilized labeled bags stored at 4 °C. Peanut seeds were collected from the local markets from different regions.

### Isolation of fungal strains

### Isolation of strains from soil

### Soil Dilution Plate Method

1g of soil from each sample was suspended in 10ml of sterile distilled water to make soil dilutions. To avoid overcrowding of the fungal colonies, dilutions of $10^{-3}$, $10^{-4}$, and $10^{-5}$ were utilized to isolate fungus. In triplicates of each dilution, 1ml of each concentration's suspension was applied to sterile Petri dishes containing sterile Potato Dextrose Agar medium. Before pouring the medium into Petri plates, a 1% streptomycin solution was added to inhibit bacterial growth. The plates were then incubated for 4-7 days at 28$^{o}$C [9]

### Blotter methods

Seeds were plated in pre-sterilized Petri dishes with three layers of moist blotters, each containing ten seeds arranged in peripheral and middle. All Petri dishes were incubated for 7 days at 25°C. A stereomicroscope and a microscope were used to examine the seeds. The purification of fungus was accomplished by utilizing fine glass capillary tubes to transmit hyphal tips to Petri dishes with PDA for 7 days at 28$^{o}$C [10]

### Maintenance of cultures

All fungus were retained at 4$^{o}$C on Dextrose potato (potato, 20 g / 100ml; 20 g; dextrose, 10 g; yeast extract; 100ml distilled water with pH adjusted to 5.6) [11].

### Chemicals

piperine 98% fine powder has been provided from Alfa Aesar, Kandel, Germany

Analytical thin-layer chromatography was performed on precoated silica gel 60 GF254 (20 x 20 cm x 0.2 mm thick) on aluminum sheets (Merck or Machery-Nagel, Germany). Visualization under UV-lamp 254 and 366 nm (Desaga, Germany). GC analysis was performed using a Hewlett Packard 5890A Series II gas chromatograph with Colum HP-5MS (30 m × 0.25 mm id × 0.25 μm film thickness) coated with 5% phenylmethyl siloxane Helium(He) gas (flow rate: 1 mL/min) is a carrier gas. Sample injection temperature conditions: 250 -280◦C. (α- medium) (dextrose, 20 g; NaCl, 5 g; $K_2HPO_4$, 5 g; peptone, 5 g and, yeast extract, 5 g: per liter of distilled water). Then autoclaved media for 20 min at 121 °C

### Microbial Transformation

For the first screening, in 250 Erlenmeyer flasks, added 50 ml of autoclaved α-medium. Then each flask inoculates with 40 strains of fungi as a fresh spore, incubate for 48 h at 28°C. After 48 h, 0.025 mg of piperine dissolved in 20 ml DMSO, divided equally to each flask, then Incubate at 28 for 14 days at 140rpm on a rotary shaker at 28°C [12].

Two types of control were run in the same way, one containing autoclaved α- medium and fresh spores, the second containing α-medium and substrate, then incubating for 14 days at 28°C in a shaker incubator at 140 rpm [13].

### Extraction

Extraction of culture achieved by using 3 equal volumes of ethyl acetate in separating funnel for 1 min, dried over anhydrous$Na_2SO_4$ evaporated under reduced pressure using rotary vacuum evaporator, then drying at room temperature in vacuum oven [14].

### Metabolites screening

TLC applied for screening of transformation of piperine by fungi. The parent substrate, control and, extract; were spotted on TLC silica plates and. The transformed products were noted at regular intervals until the complete transformation was monitored. In a glass jar, the samples were eluted with a 6:4 ratio of ethyl acetate to hexane as the mobile phase. After air

drying, the plates were seen under UV light at 254nm and 363nm in a UV light room [15].

**Biotransformation in the preparative scale and isolation of the metabolites**

The fungus which shows positive results in the biotransformation procedure was used in large-scale fermentation procedures. This was performed in the same manner as the first screening. 24 (500 ml) Erlenmeyer flasks contain 100 ml autoclaved α- medium, inoculated with *Aspergillus herberiourm* then incubate at 28°C for 48 h. After two days (1.200mg) of piperine was dissolved in 60 ml DMSO and equally divided among each flask then incubate at 28°C for 14 d at 140 rpm on a rotary shaker. Then extracted with three equal volumes of ethyl acetate "EtOAc". Dried with sodium sulfate and, evaporated under reduced pressure. The residue was monitored by silica gel TLC plates using solvent system EtOAc: n-hexane "6:4" in a glass jar then spraying with p-anisaldehyde reagent and heated for 1min [16].

