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Mutational Effect of *Penicillium griseoroseum* on Genetic Composition and Secondary Metabolites Production

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Abstract

Spores of *P. griseoroseum* were gathered from the atmosphere, this facilitated the identification of the fungus by looking for a internal spacer (ITS) that was documented by NCBI, PQ721627. Following the application of ultraviolet light at a wavelength of 253nm to the conidial suspension of *P. griseoroseum* at different distances of 15 cm from the source (UV1=2, UV2=4, UV3=6) min., the extracts were evaluated by GC-MS with regard to their secondary metabolites. 1-O-p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-.beta.-d-galactose, -Hexadecenoic acid, methyl ester, (Z)-, Triacontane, 11,20-didecyl-,d-Arabino-hexonic acid, 2-deoxy-3,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester, bis, and Androsta-3,5-diene-3,17-diol, 3-(heptafluorobutanoate) 17-(pentafluoropropanoate) are among the new chemical compounds that were identified as a result of the mutations, there are also some compounds that have disappeared due to mutation such as 2- (N-Methyl-N-nitroamino)-4-[2,2,3,3,3-pentafluoro-1,1-bis(trifluoromethyl)propyl]-6 and Octatriacontane, 1,38-dibromo- . To determine how much radiation altered the genetic composition of the fungus, six primer pairs were inserted into the DNA - OPC-08, OPL-07, OPA-10, OPA-18, and OPB-02 . There was a clear impact on the following primers : OPC-08, OPA-18, and OPB-02.

Keywords:-

تاثير التطفير على التركيب الجيني وانتاج الايض الثانوي لفطر *Penicillium griseoroseum*

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الخلاصة

تم جمع سبورات الفطر *P. griseoroseum* من الهواء، شخص الفطر من خلال التعرف على مناطق (ITS) وتم توثيقه في بنك الجينات (NCBI)، بالرقم PQ721627. بعد تعريض المعلق البوغي للفطر *P.griseoroseum* للاشعة الفوق البنفسجية بطول موجي 253 نانومتر على مسافة تبلغ 15 سم من المصدر وبأوقات مختلفة (UV1 = 2، UV2 = 4، UV3 = 6) دقيقة، تم تقييم مستخلصات الايض الثانوي بواسطة GC-MS. ومن بين المركبات الايض الثانوي الجديدة التي تم تحديدها بعد عملية التطفير: 1-O-p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-.beta.-d-galactose, -1 Hexadecenoic acid, methyl ester, (Z)-, Triacontane, 11,20-didecyl-,d-Arabino-hexonic acid, 2-deoxy-3,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester, bis, and Androsta-3,5-diene-3,17-diol, 3-(heptafluorobutanoate) 17-(pentafluoropropanoate). كما ان بعض المركبات اختفت بعد التطفير مثل 2-(N-Methyl-N-nitroamino)-4-[2,2,3,3,3-pentafluoro-1,1-bis(trifluoromethyl)propyl]-6 and Octatriacontane, 1,38-dibromo- ولتحديد مدى تأثير الإشعاع على التركيب الجيني للفطر، تم إدخال ستة أزواج من البادئات في الحمض النووي - OPC-08، وOPL-07، وOPA-10، وOPA-18، وOPB-02.

1. Introduction

Penicillium is a popular genus with more than 300 species. Typically, it can be found in wood, dry products, aromatics, cereals, soft fruits, vegetables, soil, decomposing plants, and organic waste. It can also be found in your home's dust and air, as well as in the humidity of construction materials [1].

The consistent shape of the spores and the development of long chains of green spores are both recognized as features that distinguish Penicillium species [2]. They are typically associated with the restoration of organic materials and the production of mycotoxins in food and cereal products, including patulin, ochratoxin A, penicillin and patulin A. They also have an important role in the creation of Camembert and other cheeses. Also, phosphorus in the soil is accumulated by other species, this release benefits plant growth[3],[4].

Proteins, carbs, and lipids are primary metabolites that are vital for development and for generating energy used in intricate metabolic processes such as fermentation and respiration[5] . The last phases of growth involve stationary mycelium cells that start secondary metabolism. These are chemical pathways and biological processes that add complexity to the outcomes of the main metabolic processes[6].

