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Diagnosis of Some Species of Fungi Growing on Wall Paint Using Genetic Indicators

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ABSTRACT

The problem of damaging building wall paint with biological components is a widespread economic problem, so the study was conducted to determine some of the most important fungi that cause damage to building wall paint. A group of isolates of fungal colonies growing on the painted walls surfaces of some neighbourhoods in Samarra city, Salah-al-Din, Iraq were taken. Isolation techniques and phenotypic diagnosis were used for the purpose of preliminary identification of the isolates that were taken from the walls of painted buildings, which showed the presence of the fungus *Aspergillus nig*er and *Alternaria alternata*. Genetic indicators and molecular diagnosis were used to confirm the genera that were isolated using a number of genes, which are the *ITS1*, *ITS4* genes. As a general diagnostic gene for the fungal genera, two specialized diagnostic genes were also used, namely, the *lipase* gene for the diagnosis of *A. nig*er and the *β-tubulin* gene for the diagnosis of *A. alternata*. Molecular diagnostics results revealed that the appearance of a 500 bp band of both fungi in relation to the *ITS1, ITS4* gene primers, and the appearance of a 184 bp band of the primers of the *β-tubulin* gene for the detection of the genus *A. alternata*. As for the primers of the *lipase* gene, it did not indicate the presence of any genetic bundle, which meant that there was no genetic match.

Key words : Paint, *Alternaria alternata*, *Aspergillus niger*, PCR

INTRODUCTION

About 80000 to 120000 species of fungi have been described to date, although the total number of species is estimated at around 1.5 million. This would render fungi as one of the least-explored biodiversity resources of our planet. It is notoriously difficult to delimit fungi as a group against other eukaryotes, and debates over the inclusion or exclusion of certain groups have been going on for well over a century. With photosynthetic pigments being absent, fungi have a heterotrophic mode of nutrition. In contrast to animals which typically feed by ingestion, fungi obtain their nutrients by extra cellular digestion due to the activity of secreted enzymes, followed by absorption of the solubilized breakdown products. The combination of extracellular digestion and absorption can be seen as the ultimate determinant of the fungal lifestyle. In the course of evolution, fungi have conquered an astonishingly wide range of habitats, fulfilling important roles in diverse ecosystems (Fernando *et al.*, 2022).

Paint is a liquid used to protect the surfaces of objects and to create a coloured coating to keep walls from disintegration and damage. It is a liquid with viscosity, drying capabilities and properties developed by various chemical formulations. The painted surface is subject to damage or discolouration due to natural weathering and the growth and activity of living organisms (Parna and Sarabhi, 2021). The coating contains a variety of organic and inorganic elements and provides different living areas that a large number of microbial species can use. It was found that the most common species of fungi are *Alternaria alternata* and *Aspergillus niger*, which cause damage and decomposition of paints (Salleh *et al.*, 2022). In order to ascertain the most common fungal genera present on dyes, several diagnostic and taxonomic methods were used to determine the genera and species of these fungi. Among these diagnostic

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methods best one is the molecular diagnosis. Therefore, the current study aimed at performing the phenotypic and molecular diagnosis of the most common fungi through the use of specialized primers.

MATERIALS AND METHODS

Ten random samples were taken from a group of buildings (Samarra University buildings, hospitals, shops, schools, paint stores, residential houses) in the city of Samarra for the period from 1-11-2020 to 31-5-2021, where the samples taken included colonies growing on painted walls and roofs of buildings.

Direct microscopic examination was used to ascertain the presence of spores and fungal hyphae. Samples were cultured and non-growing samples were considered negative after 10 days of incubation (Thippeswamy *et al.*, 2014). Microscopic examination was used to study the fungal hyphae, their divisions and branches, spores, vesicles and conidiophores (Kidds *et al.*, 2016). The fungal isolates were purified using the single spore technique for purification, and decadal dilutions of the conidial suspension were prepared. One spore was selected and transferred to a flask containing SDB medium for later use in molecular tests and according to the methods used by the fungal isolates were diagnosed based on the taxonomic keys used by Prathima (2018).

Genetic tests were conducted in the laboratories of the Department of Biology, College of Education, Samarra University. DNA was isolated using an isolation kit manufactured by Wizard®Genomic. DNA Purification Kit manufactured in the United States of America provided a fast and pure way to obtain DNA that was used in amplification processes of genes using polymerase chain reaction (PCR). The instructions that came with the kit were followed. For the purpose of identifying and diagnosing previously collected isolates used were : *ITS1*, *ITS4*, *β-tubulin*, *Lipase* and their sequences (Table 1).

