Two ascomycetes from different aquatic habitats

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INTRODUCTION

In lakes with a typical littoral zone, macrophytes form three characteristic zones starting from the coast, emergent plants, floating leaved plants and submerged plants (Bernatowicz and Wolny, 1974; Fare et al., 2001; Hutchinson, 1975).

Fungi are common colonizers distributed on macrophytes submerged in fresh water habitats. In particular, ascomycete fungi occur on a variety of submerged substrates in both lotic (creeks, rivers, and streams) and lentic (bogs, lakes, ponds, and swamps) habitats (Shearer, 1993; Cai et al., 2003; Shearer et al., 2004, 2007; Raja et al., 2009).

There are a number of fungal species appearing in marine and terrestrial environments. Meanwhile the same fungal species may also occur in both saline and freshwater environments (Gessner, 2001; Shearer, 1993). Studies on freshwater ascomycetes have been conducted at spatially limited sites in lentic habitats (Cai et al., 2003).

Halosphaeriaceae represents the largest and most diverse lineage of marine ascomycetes till date as proposed by Müller and von Arx (1962) and Eriksson (1984). Pinruan et al. (2004) isolated Halosphaeria appendiculata Linder from decaying palm leaves submerged in a fresh-water environment.

The present study focused on isolation of fungi from dead plant segments submerged in lentic habitats in two different aquatic ecosystems, in particular, fungi with the phenotypic characteristics of Halosphaeria fungi.

MATERIALS AND METHODS

Description of study area

Al-Soda marsh is a lentic aquatic ecosystem (shallow lake) located in the Al-Huwaizah marshes in Southern Iraq. It is characterized by high salinity (1.6%) and is rich with the common reed Phragmites australis Trin. (Scott, 1995).

Lake Sevan is an aquatic ecosystem (natural fresh water lake) located in the central part of the Republic of Armenia. It is the largest lake in Armenia and the Caucasus region. It is one of the largest high-altitude lakes in the world. The lake is part of the Sevan National Park which consists of 4 protected reserve zones as well as recreational and economic zones.

The flora of the basin is highly diverse and about 1,600 species of vascular plants have been described. The dominant vegetation of the basin is mountain steppe, sub- alpine and alpine vegetation with different species of Astragalus and Acantholimon. On Lake Sevan, emergent vegetation exists only in a limited calm area and...
pondweeds (*Potamogeton* spp.) are abundant to depths of 2-5 m.

**Sampling and morphological study**

Thirty (30) submerged plant samples were collected from Al-Soda Marsh Lake in Iraq; and another 30 submerged plant segments samples were collected from Sevan lake in Armenia, both of which were collected in 2008. The samples were randomly collected from freshwater and salty water habitats following the procedures described by Shearer et al. (2004).

The samples were placed in Ziplock® plastic bags lined with moist filter paper and were transported to the laboratory. In the laboratory, the samples were placed in moist chambers and were incubated at ambient temperature (~25°C) at 12/12 h light and dark conditions. Afterward, they were subsequently examined with a dissecting microscope within one week of collection and periodically over 6–12 months (Shearer, 1993; Shearer et al., 2004).

For isolation of ascomycetous fungi, the samples were incubated for more than 1 month in the moist chamber to permit ascocarp development. Ascocarps were picked out from the substrate under dissecting microscope, crushed on glass microscope slides to open the ascocarp and the dispersed ascospores properties were examined.

**Identification process**


Note: Practically, it was observed that the gelatinous appendages cannot be diagnosed by using Lactophenol stain due to dissolving of gelatin material, so we used water instead of lactophenol to clarify appearance of the appendages.

**RESULTS**

**Description**

*Halosphaeria cucullata* (Kohlm.) (Kohlmeyer, 1972):

Ascocarp is spherical with diameter of 150-258 µm, completely submerged, ostulate, grey to brown color, surrounded by a thick wall, distinguished by the presence of long neck of 200-250 µm and diameter of 30-50 µm, centric, cylindrical and straight. Asci are of size range 50-70 µm and contain 8 ascospores. Ascospores’ size range from 8-10×18-20 µm, unitunicate, septate, divided in two cells by transverse septum, with hyaline colour, supplied with a thin gelatinous appendage, attached in the terminal end of the ascospore from the apex. The isolate resembles the species isolated by Kohlmeyer (1972); however the ascocarp neck in this new isolate is longer. The fungus is saprobic and has been previously isolated from *Tamarix aphylla* plant in California saline lake in Ohio, Brazil, India, Southern Africa, Australia, Japan and Mexico. This new isolate is the first record of this fungus in Iraq, isolated from dead reed stem (*P. australis*) submerged in Al-Soda Marsh Lake. The isolated fungus was kept in Basrah herbarium under the number BSRA 9001 (Figures 1 and 2).
**Halosphaeria species**

Ascocarp is spherical with diameter of 200-300 µm, completely submerged, ostiolate black grey color, surrounded by a thick wall, distinguished by the presence of long neck of 120-200 µm and diameters of 20-30 µm, cylindrical and straight.