One of the most important functions of the microbe world is to make the fungal environment as conducive as possible to the development of new chemicals. The numerous synthetic clusters of genes in the fungal genome that is in a state of flutter suggests that the secondary metabolite reservoir is still unexposed[7]. Fungi may also release other chemical compounds from their filaments that are released into the culture medium, or they may be incorporated into structural components of cellularity, or they may remain cell-bound. The term "extrolite" has been proposed as a replacement for the term "biomimetics", the concept was initially derived from the idea that the biological effects of these components are regulated [8]. However, the mechanism and location of the cellular biosynthesis of many of these components is still unknown, and has a variety of random effects, therefore, we chose for the traditional term "metabolic waste"[9]. The most common approach to achieving high yields is to utilize specific genetic tools to increase the mortality rate of pathogenic microorganisms, or to produce goods that have a useful purpose in industry, such as random collections of colonies that were created in bottles after the mutation. Another common strategy is to choose mutants with singular genetic diversity in their physical characteristics, essential genes are responsible for creating complex compounds that serve as secondary metabolites in living organisms or cells, in addition genes that regulate the levels of chemical precursors and necessary chemicals for the creation of secondary metabolites [10].

One of the best techniques in molecular biology is the in vitro mutagenesis process, which is essential for accomplishing research objectives. This process is crucial for observation and is dependent on the formation of mutations in a DNA helix that are meant to impact the gene responsible for the mutagenic process[11].

Numerous physical processes, including radiation, gamma rays, genetic transformation, transduction, T-DNA, and the activation of transposons in the same DNA strands, can alter DNA[12].

In addition to occurring spontaneously, natural mutations can also be brought on by biological entities including bacteria, fungus, viruses, and similar organisms. Interestingly,reactive oxygen species (ROS) can be produced during DNA repair or during the natural process of hydrolysis, which can yield oxygen species with a reactivity similar to that of replication[13].

These mutations can also cause proteins that have a peculiar composition, alter the composition of these proteins, or create new functions for them that are intended for practical application in business. The creation of transgenic strains can also have practical applications or serve as a molecular framework for studying specific cellular properties [14].

2. Material and Methods

2.1 *Air-borne Fungi*

The dishes that contained (PDA) medium were exposed to air for half an hour then incubated at 25 degrees Celsius for five days in order to collect spores from the atmosphere [15].

2.2 *Fungi diagnosis*

The genetic material was extracted using (zymo-ecological/DNA™ Miniprep Plus Kit), used specific primer (Table. 1) and certain program (Table.2)[16].

Table 1- Specific prime

Primer	Sequences
Forward	TCGATGATGACCTCACTCTTTGC
Revrse	TTCCTATATACTTTCAGCTTATAGT

Table 2- Certain program

No.	Phases	Temp.	Time	Cycles
1	Initial denaturation	97	4 min.	1
2	denaturation	92	40 sec.	
3	Annealing	55	2 min.	40
4	Extension	75	2 min.	
5	Final extension	75	8min.	1

2.3 *Exposing Fungus to Ultraviolet Rays and extract of secondary metabolise*

The volume of the suspension containing *P. griseoroseum* conidia was approximately 107 CFU/ml. All three vials were placed on a shaker and exposed to light of the appropriate wavelength at a distance of 15 cm from the source of radiation. However, the amount of time each vial was exposed to the light was different: (UV1 2, UV2 4, and UV3 6) min. (paraphrased) After the conidia were exposed to 253 nm UV radiation, 1 ml of the suspension was added to three bottles, each containing 100 ml of nutrient solution. Next, the voltage of *P. griseoroseum* was incubated on a rotary shaker at 25 °C and 150 rpm for 10 days (1 V: 100 V) before and after mutation. After traversing the culture medium through the filter, the fungal hyphae were gathered and dried with a filter and a cloth. Other metabolites were transferred to the GC-MS instrument following the dissolution of the substance in 99% ethanol.

2.4 *Gas Chromatography-Mass Spectrometry (GC-MS)*

Gas chromatography (GC-MS)-ultraviolet light emission scans of extracts from *P. griseoroseum* were conducted to eliminate the complexes. The 7022A technology was employed. It had a American spectrometer that measured mass spectra (5870E). A poetic column (length 35 m, inner diameter 230 micrometers, program width 0.30 micrometers) of the prototype DPS_MS was employed, the column was coated with 10% biphenyl and 99% dimethyl polysiloxane. High quality helium (99.999%) was employed as the gas flow, its flow order was 70.00 degrees Celsius, the feed's temperature was 270.00 degrees Celsius, the pressure was 96.1kPa, and the total flow was 550ml/min[17].