The DNA concentration was estimated using the Nano-drop device. After running the device connected to the computer, the program for the Nanodrop device was turn on. After zero adjustment of the device, the absorbance of the ultraviolet spectrum was measured at the wavelength of 280-260 nm by taking 1 µl of the DNA sample to be measured and adding it to its designated place in the device chamber. Then the option Measure was pressed. The result showed that the DNA concentration in each microliter, as well as the purity of the sample.

The concentration was calculated by assuming that one amount of optical density at 260 nm is equal to 50 µg/ml of DNA. Samples of different concentrations were diluted using sterile distilled water to reach a concentration of 50 ng/µl, and a concentration of 2 (ng/^l) for DNA was obtained from the *A. niger* sample, and at a concentration of 1.5 (ng/^l). From a sample of *A. alternata,* to amplify the DNA fragments with the sizes mentioned in Table 1, reaction components were mixed (Table 2).

The PCR reactions were applied using the program and according to the recommendations of Kordalewska *et al.* (2015), Prathima (2018), Hussein and Voigt (2019) and Alabdalall *et al.* (2020). Electrophoresis was performed for the detection of genomic DNA and PCR products following Samaila *et al.* (2014) for the purpose of detecting the bundles resulting from the union of primers with the study samples (Table 3).

RESULTS AND DISCUSSION

The isolation and phenotypic diagnosis showed the presence of *A. niger* and *A. alternata,* which are the most prevalent on building walls, as shown in Figs. 1 and 2, which showed the

Table 1. The sequences of the primers of genes within the study with the bundle sizes and temperatures for each primer

Primer name	Seq.	Annealing temp. (°C)	Product size (bp)
ITS1	5' -TCCGTAGGT GAACCT GCGG-3'	55	600
ITS4	5' -TCCTCCGCTT ATT GAT AT GC-3'		
β-tubulin-F	5' -GT GCCTTCCCCCAAGGT CTCCG-3'	62	184
β-tubulin-R	5' -CGGAAACGAGGT GGTT CAGGTC-3'		
Lipase-F	5' - AT GTTCTCT GGACGGTTT GGAGT G-3'	64.7	894
Lipase-R	5'-TTATAGCAGGCACTCGGAAATC-3'		

Table 2. Master mix components

Master mix components	Stock	Unit	Final	Unit	Volume 1 Sample
Master mix	2	X	1	X	12.5
Forward primer	10	pM	1	pM	1.0
Reverse primer	10	pM	1	pM	1.0
Nuclease free water					7.5
DNA		ng/pl		ng/pl	3.0
Total volume					25.0
Aliquot per single rxn	23pl of Master mix per tube and 3pl of template				

