First record of three Endophytic fungi isolated from xerophyte plants in Basra, Iraq

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Abstract

This study was conducted with the aim of isolating endophytic fungi from some xerophyte plants. In this study, three fungi were isolated from the internal tissues of some plants spread in the city of Basrah, *Xenomyrothecium tongaense* from the stem of *Suaeda aegyptiace*, *Chaetomium megalocarpum* from the roots, stem and leaves of *Caroxylon jordanicola* and *Cephaliophora irregularis* from the leaves of *Cressa cretica*. Fungus was identified based on its morphological features and molecular based on the amplification of the ITS gene region and the nucleolide sequence of the fungi was deposited in the genebank under accession number NR-154511.1, KT371335.1 and OM245865.1 for fungi *Xenomyrothecium tongaense*, *Chaetomium megalocarpum* and *Cephaliophora irregularis*, respectively. This is the first study in which these fungi are isolated from desert in Iraq.

Keywords: *Cephaliophora irregularis, , Suaeda aegyptiace* , salinity, endophytic fungi, Xerophyte plant.

Introduction

The desert is one of the ecosystems in the natural geography of central and southern Iraq. Shrubs grow in the desert Environment (xerophyte plant) are resistant to drought and salinity (20). It is believed that the resistance of desert plants to drought is due to the coexistence of these plants with a group of endophytic fungi (19). The term

epiphytes is given to microorganisms that live on the surfaces of plant parts, while endophyte is applied to microorganisms that live in plant tissues (15). Endophytic microorganisms that live inside plants without causing pathological symptoms depend largely on the location of the plant in which they live and on the climatic conditions of the area in which this plant lives, these include endophytic microorganisms (fungi, bacteria and actinomycetes) that live between or within plant cells and are associated with it in a symbiotic relationship (12). Drought and salinity are key environmental factors that limit plant growth and reduce the productivity of many plant species (1). Salinity is an ancient problem that began from the depths of history about 3000 years BC, in Mesopotamia (Iraq) (18). That is, many plants are unable to adapt to environmental variables such as drought, lack of nutrients and salinity, but the presence of endophytic microorganisms that have the ability to withstand stress conditions makes the host plant more flexible in tolerating such conditions and this association between the plant and endophytic is present only when the chemical balance between them is maintained (9). Although different types of bacteria have been isolated, fungi are also among the most frequently isolated endophytes from the tissues of their host plants (14). These fungi increase the resistance of host plants to stress conditions and stimulate them to form defenses against various pathogens by altering gene expression levels and modifying vital pathways (7).

The desert area in the city of Basrah in southern Iraq is characterized by sandy soil, high temperature, low air humidity and lack of rain, this dry climate in the region is reflected in the increase in soil salinity, which is the predominant form of soil in the southern desert. Several species of plants grow in these soils, such as *S. aegyptiace* (Hasselq) Zohary Which belongs to the family Chenopodiaceae which is one of the plants that grow in saline soils and roadsides, which are spread in all areas of Basrah in southern Iraq, and it is an annual herb up to 60 cm and blooms in the Summer and Autumn. *C. jordanicola* plant belong to Chenopodiaceae family its predominant in Zubair, Jarishan and Shaib Al-Batin region in the desert with saline sandy soils, it is an annual herb 10-40 cm tall and blooms in Summer and Autumn, while *C. cretica* plant belongs to the family Convolvnlaceae its

perennial herb that grows in saline alkaline soils, blooms at the end of Spring or Autumn (4). The current study aimed to isolate and characteristics endophytic fungi from the most prominent desert plants and plants growing in saline soil in the city of Basrah.

Materials and methods

Sample collection:

Two sites were chosen to collect plant, *S. gyptiace* and *C. jordanicola* plants belongs to Chenopodiaceae family from the Zubair area, southwest of Basrah, and *C. cretic* plant belongs to the family Convolvnlaceae from the site of the University of Basrah / Karma Ali, north of the Basrah city (4) during the month of October, and these plants appeared healthy and did not show any symptoms disease, Plants were uprooted from the soil. Soil samples were also collected from the growth area of these plants from a depth of 1-30 cm with the removal of the surface layer within 1 cm, each sample was placed in a sterile plastic bag, recorded full information on it and brought to the Biocontrol Laboratory at the College of Agriculture.

pH and Ec of soil samples were measured by 3405 pH-meter, JEWAY 4510 Conductivity Meter respectively.