Asci are of size range 10-20×25-35 µm, and contain 8 ascospores. Ascospores’ size range from 8-10×20-28 µm, unitunicate, septate and divided in two cells by transverse septum. The ascospore is surrounded by a thin gelatinous appendage which covers the whole body of the ascospore.

The isolate of the present study resembles *Halosphaeria galericita* isolated by Tubaki (1968), except that this new isolate has a bigger ascocarp. The fungus is saprobiic and also has been previously isolated from woody segment in Denmark, Mexico, Spain, United States, Germany and France. The isolated fungus was kept in Basrah herbarium under the number BSRA 9002 (Figures 3 and 4). Table 1 gives a comprehensive list of the fungal species isolated from Al-Soda Marsh Lake, Iraq and Sevan Lake, Armenia.

**DISCUSSION**

The Halosphaeriales fungi are saprobiic on decaying woody and herbaceous substrata in aquatic environments. The biodegradation process of submerged substrates occurs via fungal enzyme secretion onto the substrate. In particular, cellulases degrade cell-tissue structures and pectinas decompose pectin and protease which degrade cytoplasmic proteins (Chamier and Dixon, 1982; Chamier, 1985).

Molecular phylogenetic data clearly shows that the Halosphaeriales evolved independently from a terrestrial origin (Spatafora et al., 1998) and marine ascomycetes could have migrated from terrestrial habitats to freshwater and brackish water, and then to marine environments (Shearer, 1993; Jones, 1995).

Jones et al. (1986) and Jones (1995) suggested that ascospore shape and ascospore appendage ontogeny is of primary importance as taxonomic characters in the delineation of genera in the Halosphaeriaceae. Ascospore septation is also variable; therefore there is no clear pattern for ascospore morphology within the Halosphaeriaceae.

In the present study, two *Halosphaeria* species were isolated from different aquatic environments and revealed different morphological characteristics of the ascospore appendages. *H. cucullata* isolated from salty habitats in Iraq is distinguished by a long neck 200-250 µm and ascospores of size range of 8-10×18-20 µm, ununiticate, septate, divided in two cells by transverse septum, with hyaline colour, supplied with a thin gelatinous appendage, attached in the terminal end of the ascospore from the apex.

The second isolate, *Halosphaeria* sp., was isolated from fresh water habitats in Armenia. It is distinguished by having completely submerged ascocarp surrounded by a thick wall and the presence of long neck 120-200 µm and unitunicate ascospores 8-10×20-28 µm, divided in two cells by transverse septum. The ascospore is surrounded by a thin sheath of gelatinous appendage which covers the entire ascospore.

The results of the present study are similar to those of Gregory and Darryl (1979) who isolated ascomycetes including two *Halosphaeria* species from wood blocks submerged in salty habitat of Minas Basin salt marshes and fresh water in habitat of Wallis River, also in the month of August.

Freshwater ascomycetes appear to have adapted morphologically to aquatic habitats in a variety of ways.
One type of modification involves the presence of ascospores on viscous sticky appendages that may enable the ascospores to stick onto substrates in moving water (Kohlmeyer and Kohlmeyer, 1979).

Kohlmeyer (1972) assigned 12 marine species to *Halosphaeria* Linder but revision resulted in splitting up of *Halosphaeria* into a number of genera leaving only two species in *Halosphaeria* genus: *H. appendiculata* and *H. cucullata*. And recently, *H. cucullata* (Kohlm.) was transferred to a new genus, *Okeanomyces*, based on morphology and DNA sequence analysis (Pang et al., 2008).

According to Kohlmeyer (1979), there are some fungal species which is distinguished to be marine fungi and have adapted completely to appear exclusively in salt water; however, these species belongs to the family Halosphaeriaceae which are characterized by the fact that most of them are marine fungi and some species are endemic in fresh water (Cai et al., 2003; Kohlmeyer and Kohlmeyer, 1979).

The Halosphaeriaceous fungi constitute the largest group of marine ascomycota found predominantly in marine environments with few transitional species found in freshwater and brackish water habitats (Sakayaro et al., 2011).

**Conclusion**

Fungi like other completed microorganisms in nature can
Table 1. Fungal species isolated from Al-Soda Marsh Lake, Iraq and Sevan Lake, Armenia.