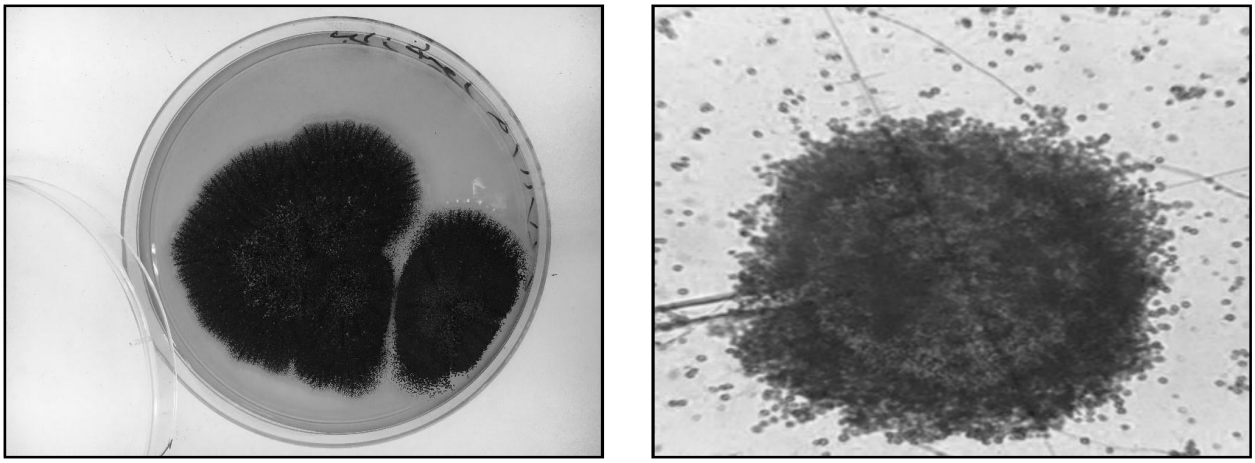

Fig. 1. The shape of the spore and colony of *Aspergillus niger.*

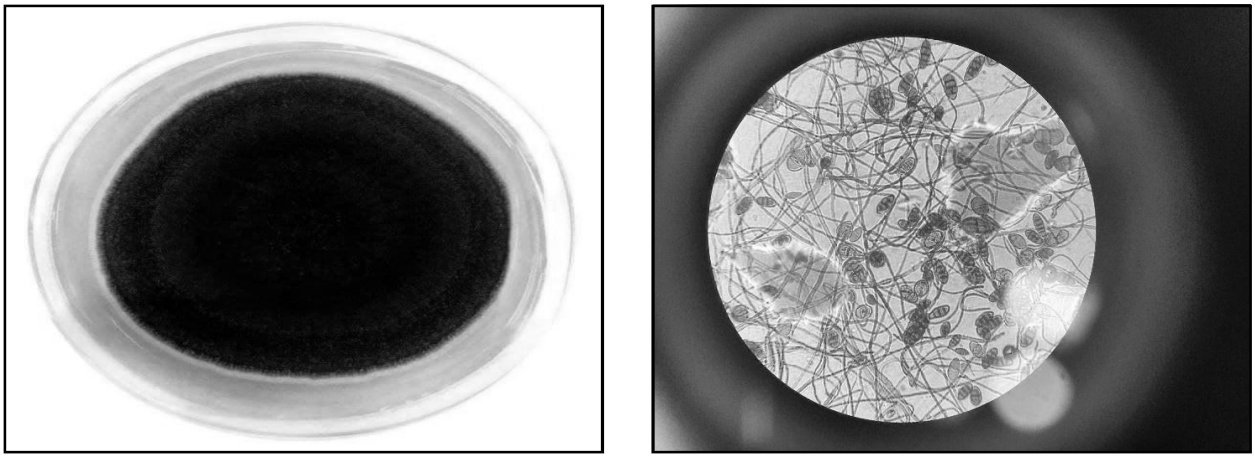

Fig. 2. The shape of the spore and colony of the fungus *Alternaria alternata.*

results of the phenotypic diagnosis of the two isolated fungi. As for the results of the molecular diagnosis, it was shown that the gene primers *ITS1, ITS4.*

For both fungi, it showed a bundle with a molecular weight of 600 bp, while the primer of the *β-tubulin* gene for *A. alternata* showed a bundle with molecular weight of 184 bp, while the primer of the *lipase* gene did not record any appearance of any genetic bundle, which meant that there was no genetic match with the isolated fungi. It was a diagnostic primer for the presence of *A. niger* (Fig. 3).

Results of the amplification of *ITS, Lipase and β-tubulin* gene of species of fungi were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M : 100 bp ladder marker. The image on the right of Fig. 3 showed the results of the molecular diagnosis of the primer of the *β-tubulin* gene of *A. alternata.* The image in the middle of Fig. 3 showed the results of the molecular diagnosis of the primer of the *lipase* gene of *A. niger.* The image left of Fig. 3 showed the results of a molecular assay for the primer of the *ITS1, ITS4* gene for both *A. niger* in line 1 and *A. alternata* in line 2.