**2.Metabolite identification**

GC was used to determine the composition of the product mixture. the solutions were dissolved in methanol, diluted to 10 μg/ml[17]. Firstly, injection of the sample into the GC inlet, where the carrier gas (mobile phase) (helium) vaporizes it and pushes it into a capillary column. The sample passes through the column, and the chemicals that make up the mixture of interest are separated by their interactions with the column's coating (stationary phase) and the carrier gas. The column's final section flows via a heated transfer line before terminating at the ionization source, where substances eluting from the column are transformed to ions. This separated in a mass analyzer after that. The ions then pass via a detector. The detector delivers data to a computer, which saves all of the information and turns electrical impulses into visual and hard copy displays [18].

**3. Results and Discussion**

**Identification of isolated fungi**

The isolated fungi were identified based on macro-morphology and characteristics by interval microscope according to the following references: [19][20][21]

**Screening procedure**

After 14 days, incubation of fungi in α media with piperine, extraction with ethyl acetate, then dried over anhydrous $Na_2SO_4$, the extract was monitored on TLC, comparing the extract with control and parent product (piperine) prove that only 4 fungi show several spots at different retention factors (Rf), these spots at 245nm didn't show any quenching, but give violet color when spraying by p anisaldehyde/$H_2SO_4$ reagent then heating for 1 min at 105°C. This result indicates that piperine has been transformed (Figure 1).

**Table (1)** Identification of the isolated fungi

| | |
|---|---|
| **I.zygomycetes** | Genus: Penicillium |
| Abasidia corymbifera Tigh | P. chrysogenum Thom |
| Genus: Mucor | P. citrinum Thom |
| Mucor sp Hagem | P.fellutanum Keith |
| **II. Dothideomycetes** | P. notatum Thom |
| Genus: Alternaria | P. purpurogenum Stoll |
| Alt.alternat Keissler | Genus: Rhizopus |
| Alt.solani Sorauer | R. azygosporous |
| Genus: Cladosporium Pers | R.microsporou Tiegh |
| **III-Eurotiomycetes** | R.solanifer Vuillemin |
| Genus: Aspergillus | **IV.mortierellomycetes** |
| A.aculeatus Izuka | Genus: Mortierella |
| A. carbonarius | M.polycephala Coeans |
| A. carneus Blochwitz | **IV.Sordariomycetes** |
| A. herbariorum Fisch | Genus: Chaetomium |
| A. flavus Link | Chaetomium sp |
| A. flavipes Bainer | Genus: Fusarium |
| A. niger Tieghem | F. incarnatum Sacc |
| A. nidulans G.winter | F.oxysporum Schlech |
| A. ochraceus Wilhelm | F. poae |
| A. oryzae E.cohn | F.semitectum Berk |
| A. parasiticus Speare | F. iricum |
| A. sclerotiorum Huber | F. solani Mart(Sacc) |
| A.sydowii Thom | Genus: Trichoderma |
| A. terreus Thom | T. hamatum Bainier |
| Genus: Paeciloyces | T. harzianum Rifar |
| P.variottii Bainier | T. viride Pers |
| P. rugulosum Thom | Genus: Stachybotryssp Corda |
| P. wortmannin | |

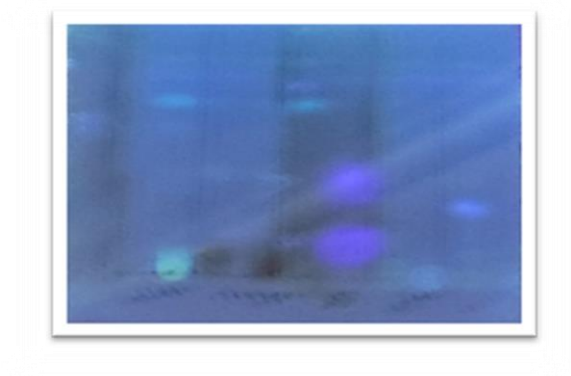

**Figure (1):** TLC of the metabolized piperine

**Large scale production**

Among the four fungi, only *Aspergillus herbariorum* was used in preparative scale fermentation, identified by GS/MS. *Aspergillus herbariorum* shows a partial transformation of piperine. Metabolisms of piperine yielded 5 metabolites, only 5 metabolites were shown to have resulted from transformation, while other metabolites were obtained from multistep synthesis reactions. The characteristic patterns of detected components were given in (Table 2).