2.5 *Random Amplified Polymorphic DNA (RAPD)*

As shown, this experiment used six distinct random primers obtained from Operon Biotechnology (Table 3) [18]and [19].

Table 3- Random primers, their binding sequences and binding temperatures (TM).

ranking	Primers	Sequence of Primers (5' to 3')	TM
1	OPC-08	TGGACCGGTG	39
2	OPL-07	AGGCGGGAAC	39
3	OPL-18	ACCACCCACC	39
4	OPA-10	GTGATCGCAG	32
5	OPA-18	AGGTGACCGT	36
6	OPB-02	TGATCCCTGG	32

Before employing a thermal cycler to enhance the quality of primers, samples must be prepped for this.

- Increase the volume of water in a suitable container by 1-23 µL.
- Add 2-1 ml of random nucleotides.
- Add 3-6 µl of the DNA from *P. griseoroseum.*
- Add 4-20 ml of the master mixture to the mixture and stir well.

3. Results and Discussion

The fungal spores were isolated after leaving the culture medium plates exposed to the air. After isolation more than once, a pure culture of the fungus was obtained (Figure 1).

A **B**

Figure -1 (A) *P. griseoroseum* colonies on PDA media, *P. griseoroseum* under microscope 40x

P. griseoroseum was recognized by utilizing the internal transcribed spacer (ITS) in the fungus's DNA via PCR technology (Figure 2, and 3) and was documented in (NCBI) PQ721627.

CGGGGGGCNTGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCT
TGTGTGTNGGGCCCCGTCCTCCGATCCCGGGGGACGGGCCCGAAAGG
CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCAC
CCGCTCTGTAGGCCCGGCCGGNGCTTGCCGATCAACCCNAATTTTTA
TCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCA
TATCAATAAGCGGAGGAAAAGAAAA

Figure -2 DNA sequences

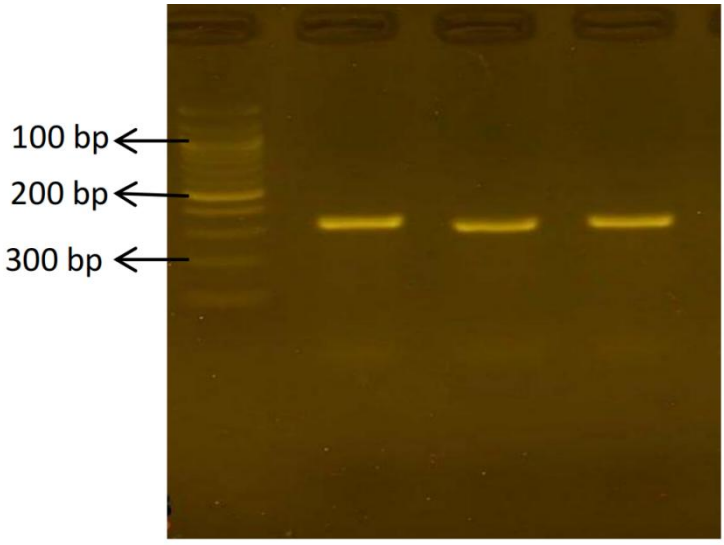

Figure -3 DNA extraction

When the metabolite extracts from R. griseus were evaluated using GC-MS, dissimilarities in the mass and volume of compounds produced by the fungus before and after the mutation UV_3 = 6 min. were observed (Figure 4), (Table 4) and (Figure 5), (Table 5).

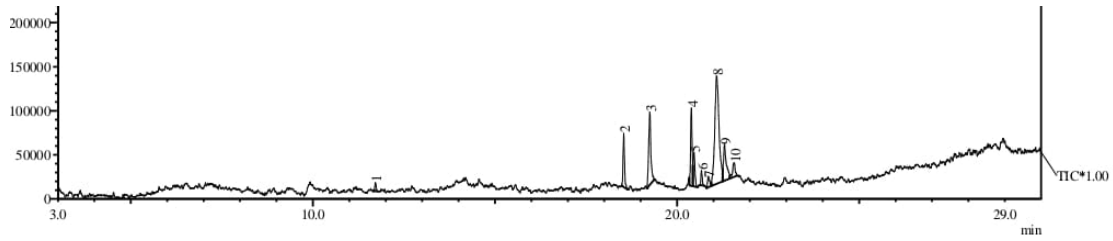

Figure -4 Chromotogram of secondary metabolities of *P.griseoroseum* before mutation.

