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First report on *Fusarium virguliforme* in Persian Gulf Beach soils

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Abstract:

Fusarium species are cosmopolitan species that frequently isolated from soils and plant debris and can seriously damaged plant, animal and human. Persian Gulf beaches in Iran are the ideal habitat for a variety of *Fusarium* species. Therefore, the study is conducted to gather some information on the population of *Fusarium* species which could be found in Persian Gulf beaches in Iran. The study involved the isolation and identification of *Fusarium* species from beach soil samples in Persian Gulf beaches. The direct isolation technique from soil particles was used to isolate *Fusarium* species from beach soil samples. *Fusarium* species were identified by the observation of macroscopic and microscopic characteristics. *Fusarium solani* is the most common species found on the beach samples which had the highest percentage of abundance (58%). The percentage of abundance for other *Fusarium* species is relatively low, where *F. proliferatum* consists of 12%, followed by *F. equiseti* (10%), *F. falciforme* (10%), *F. oxysporum* (7%) and *F. virguliforme* (3%). Molecular identification using translation elongation factor 1α (*tef*1) gene and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences were conducted to confirm morphological data. Numerous *Fusarium* species associated with soil and different plants have been reported from Iran. Until today, there are no reports on *F. virguliforme* in Iran. The study on the *Fusarium* species on beach soil samples of Persian Gulf in Iran will provide insight on the influence of environment on occurrence of *Fusarium* in Persian Gulf.

Keywords: *Diversity, Fusarium, Persian Gulf, Iran.*

Introduction

The genus *Fusarium* is well-known important plant, human and animal pathogens. *Fusarium* species are distributed across the globe and frequently isolated from soils and organic substrates. *Fusarium* species can be found in different bioclimatic regions of the world including tropical and subtropical grasslands, forests, Gulf beaches, as well as harsh desert and alpine environment (Leslie and Summerell, 2006). The efficient mechanisms for dispersal of *Fusarium* spp. and the ability of these fungi to grow on a wide range of substrates can be influenced on widespread distribution of *Fusarium* spp..

Therefore, *Fusarium* occurs in nearly all ecosystems worldwide (Leslie and Summerell, 2006; Zhang *et al*., 2006). Climate conditions are the most important factors which can influence on the occurrence and distribution of *Fusarium* species on a broad, regional scale (Sangalang *et al*., 1995). Also, soil texture determined the availability of minerals and moisture in the soils that soil fungi such as *Fusarium* require moist environment to live (Coyne and Thompson, 2006).

The Persian Gulf lies between Iran to the northeast and the Arabian Peninsula to the southwest. Persian Gulf beaches are located in the tropical region which has a hot wet equatorial climate with sandy soils. The average daily temperature in Persian Gulf throughout the year is ranging from 18ºC to 40ºC (Daryaee, 2003).

There is some information concerning *Fusarium* spp. in different areas in Iran (Darvishnia *et al*., 2006; Chehri *et al*., 2010; Chehri *et al*., 2014); however, there is no report on the occurrence of *Fusarium* species on Persian Gulf beaches in Iran. Therefore, the present study was carried out to determine the occurrences and diversity of *Fusarium* species on Persian Gulf beaches in Iran.

Materials and Methods

Isolation and Identification of *Fusarium* spp.

The samples were collected from 12 sampling sites (Table 1) in Persian Gulf beaches in Iran from 2013 to 2014. *Fusarium* spp. was recovered from sandy beaches in Persian Gulf in Iran. Isolation of *Fusarium* was done by directly plating the sandy soil samples onto a semi-selective media, peptone-pentachloronitrobenzene agar (PPA) (Nash and Snyder, 1962). Plates were incubated under 12 h alternating light (black/white) for 48 h and emerging colonies were transferred to potato dextrose agar (PDA) plates. For microscopic and macroscopic observations, all the strains of Fusarium were transferred to PDA, Carnation leaf agar (CLA) (Fisher *et al*., 1982) and Water Agar (WA) media as described in The Fusarium Laboratory Manual (Leslie and Summerell, 2006). The species were identified on the basis of microscopic and macroscopic characteristics as described in the manual (Leslie and Summerell, 2006). Identification of species was based on species description of Leslie and Summerell (2006), Summerbell and Schroers (2002), and Aoki *et al*., (2003, 2005, 2012).

