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Cloacal fungal flora in rock lizard *Laudakia nupta* (Sauria: Agamidae) in Iran

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ABSTRACT

Fungal species are the common pathogen on wildlife animals. Reptiles usually bear microbial flora in the cloaca. Native microflora of reptiles is important and cloacal fungal flora in *Laudakia nupta* has not yet been reported. In Iran usually agamid lizards, especially *Laudakia nupta,* live in rural areas in close vicinity to humans, so they play an important role in the spread of microorganisms and fungi in the environment and humans. The aim of this study was to characterize the cloacal fungal flora in *Laudakia nupta* using molecular studies. The cloacal fluids were collected and directly placed in potato dextrose agar plates and incubated for 48 h. All

fungal isolates were purified through single-spore isolation technique. Twenty-three isolates were obtained from lizard samples and used in molecular studies. All fungal isolates were identified as *Aspergillus niger*, *Fusarium solani*, and *Botryotrichum atrogriseum* based on partial sequences of the*tub2*gene. This is the first report of fungal spp. associated with cloaca of *Laudakia nupta* using molecular studies. In this research, native fungal flora in the cloaca of *Laudakia nupta* was identified.

Keywords: Fungi, Cloaca, Microflora, Lizard, *Laudakia nupta*

1. INTRODUCTION

Fungi are considered ubiquitous organisms in nature and they are opportunistic invaders of the integument, respiratory system, and gastrointestinal tract (Mitchell and Tully, 2009). Many fungal species have been isolated from reptiles (Rosenthal and Mader, 1996; Nichols et al.1999; Cheatwood et al. 2003). Fungal organisms have been isolated from the oral cavity, through the intestinal tract, stomach and into the cloaca. Although most of the fungi are normal residents of reptile gastrointestinal tract, they can cause secondary infections under suboptimal conditions and can play an important role as disease-causing agents in reptiles (Jacobson 1980; Hernandez-Divers 2001; Miller et al. 2004; Orós et al. 2004). Infection of reptiles by fungi has been regarded as opportunistic, caused by normally saprophytic organisms that invade living tissue strictly under favorable circumstances (Austwick and Keymer 1981; Migaki et al. 1984). Most fungi may become pathogenic for their animal hosts (Jacobson 2007). Fungal disease may present as dermatomycosis (cutaneous infection) or as disseminated (systemic) mycosis. Fungal species are true pathogens of mammals but, until now, none had yet been clearly identified as a true pathogen of reptiles (Migaki et al. 1984). The Large-Scaled Rock Agama, *Laudakia nupta,* is found in mountainous areas, among large rocks and around human habitats as well as rural and urban areas (Fig.1), (Rastegar-Pouyani and Nilson 2002; Rastegar-Pouyani et al. 2006). This species has a wide distribution in Iran (Anderson 1999). In this research, native fungal flora in the cloaca of *Laudakia nupta* was identified as *Aspergillus niger*, *Fusarium solani*, and *Botryotrichum atrogriseum*. *Botryotrichum atrogriseum* is a terrestrial fungus and opportunistic human pathogen reported in freshwater leeches mycoflora (Khalilli et al. 1991) as well as the genus *Aspergillus* are usually saprophytic and fast-growing fungi isolated from soil (Phillott 2002). Many species of genus *Aspergillus* have been recognized as human and plant pathogens (Dagenais and Keller2009) and cause opportunistic invasive infections such as *Aspergillus niger, Aspergillus flavus, Aspergillus versicolor,* etc. (Denning 1998; De Hoog et al. 2000). Also, the genus *Fusarium,* like many soil fungi, is widely found in soil and organic substrata (Burgess 1981; Booth1985; Rodrigues and Menezes 2006). Also, a number of *Fusarium* species such as *Fusariumsolani*, *Fusarium proliferatum* have been the cause of infection, disease and mortality in some species of reptiles and humans (Desjardins et al.2006; Nardoni et al. 2008; Chehri et al. 2015). Based on previous studies, these fungi are common allergens and may cause opportunistic invasive infection. *L. nupta* is abundant in residential areas in contact with humans. Therefore, identification of the cloacal fungal flora is important in this rock-dwelling lizard and we found that *L. nupta*is a carrier of microorganisms that can transfer fungi to other animals and humans. This lizard usually lives in close vicinity to humans and identification of microbial flora is important for further studies.

2. MATERIAL AND METHODS

This research was approved by the ethics committee of Razi University.

Isolation of fungal species

Fifty specimens of *Laudakia nupta* (male and female) were collected from Kermanshah Province, Western Iran. The outer part of cloacal region of samples was disinfected by ethyl alcohol (70%). The cloacal fluids (Fig. 2) were collected by syringe and directly placed in the potato dextrose agar (PDA) plates and incubated at 25±2ºC for 48 h. All fungal isolates were purified through single-spore isolation technique. The resulting fungal colonies were transferred to fresh potato dextrose agar (PDA) plates for further studies. The specimens were released in the environment after examination.

DNA extraction, PCR amplification, and Sequencing alignment

All fungal isolates were grown on potato dextrose broth (PDB) for 5 days. The mycelia were harvested and ground in a sterile mortar with liquid nitrogen to a fine powder. The DNA extraction was done by using The DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Amplification of the *tub2* gene was conducted using primer pair T1 and T2 for *tub2* gene (O'Donnell and Cigelnik 1997). The PCR was performed in a Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) in a total volume of 25 µl for

each strain. The PCR mixture contained 4 µl 5X buffer (Promega, Madison, WI, USA), 4 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate (dNTP) (Promega), 0.8 µM each primer, 0.75 units of Taq DNA polymerase (Promega), and 6 ng of template DNA. The PCR for the *tub2* gene was performed with an initial denaturation of 1 min at 94°C followed by 39 cycles of 30 sec. at 94°C, 30 sec. at 58°C and 1 min at 72°C, and a final extension of 5 min at 72°C. PCR products were purified using Qiagen columns according to the manufacturer's protocol and sent for sequencing to a service provider. Nucleotide sequences were edited and assembled with BioEdit software version 5.0 (bioedit.software.informer.com). The aligned sequences were BLAST in genome database of GenBank to identify all the selected isolates. The edited *tub2* gene sequences were compared with other available fungal species sequences in the GenBank.