Isolation of endophytic fungi:

The root, stems and leaves of xerophyte plant samples were thoroughly washed in running tap water to remove the dust stuck to them. The plant parts were cut into small pieces (1 cm) and sterilized superficially, with 70% of ethanol 1-2 minutes and then transferred to a 5.25% sodium hypochlorite solution NaOCl at a concentration of 6% for 2-5 minutes and then sterilized again in 70% ethanol for 30-60 seconds. The pieces were washed with sterile distilled water three times to dispose of the remnants of the sterilization material and dried by placing them on sterile filter paper. The sterile plant parts were transferred to Petri dish (4 pieces per dish) containing the sterile potato dextrose agar (PDA) supplemented with 100 mg/L tetracycline incubated at 25±2°C for 7-14 days (This method is used in isolation because this fungus is facultatively saprophyte). Pure cultures of endophytic fungi were made by hyphal technique. Parts of mycelium were taken from pure cultures of endophytic fungi and kept at 15% (v/v) of glycerol and kept at -20°C for long-term preservation (5).

Morphological identification of endophytic fungi:

Fungi isolated from different plant parts of xerophyte plants were identified depending on morphological characteristic using approved taxonomic keys.

Molecular identification of endophytic fungi:

The fungal colonies of each 4-day-old fungus were scraped off mycelium of each fungus were crushed. Total DNA was extracted from each fungus using the Geneaid–Plant Genomic DNA Mini Kit (GP100) Taiwn, according to the manufacturer's instruction. The concentration of the eluted DNA was determined using NanoDrop from Thermo Scientific. The genomic DNA was stored at -20 ° C for subsequent analyses. To analyze the ITS gene region, the genetic DNA was amplified add the sequence of the primer ITS1F (5'TCCGTAGGTGAACCTGCGG-3') ITS4R (5'TCCTCCGCTTATTGATATGC-3')

The reaction mixture was prepared from (Table 1):

Table 1. Component and volume of mixture

Component	Volume
GoTaq Green Master Mix (Promega)	25 µl
FWD	2 µl
REV	2 µl
Nuclease-free water	16 µl
gDNA	5 µl
Total	50µl

PCR reaction conditions were as follows:

Pre-denaturation at 94 C° for 3 min; 1cycle, denaturation at 94 C° for 40 s; 35 cycles, annealing at 55 C° for 1 min; 35 cycles, extension at 72 C° for 1 min; 35 cycles, final extension at 72 C° for 10 min; 1 cycle according to (8). DNA samples were loaded into the appropriate wells of the TAE agarose gel (1.5% (w/v)) stained with 1-3 µl ethidium bromide dye. The agarose gel was run at 85V for 50 minutes. The DNA within agarose gel was visualized using UV transilluminator.

The gene amplification products were sent to Macrogen company for the purpose of determining the nucleotide sequence of gene and compared them with to the sequences in the NCBI GeneBank database.

Results

Features of samples soil:

The results of (Table 2) showed that the soil of the study area is characterized by being alkaline, while the values of electrical conductivity varied between the two study sites, as it was high salinity at the site of the University of Basrah - Karma Ali, while it was of medium salinity in the Zubair area. Plants samples collected from the two area are *S. aegyptiace* and *C. jordanicola* from Zubair area southwest of Basrah city and *C. cretica* plant from the University of Basrah site Karma Ali north of Basrah city (Fig.1).