DNA Extraction

Selected strains of *Fusarium* spp. isolates were grown on potato dextrose broth for 10 days (Table 1). The mycelium were harvested and ground in a sterile mortar with liquid nitrogen to a fine powder, and DNA was extracted of each *Fusarium* spp. by using a DNeasy® Plant Mini Kit (Qiagen) according to the manufacturers' instruction.

Polymerase Chain Reaction (PCR) Amplification and DNA Sequencing Alignment

The species identity of *Fusarium* spp. isolates determined through morphological characterization were confirmed by DNA sequencing. Two isolates of each *Fusarium* spp. based on morphological characterization were chosen as representatives which undergone the DNA sequencing (Table 1). *tef*1 gene was amplified by using primers ef1 (5'-ATG-GGT-AAG-GAG-GAC-AAG-AC-3') and ef2 (5'-GGA-AGT-ACC-AGT-GAT-CAT-GTT-3') (O'Donnell *et al*., 1998). Amplification of the internal transcribed

spacer (ITS) regions was conducted utilizing the primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990).

A total volume of 25 µl amplification reactions for each isolates were prepared containing the mixture of 0.2 mM deoxynucleotide triphosphate (dNTP) (Promega), 4 µl 5X buffer (Promega, Madison, WI, USA), 0.8 µM of each primer, 4 mM $MgCl_2$, 0.75 units of Taq DNA polymerase (Promega®, USA), and 6 ng of template DNA. The PCR products were purified by using QIAquick PCR Purification Kit (QIAGEN) according to the manufacturers' instructions. The purified PCR products were sent to First BASE Laboratories Sdn. Bhd. for sequencing, sequencer ABI 3730xl model with 96 capillary by chain termination, or Sanger methods were used by the company for sequencing process.

Table 1. Twelve *Fusarium* spp. isolates from Persian Gulf beach soils (based on morphological identification) selected for molecular study

Culture no.	*Fusarium*	[a]*tef1*	[a]ITS	Location in Iran
PGFSB-41	*F. solani*	-	-	Hormuz island
PGFSB-44	*F. solani*	-	-	Hengam island
PGFPB-45	*F. proliferatum*	-	-	Hormuz island
PGFPB-47	*F. proliferatum*	-	-	Lavan island
PGFEB-69	*F. equiseti*	-	-	Hormuz island
PGFEB-67	*F. equiseti*	-	-	Lavan island
PGFFB-76	*F. falciforme*	-	-	Hormuz island
PGFFB-87	*F. falciforme*	-	-	Kish island
PGFOB-64	*F. oxysporum*	-	-	Hormuz island
PGFOB-67	*F. oxysporum*	-	-	Kish island
PGFVB-56	*F. virguliforme*	KT033493	KT033491	Hormuz island
PGFVB-68	*F. virguliforme*	KT033494	KT033492	Hormuz island

aGenBank numbers for translation elongation factor 1-alpha (*tef1*) partial sequences, and the ITS rDNA regions

DNA Sequence Analysis of EF-1α Gene

The sequences of received *tef*1 gene and ITS regions were aligned and edited by using BioEdit version 7.0.5 (Hall, 1999). The edited alignments were used as a query to search for similarities in using two databases, FUSARIUM-ID and BLAST network services at the National Centre for Biotechnology Information (NCBI).

