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Detection and Identification of Novel Genes from Fungi Isolated from Wastewater

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Abstract

The environment in Mosul city is very rich, containing a wide variety of microorganisms which have not been recognised for a long time. Five new fungal genes were identified and registered for the first time in the gene bank. These included *Fusarium falciforme* 2020-06-MIK-F1 genes for 5.8S rRNA with Accession no. LC555741, *Nectriaceae* sp. 2020-06-MIK-F2 genes for ITS1 with Accession no. LC555742, *Trichoderma asperellum* MIK3 genes for 5.8S rRNA with Accession no. LC575020, *Penicillium* sp. MIK4 genes for 5.8S rRNA with Accession no. LC575021, and *Neurospora crassa* MIK5 genes for 5.8S rRNA with Accession no. LC575022. These fungal genes were isolated from wastewater of Khosr river in Mosul city/ Iraq, which has many contamination sources.

Keywords: wastewater, new fungi genes, Khosr river, Mosul city

الكشف والتشخيص عن جينات جديدة لبعض الفطريات المعزولة من مياه الفضلات

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الخلاصة

تعد بيئة مدينة الموصل من البيئات الغنية والتي تحتوي على مدى واسع من التنوع لائنواع الاحياء المجهرية والتي لم تشخص من قبل. تم تشخيص وتسجيل خمس جينات جديدة لأول مرة في بنك الجينات الدولي وهي جين 2020-06-MIK-F1 للفطر *Fusarium falciforme* حاملا الرقم الدولي LC555741 وجين 2020-06-MIK-F2 للفطر *Nectriaceae* sp. حاملا الرقم الدولي LC555742 وجين MIK3 للفطر *Trichoderma asperellum* حاملا الرقم الدولي LC575020 وجين MIK4 للفطر *Penicillium* sp. حاملا الرقم الدولي LC575021 وجين MIK5 للفطر *Neurospora crassa* ، حيث عزلت هذه الجينات من مياه فضلات نهر الخوصر في مدينة الموصل.

Introduction

Water fungi play an important role in biodegrading different types of pollutants, such as detergents, organic compounds, and other different types of materials. Any environment has always new strains and genes as results of continuous changes of climate, as the organisms need to adapt with these changes to survive and conserve progeny [1]. Using molecular biology tools to discover new strains is the best and most accurate method to find out if there are new organisms in various environments.

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Wastewater includes many elements and nutrients [2]. The Khosr river is a branch of the Tigris river which is running within the centre of the ancient town of Nineveh in Northern Iraq. All types of heavy, industrial and wastewater pour into the Khosr river. It is considered as a complicated environment as it contains different materials. This environment may change and offer a good chance to produce new genes and strains [3]. Fungi are microorganisms which are living in terrestrial and water environments. Most of fungi phyla live in soil, while very few information is available about fungi which are living in various types of water environments[4, 5]. In this study, samples were collected from wastewater for the detection of the varieties of fungi.

Methodology

Fungal isolation

Khosr is a tributary river that rises in Shikhan suburb and runs through Mosul city. It is used for drainage of sewage water from houses and industrial buildings along the river (Figure-1). Samples were collected by using sterile flasks from the end of Khosr river, before meeting the Tigris river, in the end of November (24 °C water temperature). Wastewater samples were analysed for the presence of fungal new strains by using the plates dilution method. A volume of 1 ml from each sample was added to a plate and then the Potato Dextrose Agar (PDA) medium with chloramphenicol (0.05g/L) were decanted on it after cooling to 45 ° C. Then, the plates were incubated for 5 days in 28 ± 2 °C [6].



Figure: 1-The satellite picture of samples location

Morphological fungal identification

All Fungal isolates were identified by using common classification keys according to references [7].

DNA extraction

The dominance fungi (5 isolates) were collected from the plate and transferred to a new PDA medium plate to obtain single fungi for DNA extraction. The mycelia were transferred to PDB (Potatoes Dexterous Broth) in 250 conical flasks and grown in an incubator shaker with agitation of 150 rpm for four days at temperature 28 °C. The mycelia were harvested aseptically via filter papers for fungal strains, which were cultured in PDB for DNA extraction using Geneaid Plant Genomic DNA mini-kit.

Fungi identification by PCR

The PCR reaction mixture consisted of ITS primers (forward ITS86:

5`TGAATCATCGACTCTTTGAACGC`3 and reverse ITS4:

5`TTTCTTTTCCTCCGCTTATTGATAT`3) [8, 9] and a master mix with DNA template. The PCR program steps involved denaturation at temperature 94 °C for 4 minutes, denaturation at temperature 94 °C for 1 minute, annealing at 54 °C for 1 minute, and extension at temperature 72 °C for 1 minute. These steps were repeated for 30 cycles with a final extension at temperature 72 °C for 7 minutes. After the end of the program, the bands were run in agarose gel by electrophoresis [10].

DAN Sequencing

The amplified DNA bands were extracted from the agarose gel and then sequenced by using the Genetic Analyzer 3130 from Macrogen biotechnology company in the South Korea. The nucleotide sequences were then submitted to DNA Data Bank of Japan (DDBJ) for further information.

Results and Discussion

PCR reaction products

The PCR reaction was conducted in a thermocycler by using the ITS primer. The size of the PCR products was 300 bp for all the 5 fungal isolates identified in the present study, as shown in Figure-2.