Table 2. Electrical conductivity and pH values for soil samples

soil samples	Electrical conductivity(EC)	pH
University of Basrah – Karma Ali	8.32	7.93
Zubair Area	3.80	7.84

Fig. (1): (A) *Suaeda aegyptiace* (B). *Caroxylon jordanicola* (C) *Cressa cretica*

Isolation and characteristics of endophytic fungi:

X. tongaense, phylum Ascomucota, class Sordariomycetes, order Hypocreales, family Stachydotryaceae, (17) appeared from several pieces of the stem of the *S. aegyptiace* plant grown on PDA, the fungus is characterized by the formation of round-shaped colonies of white color with regular edges, mycelium is white color, The fungus is an irregularly shaped sporodochial conidiomata of olive green or dark green to black that is superficially formed on the mycelium individually or in groups (Fig.2). conidia aseptate oblong to ellipsoidal in shape with a pale green color and smoth,

dimensions 4.99-6.66 X 2.49-3.33 µm it was found that this description matches the description contained in (13).

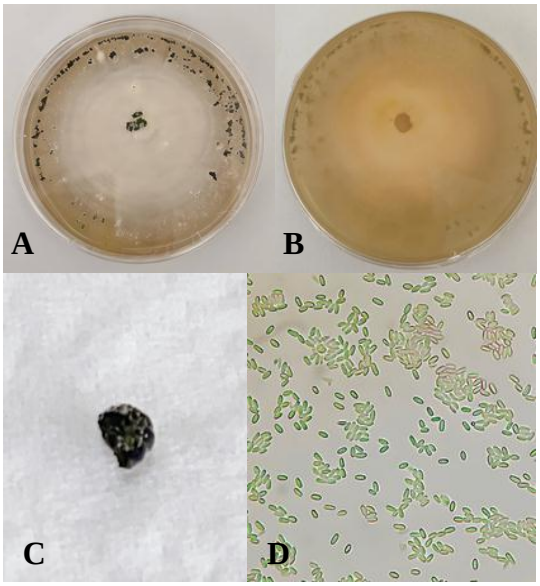

Fig. (2): Morphological features of *Xenomyrothecium tongaense*

A. Colony morphology on PDA. B. Reverse of colony. C. Conidiomata on microscope slide. D. Conidia.

C. megalocarpum phylum Ascomycota, class Sordariomyceyes, order Sordariales family Chaetomiaceae, (6) was isolated from the roots, stem and leaves of the *C. jordanicola*. The fungus forms a circular colony on PDA, mycelium is pale white and over the days turns yellow, with the color of the culture medium changing to yellow as well, ascomata are formed superficially on the mycelium and olive to gray in color and began to form from the center of the colony towards its edge with production of exudates in the form of honey-colorred droplets, ascomata is spherical to oval in shape, dark brown to black, acsomatal hairs are long, brown in color and wavy , some of the appendages are branched and the lateral appendages are short, the ascospores are spherical or almost spherical to oval with a light brown to olive color with dimensions 9.99-13.32 x 13.32µm Fig(3). This description was in agreement with (11).

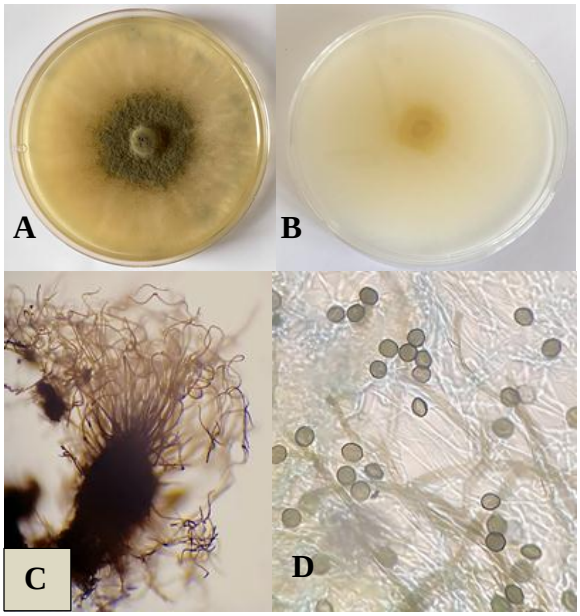

Fig.(3): Morphological features of *Chaetomium megalocarpum*

A. Colony morphology and ascomata on PDA. B. Reverse of colony. C. ascomata on microscope slide. D. ascospores.

C. irregularis phylum Ascomycota, class Pezizomycetes, order Pezizales, family Ascodesmidaceae, (16) has also been isolated from the leaves of *C. cretica*, being a fast-growing, round-shaped colony with a pink to light brown color. The fungus was characterized by the formation of an aerial mycelium extending to the edge of the