Results

A total of 100 *Fusarium* isolates were recovered from all soil samples. The direct isolation technique from soil particles was used to isolate *Fusarium* species from beach soil samples. *Fusarium* species were identified by observing the macroscopic and microscopic characteristics. For species determination, the descriptions by Leslie and Summerell (2006), Summerbell and Schroers (2002), and Aoki *et al*., (2003, 2005, 2012) were adopted. In this research *Fusarium solani* is the most common species found in the beach samples which had the highest percentage of abundance (58%). The percentage of abundance for other *Fusarium* species is relatively low, where *F. proliferatum* consists of 12%, followed by *F. equiseti* (10%), *F. falciforme* (10%), *F. oxysporum* (7%) and *F. virguliforme* (3%).

Morphological studies showed 71 out of 100 strains obtained from soil in Persian Gulf beaches were identified as *F. solani* species complex (FSSC). Features showed that all strains belonged to *F. solani* (58), *F. falciforme* (10), and *F. virguliforme* (3) as the known species among FSSC. *Fusarium solani* was characterized by the production of sparsely aerial mycelium and dorsiventral falcate macroconidia with 3-5-septates. *Fusarium falciforme* produced short dorsiventral falcate macroconidia with 3-4-septates. Three strains were identified as *F. virguliforme* that are characterized by production of pink to bluish-gray mycelium. Also, they produced dorsiventral falcate macroconidia with 3-5-septates, with a tapered and curved apical cell; and distinctly notched basal cell and microconidia were comma-shaped, short-clavate and ellipsoidal shaped with a swollen apex often rounded and mostly 0-2-septates (Fig. 1).

Features showed that 12 out of 100 strains belonged to *F. proliferatum* that are characterized by production of slender and relatively straight macroconidia with 3-5-septates. Microconidia form in chains and false heads. And, conidiogenous cells from monophialides and polyphialides. The polyphialides may proliferate extensively. Also, 10 strains of *Fusarium* spp. strains were identified as *F. equiseti* that are characterized by the production of long and slender macroconidia with tapered and elongate apical cell and foot shape basal cell. Morphological studies showed that 7 strains were identified as *F. oxysporum.* All the strains were characterized by the production of 3-5-septates macroconidia which are slender than those of *F. solani*. *F. oxysporum* usually produces a pale to dark violet pigment in the PDA plates. Conidiogenous cells form short monophialides.

The species identity of *Fusarium* spp. isolates determined through morphological characterization were confirmed by DNA sequencing. Two isolates of each *Fusarium* spp. based on morphological characterization were chosen as representatives which undergone the DNA sequencing (Table 1). According to Chehri *et al*., (2014) PCR products were purified using Qiagen columns according to the manufacturer's instructions and stored at -20°C. The purified PCR products were sent First BASE Laboratories Sdn. Bhd. for sequencing of *tef1* gene and ITS regions in both directions (forward and reverse) in order to use ABI 3730x1 model of sequencer. Forward and reversed sequences of *tef1* gene and ITS regions were edited and aligned which used BioEdit version 7.0.5 (Hall, 1999). Based on the similarities of conducted researches at NCBI database and FUSARIUM-ID, all strains were similar to *F. solani*, *F. proliferatum*, *F. equiseti*, *F. falciforme*, *F. oxysporum* and *F. virguliforme* with the percentage of maximum identity from 97% - 99%.