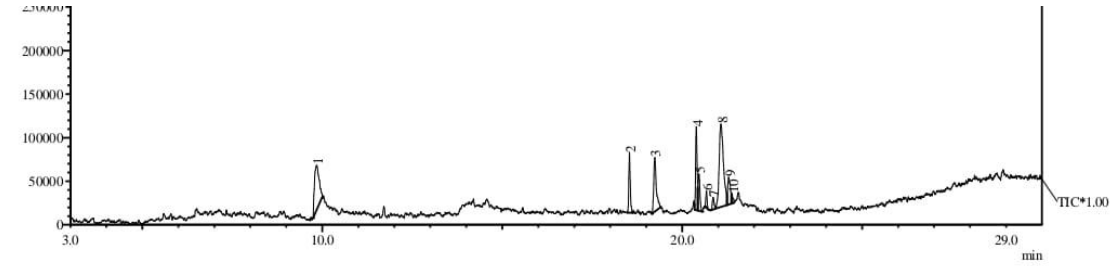

Figure -5 Chromotogram of secondary metabolities of *P.griseoroseum* after mutation by UV_3= 6 min.

Table 4- Secondary metabolities of *P.griseorodeum* before mutation

Peak	Time	Area%	Name
1	11.716	1.00	Octatriacontane, 1,38-dibromo-
2	18.535	7.33	Pentadecanoic acid, 14-methyl-, methyl ester
3	19.251	14.78	n-Hexadecanoic acid
4	20.392	11.08	10-Octadecenoic acid, methyl ester
5	20.465	5.31	10-Octadecenoic acid, methyl ester
6	20.670	1.76	2-(N-Methyl-N-nitroamino)-4-[2,2,3,3,3-pentafluoro-1,1-bis(trifluoromethyl)propyl]-6
7	20.850	2.08	Serverogenin acetate
8	21.086	43.45	cis-Vaccenic acid
9	21.301	9.73	Octadecanoic acid
10	21.564	3.48	5,9-Tetradecadiyne

Table 5- Secondary metabolities of *P.griseorodeum* after mutation by UV_3= 6 min

Peak	Time	Area%	Name
1	9.843	18.86	1-O-p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-.β.-d-galactose
2	18.534	7.82	Pentadecanoic acid, 14-methyl-, methyl ester
3	19.239	12.54	n-Hexadecanoic acid
4	20.393	11.09	10-Octadecenoic acid, methyl ester
5	20.467	5.83	7-Hexadecenoic acid, methyl ester, (Z)-
6	20.674	1.78	Triacontane, 11,20-didecyl-
7	20.863	1.91	d-Arabinic acid, 2-deoxy-3,4,5-tri-O-(trimethylsilyl), trimethylsilyl ester, bis(trimethylsilyl)
8	21.078	32.47	cis-Vaccenic acid
9	21.291	6.50	Octadecanoic acid
10	21.383	1.21	Androsta-3,5-diene-3,17-diol, 3-(heptafluorobutanoate) 17-(pentafluoropropanoate)

It was noted that compounds with a metabolic origin, like B, were produced by ultraviolet light. Hexadecenoic acid methyl ester, (Z)-triacontane, 11,20-didecyl-, d-arabinohexanoic acid, 1-O-p-nitrobenzoyl-2,3,4,6-tetra-O-benzyl-β-d-galactose, 3-(heptafluorobutyrate) 17-(pentafluoropropionate), di-andro-3,5-diene-3,17-diol, 2-deoxy-3,4,5-tri-O-(trimethylsilyl)-, and trimethylsilyl ester, there are also some compounds that have disappeared due to mutation such as2- (N-Methyl-N-nitroamino)-4-[2,2,3,3,3-pentafluoro-1,1-bis(trifluoromethyl)propyl]-6, and Octatriacontane, 1,38-dibromo.

Six primers (OPC-08, OPL-07, OPA-10, OPA-18, and OPB-02) were employed to differentiate DNA bands in the fungal genus; these primers were randomly associated with fungal DNA bands. Fungi that were not affected by genetic alteration (standard) and those that were affected by genetic alteration caused by ultraviolet light 1, 2, and 3 were evaluated. Additionally, significant discrepancies were observed between the three primers (Figure 6).
1-OPC-08's peculiar patterns (1000 and 1100)bp in the UV1 gene and different patterns (800 and 1100) in the UV3 gene were observed.

2-The primer OPL-18 exhibited clearly perceptible bands (500 and 750) bp for the mutagen UV1, as well as bands (400 and 600)bp for the mutagen UV2. While with a mutagen that creates UV3, the bands (1000 and 1250) that are supposed to be present in the DNA were removed, as is common for fungi.