Figure 1 *Laudakia nupta* in habitat

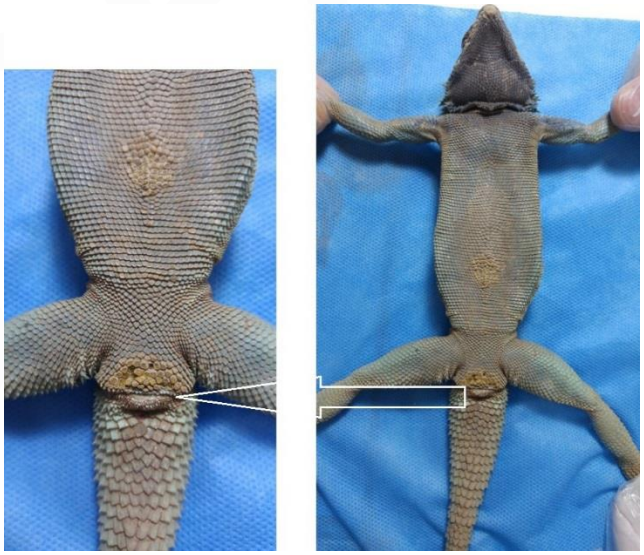

Figure 2 Cloacal area in *Laudakia nupta*

3. RESULTS

A total of 50 *Laudakia nupta* specimens were collected from Kermanshah Province, Western Iran. The presence of different fungi was observed in 23 cloacal regions of *Laudakia nupta* under the stereo microscope. All isolates were chosen for DNA sequence analysis using the *tub2*gene. All fungal isolates were identified as *Botryotrichum atrogriseum* (12), *Aspergillus niger* (8) and *Fusarium solani* (3)

using molecular studies. The obtained sequences were compared with those on the NCBI. From similarities searched at NCBI database, identification of all species was confirmed with statistical significance.

4. DISCUSSION

Fungi have been recognized as main agents of human infections (Enweani et al. 1997). The opportunistic pathogenic role of most of the present fungal isolates has been well documented in reptiles and humans (Nardoni et al. 2008). Fungal organisms have been isolated from the oral cavity, through the stomach, intestinal tract and into the cloaca (Milena et al. 2013). There are hypotheses about the presence of the same fungal isolates in the cloacal area (Phillot et al. 2002), including: contact with feces of other infected animals or contact with the infected substrate, as well as the distribution of similar fungal isolates in different hosts, suggesting that reptiles may act as effective animal carriers for fungi and yeast in their cloacae (Nardoni et al. 2008).

In this research in the cloacal region of *Laudakia nupta, Aspergillus niger, Botryotrichum atrogriseum* and *Fusarium solani* were isolated. Some *Aspergillus* species were reported as causative agent of numerous infections in reptiles and has been firmly incriminated in a disproportionately high number of reptilian mycoses in lizards, snakes, and crocodiles (Paré et al. 1997; Nichols et al. 1999; Thomas et al. 2002; Bertelsen et al. 2005) suggesting it carries a substantial pathogenic potential for reptiles. The *Aspergillus* and *Candida* species have been isolated from pulmonary lesions of lizards and chelonians (Milena et al. 2013). *Aspergillus terreus*, *Aspergillus niger*, and *Fusarium solani* are known to be the cause of mortality in embryos of some species of sea turtles (Nardoni et al. 2008). *Botryotrichum atrogriseum* is a terrestrial fungus common only on cellulose agar medium and reported in freshwater leeches mycroflora (Emmons *et al*., 1977; Khalilli et al. 1991).

Also *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae*, and *Aspergillus versicolor* can cause allergic states, toxicosis, and opportunistic invasive infections in humans, though with different frequency (Nardoni et al. 2008).

The prevalence of *Aspergillus* spp., *Candida* spp., *Fusarium* spp. and *Mucor* spp. is considerable as these fungi are known to cause infections with varying degrees of severity (Emmons et al. 1977).

It is likely that *L. nupta* cloacae transmit fungi by contact with environment and substrate (Phillot et al. 2002) and also this species can be animal carriers for fungi and yeast in their cloacae for the transmission of microorganisms (Nardoni et al. 2008). In this study, we identified the fungal flora colonizing the cloaca of *L. nupta* using morphological and molecular studies. This report is one of the foremost and recent findings of the fungal flora of the cloaca of this lizard in Iran. Living in close vicinity to man, reptiles could play an important role in the transmission of fungal agents to human beings (Emmons et al.1977).

5. CONCLUSION

In this study, fungi did not have any negative effects on lizards and they were found in all species studied as well as are part of the natural flora of the cloaca in *L. nupta.* By doing this research, we found that *L. nupta* is a carrier of microorganisms which can transfer fungi to the environment, other animals, and humans.

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Authors' Contribution

All authors participated in the research design and contributed to different parts of the research.

Conflict of Interests

The manuscript has been seen and approved by all authors. The authors declare that there was no conflict of interest to publish this article. All authors are satisfied with this study and are aware of it.

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