growing dish in which the color of growth at the edges of the colony turned brown, the conidiophores are short and unbranched, and the apical cell is an enlarged conical that is colorless or pale, carrying on its surface in a circular manner several asexual spores formed by budding method called blastoconidium, The fungus produces many spores and each spore consists of two or three cells, one or two small basal cells that are transparent or light yellow in color separated by transverse septa from the apical cell, which is large in size and of a light brown or olive color. These spores are either pear, elongated, fan or Y-shaped, dimensions 12.83-43.29 x13.32-27.47 µm (Fig. 4), this description was found to match the description in (10).

Molecular identification of endophytic fungi:

Molecular identification based on ITS area amplification showed that the fungi *X. tongaense*, *C. megalocarpum* and *C. irregularis* isolated from the plants *S. aegyptiace*, *C. jordanicola* and *C. cretica* respectively, It matched the isolates in the NCBI genebank with samilary rate of 95.62%, 95.54% and 99.82%. The nucleotide of the fungi sequence was deposited in the genebank under accession numbers NR-154511.1, KT371335.1 and OM245865.1 (Table 3).

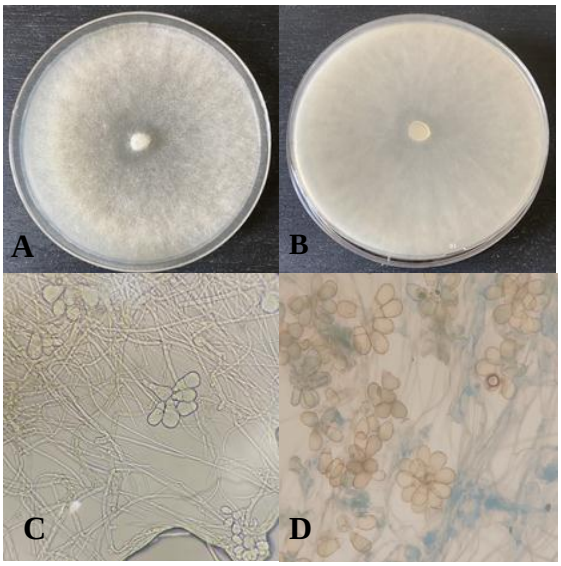

Fig.(4): Morphological features of *Cephaliophora irregularis*

A. Colony morphology on PDA. B. Reverse of colony. C. mycelium and Conidia. D. Conidia.

This study is the first in which this fungus has been isolated from the internal tissues of xerophyte plants in Iraq.

Table 3. Accession number and Identity ratio of endophytic fungus isolated from Xerophyte plants

xerophyte plants		endophytic fungus		
Plant Species	Family	Endophytic fungi	Accession number	Identity (%)
Suaeda aegyptiace	Chenopodiaceae	*Xenomyrotheciu m tongaense*	NR-154511.1	95.62
Caroxylon jordanicola	Chenopodiaceae	*Chaetomium megalocarpum*	KT371335.1	95.54
Cressa cretica	Convolvnlaceae	*Cephaliophora irregularis*	Om245865.1	99.82

Discussion

This study showed that many endophytic fungi were isolated from plants grown naturally in desert areas and areas with saline soils and that are exposed to extreme environmental conditions such as high temperatures, salinity and drought. Many fungi was associated with plant naturally grow under stress conditions (9). Previous studies showed *X. tongaense* isolated from the green algae *Halimeda* sp. in Tonga (13).

C. megalocarpum isolated from the stem of the wheat plant in the city of Salmas- Iran (6). *C. irregularis* was isolated from the zone of the Khor al-Zubair canal in southern Iraq (2). Referred (3) isolated fungi from school soils in the city of Diwaniyah in Iraq with a frequency rate of 0.04%.

Conflict of Interest

The authors have no conflict of interest.

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