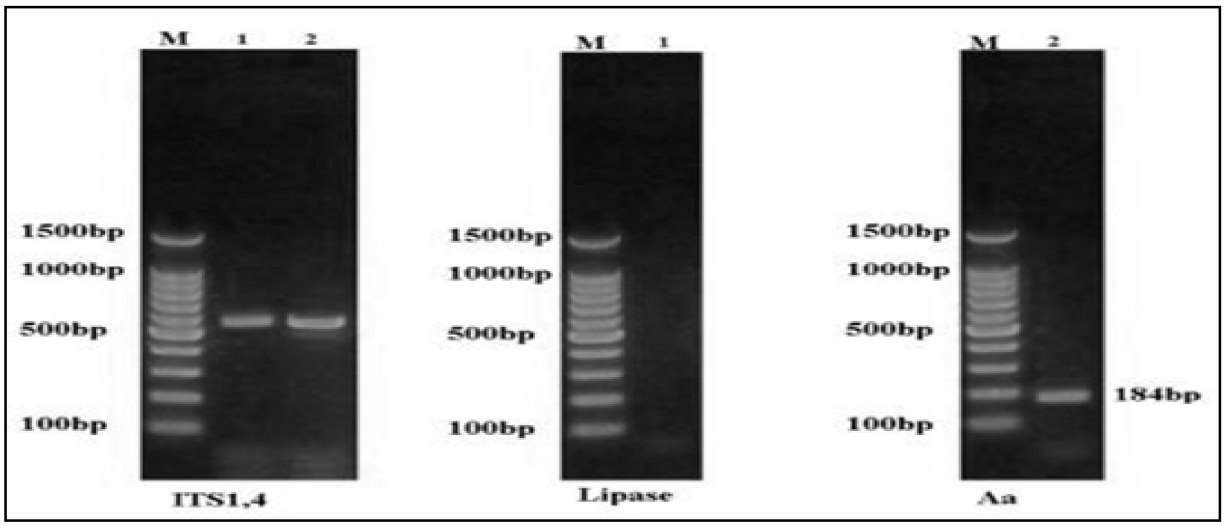

Fig. 3. Results of the PCR assay for primers.

Various organisms such as bacteria, fungi and insects can inhabit building materials, as the primary compounds that microbes feed on are likely to be available, as microbes need to have certain environmental conditions that suit their growth requirements. It was recorded that the fungi need different conditions to grow on the surfaces of the walls and to feed on the components of the paints. The results of phenotypic diagnosis of spores and fungal colonies isolated from ceiling and roofs of painted buildings showed the presence of *A. niger* and *A. alternata*, which corresponded to the taxonomic keys used. The results also showed the spread of fungi *A. niger* and *A. alternata* on building walls paint. This was due to their production of large numbers of conidia, which were considered asexual reproduction methods that helped their spread. They also contained compounds and enzymes such as lipase, protease and cellulase, which enabled them to analyze the main components in the composition of the paint. The results of the molecular diagnosis also showed the emergence of a 600 bp molecular weight bundle of both *A. niger* and *A. alternata* in relation to the *ITS1, ITS4* gene primer. This was supported by Kordalewska *et al.* (2015) who indicated the emergence of the same 600 bp molecular weight bundle when identifying *A. alternata* using real-time PCR technology. Hussein and Voigt (2019) indicated the appearance of the same aforementioned bundle, which had a genus identification of Alternaria in the Arab Republic of Egypt. The reason for this difference may be due to genetic variation between strains resulting from variation in the environmental conditions in which those strains grew.

Table 3. PCR program used to amplify primers++

Steps	°C	m : s	Cycle
Initial denaturation	95	05 : 00	1
Denaturation	95	00 : 30	30
Annealing	55, 62, or 64.7	00 : 30	
Extension	72	00 : 30	
Final extension	72	07 : 00	1
Hold	10	10 : 00	

The results of the primer of the *β-tubulin* gene for the detection of *A. alternata* showed the appearance of a bundle with a molecular weight of 184 bp, which was consistent with the results obtained by Prathima (2018).

As for the results of the *lipase* gene primer, it did not indicate the appearance of any bundle of molecular weight when applying the PCR program for the detection of the *A. niger* fungus. This indicated a genetic variation between the strains of this fungus as a result of changing the nature of the food available in its environment and affecting the growth and spread activities.

A. niger is one of the most effective *lipase* producers and is a diagnostic trait for this fungus. Moreover, studies showed that the extracellular production of *lipase* enzyme in *A. niger* varied in different strains of the same species. Molecular studies are necessary to understand the factors that contribute to differences in enzymatic production between *A. niger* strains. In this study, the *lipase* gene was amplified in *A. niger*. For the purpose of

detecting it, after conducting a PCR test and applying the interaction program, the result of the bundle did not appear in the scale of weights of the bundles, and this differed from what was reached by Alabdalall *et al.* (2020).

CONCLUSION

It was found that the fungi *A. niger* and *A. alternata* spread widely on the roofs of buildings painted with water-diluted paints. It also indicated its high ability to decompose the materials that make up the paint and the use of its chemical components as food for its growth. It was noted in the fungi genera used in this study that the presence of genetic variation when using molecular diagnostic indicators based on specialized primers used in the diagnosis of these genera, necessitated the continuation of molecular diagnostic studies to identify genetic variations in these and other species.

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