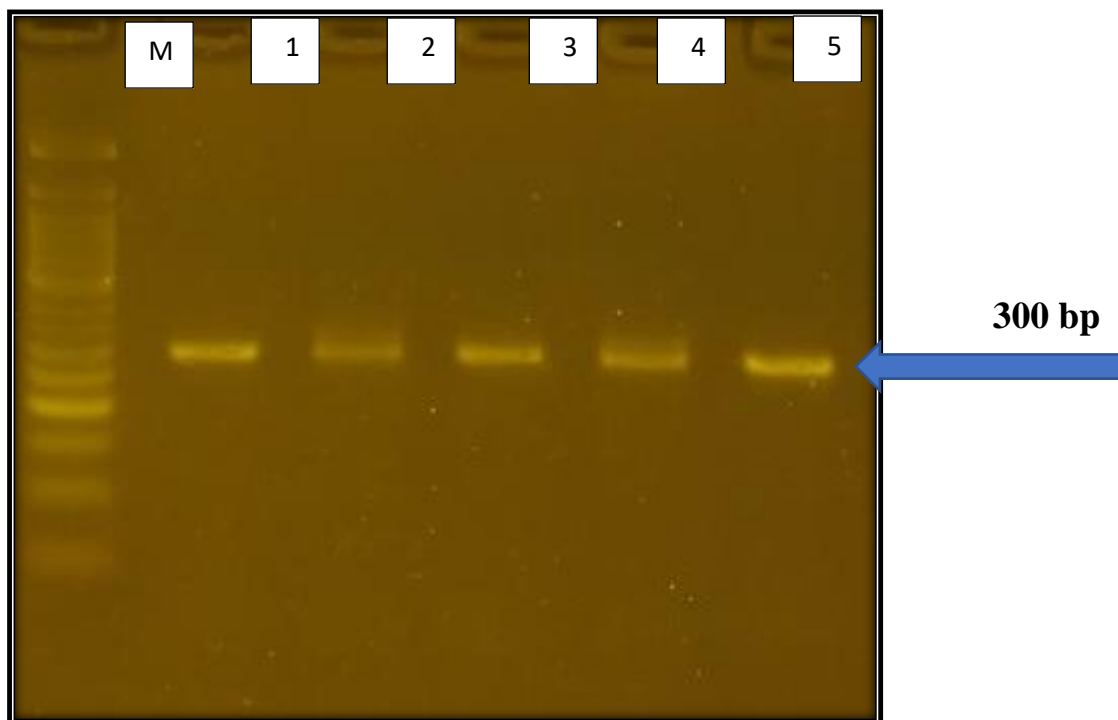


Figure 2-Agarose gel electrophoretic profile of ITS-PCR amplification of The 5 isolates. Lane M: 50 bp ladder (Promega, U.S.A); Lanes 1- 5: PCR amplicon.

DDBJ submission

The results of the five fungal sequence submissions confirmed that the sequences belong to uncultured strains and their genes, which will be given new accession numbers; these isolates included *Fusarium falciforme* 2020-06-MIK-F1 genes for 5.8S rRNA with Accession no. LC555741, *Nectriaceae* sp. 2020-06-MIK-F2 genes for ITS1 with Accession no. LC555742, *Trichoderma asperellum* MIK3 genes for 5.8S rRNA with Accession no. LC575020, *Penicillium* sp. MIK4 genes for 5.8S rRNA with Accession no. LC575021, and *Neurospora crassa* MIK5 genes for 5.8S rRNA with Accession no. LC575022, as shown in Figures-3, 4, 5, 6 and 7 , respectively.

These genes are supposed to encode for hypothetical proteins, as their functions have not been yet identified, in addition to the lack of evidence that they are expressed *in vivo*. There is a need to study the proteins they are likely to encode along with their roles in the cell [11]. Genomes of eukaryotes always needs a new protein to be able to survive under different condition by changing the genes sequences as a results of mutation; these new genes can be formed and created by organism itself through the *de novo* pathway or by the repeating the large regions in genomes which is called non-coding genes . The importance of new genes is to secure rapid adaptation among genes and species to

survive extreme or new environmental conditions. A high percentage of fungi (> 90%) are reported to be still undiscovered [12].

New fungal species and genes can be identified and found anywhere; from air, water, animals, and sand sources. They are discovered every year in high numbers. However, many species are still unclassified and unnamed because of the deficiency of samples, thus remaining in the shadowed "dark taxa" [11].

Wastewater is considered as a rich environment for different microorganisms, since it contains different components, such as heavy metals, pharmaceutical compounds, nutrients, detergents, and other wastes. All these components create a special environment for microorganisms and induce them to develop and adapt to the new condition. For every ecosystem, there are new strains that still need to be discovered and registered [13].

Understanding the biological complexity is very important to study the activity and development of microorganisms in parallel with environmental changes. Such an understanding is necessary to keep these organisms under control, as they may cause some epidemiological problems [14].

Wastewater is a very rich environment for many components which play an important role in changing a DNA sequences of many organisms as a result of expose to pollution and mutagen elements like heavy metals, antibiotic, nutrient and other sources of antagonisms of other living organisms, [15-19].

Conclusions

Five fungal genes were discovered and registered in the gene bank, isolated from fungi collected from the wastewater of Khosr River in Mosul city. The utilization of ITS primers for PCR amplification and sequencing is the best and most accurate method to discover new strains and genes in different environments.

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