<table>
<thead>
<tr>
<th>Species of fungi</th>
<th>Sevan Lake</th>
<th>Al-Soda Marsh Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata (Fr.) Keissler</td>
<td>+</td>
<td>-*</td>
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<tr>
<td>Alternaria raphani J. W. Groves &amp; Skolko</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspergillus flavus Link</td>
<td>+</td>
<td>+</td>
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<tr>
<td>A. fumigatus Fresen</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. niger van Tieghem</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. terreus Thom</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. spergillus sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. ureobasidium pullulans (De Bary) G. Arnaud</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bipolaris spicifera (Bain) v. Arx Syn. of Curvularia spicifera (Bainier) Boedijn</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chaetomium sp.</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Cladosporium cladosporoides (Fresen) G. A. de Vries</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Curvularia pennisetii (Metra) Boedijn</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Emericella sp.</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Eurotium sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exerohilum sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium stoveri, Booth Syn. of Microdochium stoveri (C. Booth) Samuels &amp; I. C. Hallett</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gilmaniella humicola G. I. Barron</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Halosphaeria cucullata (Kohlm.) Kohlm.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Halosphaeria sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Leptosphaeria sp.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Monodictys glauca (Cooke &amp; Harken) S. Hughes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Penicillium sp.</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Phoma glomerata (Corda) Wollen.&amp; Hochapfel</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Rhizopus stolonifer (Ehrenb) Vuill.</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Savoryella lignicola E. B. G. Johnes R. A. Eaton</td>
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<td>-</td>
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<tr>
<td>Sclerotium hydropilum Saccardo</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Sepedonium sp.</td>
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<td>+</td>
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<tr>
<td>Sphaerodes sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Stachybotrys atrapra Corda</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stemphylium sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Torula herbarum (Pers.)Link</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Trichocladium opacum (Corda)Hughes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichurus spiralis Hasselbring</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ulocladium botrytis Preuss</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Absent, ·: present, +*.

survive under different physical and chemical parameters. In particular, salinity is considered one of the important physical factors that influence limited specific fungal species in marine and freshwater habitats. Fungal species belonging to the same genus keep their prolong adaptation and modified significant features to be familiar with new environment and changed morphological characterization to keep species survival in nature.

ACKNOWLEDGEMENT

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with their facilities during sample collection process.

REFERENCES


APPENDIX

Key to species identification of the genus Halosphaeria

<table>
<thead>
<tr>
<th>1</th>
<th>Extended appendages of mature ascospores apically or subapically attached only</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Apical and lateral ascospore appendages present</td>
<td>11</td>
</tr>
</tbody>
</table>
2(1) one subglobose cap at one end of the ascospore only........................................... *H. cucullata*
2(1*) Appendages at both ends of the ascospore.......3
3-(2*) Two spurlike, stiff appendages at each end of the ascospore; these pairs at right angles to one another .......... *H. quadricornuta*
3*(2*) Appendages not in such pairs..................4
4(3*) Appendages starlike, composed of three or more distinct arms radiating from the ascospore apices ........5
4*(3*) Appendages not composed of radiating equal apical arms...........................................7
5(4) Generally three or four, subterminally attached appendages at each ascospore apex; species of tropical waters......................................................... *H. salina*
5*(4*) Four or more terminally attached appendages at each ascospore apex; species of temperate waters........6
6(5*) Generally four radiating appendages at each ascospore apex....................................... *H. quadriremis*
6*(5*) Generally six appendages at each ascospore apex ...................................................... *H. stellata*
7(4*) Young ascospores completely surrounded by an exosporic sheath that unfolds and develops into pleomorphic, long appendages.................................8
7*(4*) Appendages covering only part of the ascospores, near the apices; or, if young ascospores are enclosed by a sheath, they disappear at maturity leaving subglobose apical appendages........9
8(7) Ascospores thick-walled, rhomboid, up to 20.5 µm in diameter, appendages usually simple, not yoke-shaped................................................................. *H. pilleata*
8*(7) Ascospores thin-walled, ellipsoidal, up to 13 µm in diameter, appendages mostly germinate and yoke-shaped......................................................... *H. martima*
9(7*) Ascospores appendages subapical, hooklike, eventually gelatinizing, stretching and dissolving ................................................................. *H. hamata*
9*(7*) Ascospores appendages apical, persistent or gelatinizing ........................................................................10
10(9*) Ascospores diameter generally less than 13 µm, appendages at apertures of subglobose cap with faint striae......................................................... *H. galera*
10*(9*) Ascospores diameter generally more than 13 µm, appendages at first scoop-shaped then gelatinizing and dissolving ........................................ *H. trullifera*
11(1*) Lateral ascospore appendages crescent-shaped, at each apex, a small inconspicuous cap................................. *H. mediosetigera*
11*(1*) Lateral ascospore appendages not crescent-shaped, apical appendages, long, subcylindrical or obclavate.................................................................12
12(11*) Lateral and apical ascospore appendages, identical, obclavate........................................ *H. appendiculata*
12*(11*) Ascospores with a tubular annulus around the septum at each end with a subcylindrical thorn................................. *H. torquata*