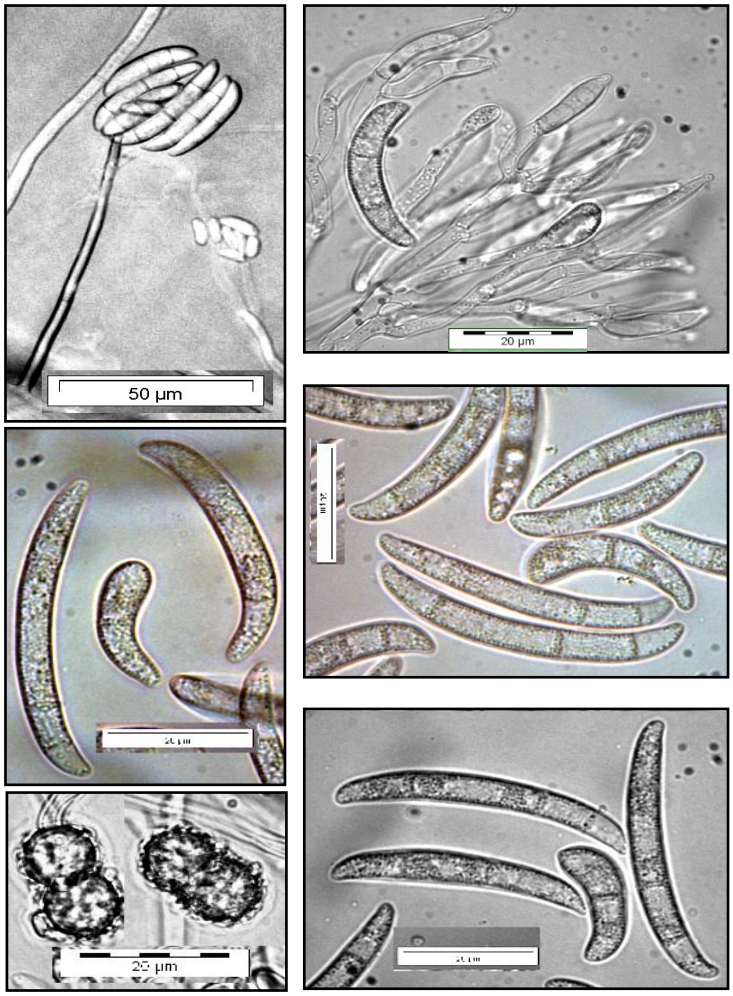

Fig. 1. *Fusarium virguliforme* grown on CLA, two weeks, 25°C, cultured in the dark. a, Slender, unbranched aerial conidiophores. b, Branched sporodochial conidiophores forming falcate to curved cylindrical conidia. c, d, f, Falcate macroconidia and comma-shaped microconidia. e, Terminal and intercalary chlamydospores,. Scale bars: b-f= 50 µm, a= 20 µm.

Discussion

Comprehensive information about the diversity of *Fusarium* spp. has not yet been well documented in Iran especially in Persian Gulf. There is some information concerning *Fusarium* spp. in tempered regions in Iran (Darvishnia *et al*., 2006; Chehri *et al*., 2010; Chehri *et al*., 2014); however, there is no report on the occurrence of *Fusarium* species on Persian Gulf beaches as one of the tropical regions in Iran. Thus, this study focused on isolation and identification of *Fusarium* spp. based on the morphological and molecular characteristics.

According to Nucci and Anaissie (2007), 12 species of *Fusarium* have been isolated from human and animal sources that had caused different types of infections in their hosts. *Fusarium solani, F. falciforme* (O'Donnell *et al*., 2008; Rosa *et al*., 1994), *F. Oxysporum* (Rosa *et al*., 1994), *F. proliferatum* (Bodey *et al*., 2002; Herbrecht *et al*., 2004) are the common species associated with various types of localized and systematic infections in humans that are associated with soils around the world, and are in agreement with obtained results in this study. Hence, the occurrence of these

species on Persian Gulf beaches will be important for development of proper management strategies in order to control fusarial keratitis in different sensitive animals such as turtles and crab, those are living near beaches.

Fusarium virguliforme is associated with variety of devastating diseases in many economically important agricultural crops such as the disease of sudden-death syndrome (SDS) in soybean (Aoki *et al*., 2005). *Fusarium virguliforme* has been reported from different countries in the world for example Brazil (Nakajima *et al*., 1993), United States (Aoki *et al*., 2003) and Canada (Anderson and Tenuta, 1998). The first report on *F. virguliforme* in Iran indicates the need to clarify the biology of this fungus in order to provide sufficient information regarding its economic significance and to develop appropriate control measures.

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