**Table (2):** Identification of transformed products by GC/MS.

| Peaks | RT | Area Sum % | Mwt |
|---|---|---|---|
| Peak 1 | 3.093 | 14.5 | 112.11 |
| Peak 2 | 3.316 | 0.45 | 277.4 |
| Peak 3 | 3.448 | 1.33 | 92.15 |
| Peak 4 | 5.113 | 1.42 | 106.0 |
| Peak 5 | 5.336 | 4.65 | 134.2 |

M1 had retention time (3.093) corresponding to hexyl(methylidyne)ammonium with formula ($C_7H_{13}N$) (figure 2A). M2 had retention time (3.339) corresponding to dimethyldiphenyl ethyltethylidyl pyrrolidine with formula ($C_{20}H_{23}N$) (figure 2B). M3 had retention time (3.465) corresponding to (4E)-hepta-1.2.4.6-tetraeane with formula($C_7H_8$)(figure 2C). M4 had retention time (5.141) corresponding to methyllaurate with formula ($C_8H_{10}$) (figure 2D). M5 had retention time (5.284) corresponding to 6,7diazabicyclo[3,2,2]non a-3,6-diene,2-methylene with formula($C_8H_{10}N_2$) (figure 2E).

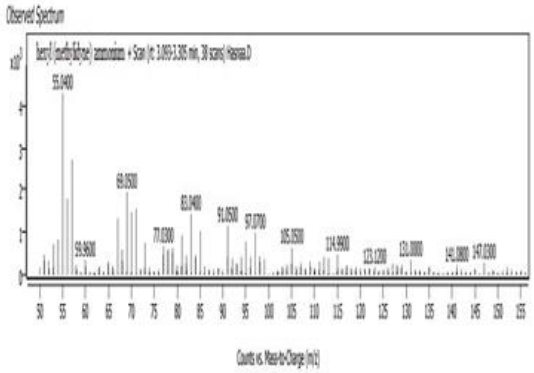

**Figure 2A:** Chromatogram of hexyl(methylidyne)ammonium

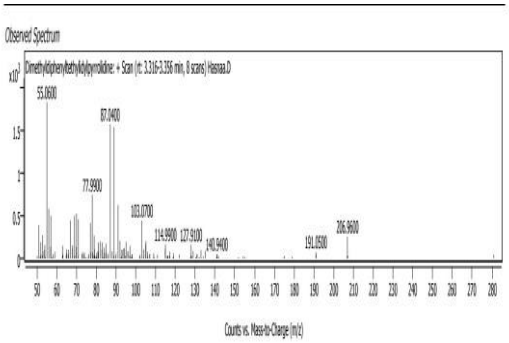

**Figure2B:**Chromatogramofdimethyldiphenyl ethyltethylidyl pyrrolidine

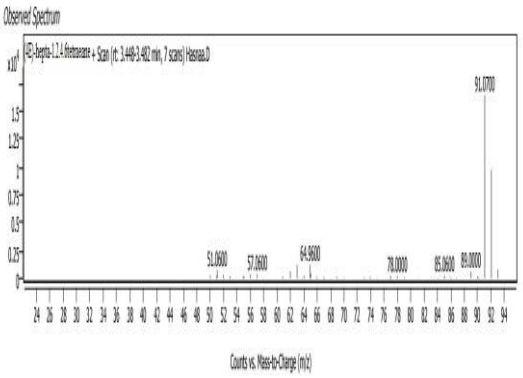

**Figure 2C:** Chromatogram of (4E)-hepta-1.2.4.6-tetraeane

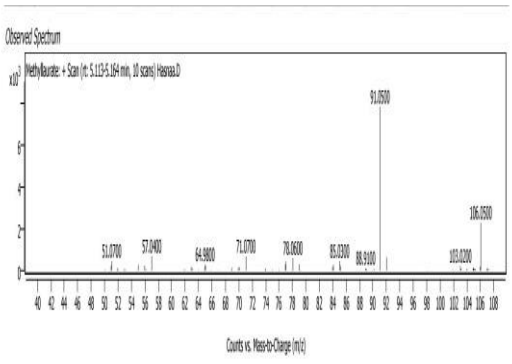

**Figure 2D:** Chromatogram of Methyllaurate

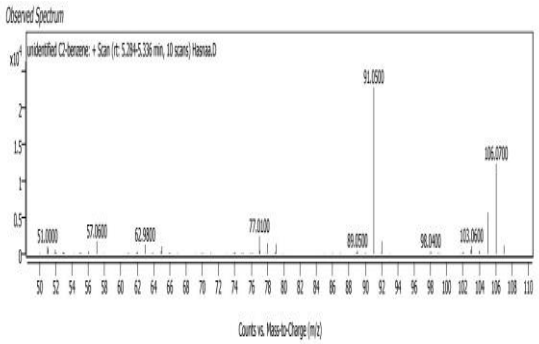


**Figure2E:**Chromatogramof6,7diazabicyclo[3,2,2]non a-3,6-diene,2-methylene

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