3-The primer pair OPB-02 showed a perceptible band (1250) bp at the ultraviolet light source UV3. On the other hand, no amassing of the mutagenic component UV1 was observed.
The effect of the mutants not only increased the production of secondary metabolites, but also led to the creation of new chemicals (11).

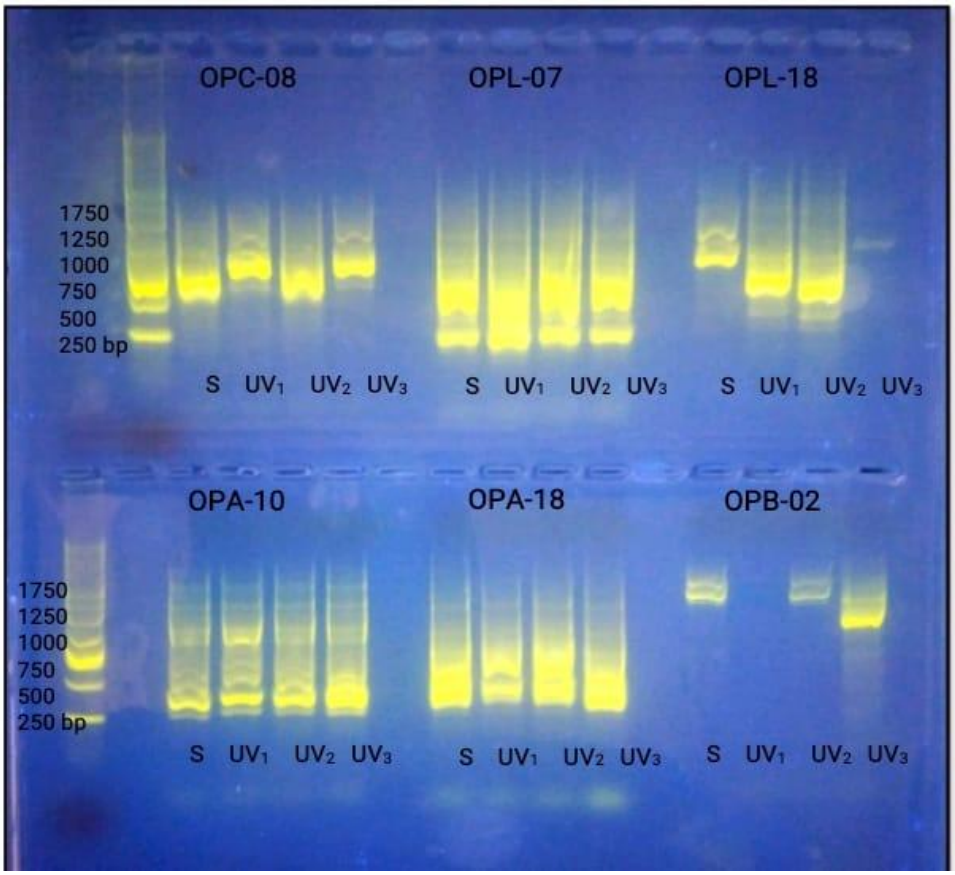

Figure -6 Random Amplified Polymorphic DNA (RAPD)

The ultraviolet light that is detrimental to DNA is said to be caused by the dimerization and mutation that occur as a result of the oxidative process. Dimerization mutations break the chemical bond that attaches to nearby pyrimidines, this creates a chemical intermediate that negatively affects the DNA structure, thus impeding its replication[20]. Mutations that are generated via oxidization have the capacity to associate with DNA bases and to oxidize them, this results in the inability to properly bind the DNA and thus causes mutations[21].The effect of the mutants not only increased the production of secondary metabolites, but also led to the creation of new chemicals[11].

4. Conclusion

A new isolate of the fungus was recorded in (NCBI), PQ721627. It was observed that it is possible to expose it to ultraviolet rays and affect the regions of the DNA, which leads to the production of some new secondary metabolites, Such as 1-O-p-Nitrobenzoyl-2,3,4,6-tetra-O-benzyl-.beta.-d-galactose, -Hexadecenoic acid, methyl ester, (Z)-, Triacontane, 11,20-didecyl-,d-Arabino-hexonic acid, 2-deoxy-3,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester, bis, and Androsta-3,5-diene-3,17-diol, 3-(heptafluorobutanoate) 17-(pentafluoropropanoate).

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