Identification of Fungi Associated With *Dermacentor variabilis* Ticks

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Abstract

Ticks are medically important arthropods because they carry numerous pathogens of humans and domesticated animals. Recent studies showed that besides pathogenic microorganisms, ticks carry numerous endosymbionts which significantly affect different aspects of tick biology. For example, endosymbiotic microorganisms can reduce tick's ability to carry pathogens. So far, studies have mostly focused on molecular identification of bacterial endosymbionts of ticks targeting prokaryotic 16S ribosomal RNA. In our study we attempted to identify fungal species that are associated with ticks. We used a molecular approach to detect fungal spp. in a *D. variabilis* tick. Using fungi specific primers, we amplified a sequence of fungal ITS region by PCR in which as a template we used DNA extracted from tick. To identify origins of fungal DNA in sample we purified amplified sequences and performed cloning in pGEM-T vector plasmid followed by sequencing around 30 clones per samples. After bioinformatic analysis of obtained sequences, which consists of using BLAST tool in Genbank, we identified 20 fungal species associated with used tick species, with at least 10 of them being possible tick endosymbionts. Future studies, focused on tick dissection and determination of which part of tick body contains fungal endosymbionts, are required to determine role of fungal endosymbionts in tick biology.

Introduction

Ticks are hematophagous arthropods of medical importance. They often feed on humans and during feeding they can transmit pathogenic microorganisms they carry, including bacteria, viruses, protozoa, and helminths. Emerging and re-emerging tick-borne diseases intensified studies of ticks in the last couple decades. Initially, studies were focused on detection of pathogenic microorganisms that ticks can transmit, but later focus switched to studies of tick microbiome, since it was shown that endosymbiotic microorganisms can affect vector capacity of ticks. Tick microbiome studies advanced with application of next-generation sequencing approach. However, these studies are mostly based on targeting bacteria specific 16S rRNA and reveal presence of different bacteria in ticks (Couper and Swei, 2018; Greay et al., 2018), while other endosymbiotic microorganisms stay unidentified. Very few data on fungi associated with ticks is obtained through traditional microbiological culturing techniques (Yoder et al. 2003, 2018; Benoit et al., 2009). The purpose of this study is to use molecular biology approach to identify fungi associated with *Dermacentor variabilis* ticks.

Material and Methods

An unfed female *Dermacentor variabilis* tick, collected by flagging in Nacogdoches area, was used in this study. Tick was washed in 70% ethanol, followed by four washes in nuclease-free water. Total DNA from tick (DV) was extracted by using DNeasy Blood & Tissue Kit (Quigen) according to manufacturer's instructions. Two controls were prepared by extraction of DNA from the water used for fourth wash (washing control – WC) and environmental control (EC) made by DNA extraction without starting material with purpose of identification of fungal spores present in the lab environment.

Extracted DNA was used as a template in a PCR to amplify ITS region of rRNA by using universal fungi-specific primers NSA3 and NLC2 (Martin and Rygiewicz, 2005). Amplified sequences from the ticks sample (DV), as well as from both control, were purified using Wizard SV Gel and PCR Clean-Up System (Promega) and cloned in pGEM-T vectors. Transformation was performed by using JM109 competent cells. A total of 30 clones per sample were randomly selected and sequences by using Eurofins Genomics service. Bioinformatic analysis was performed by using BLAST tool in the GenBank.

Results and Discussion

Targeted fungal ITS region was successfully amplified from tick sample, as well as from both controls (Fig. 1). However, intensity and estimated sizes of amplified sequences indicated that there is the difference in amplicons originated from tick sample and washing control on one, and environmental control on the other size. After random selection of clones for sequencing, bioinformatic analysis revealed different origins of amplified fungal sequences (Table 1).

Conclusions

Our study identified 20 fungal species associated with *Dermacentor variabilis* tick, with at least 10 of them being possible tick endosymbionts. Future studies are required to determine role of fungal endosymbionts in tick biology.

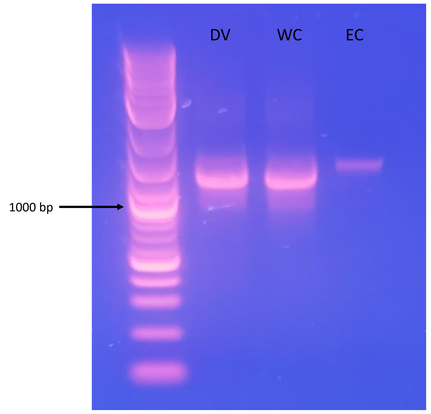

Figure 1. Amplified sequences of fungal ITS region from tick samples (DV) and controls (WC and EC).

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Table 1. Results of bioinformatic analysis revealing origins of amplified fungal sequences..

BEST MATCH IN GENBANK (ACCESSION NUMBER)	CLONES THAT MATCH (%)
Clones from Dermacentor variabilis sample	
Fungal endophyte (KR016091.1)*	6/30 (20.00%)
Didymella pinodella (MW784722.1)*	4/30 (13.33%)
Penicillium commune (GQ458026.1)*	2/30 (6.67%)
Seiridium marginatum (KT949916.1)*	2/30 (6.67%)
Coniothyrium palmicola (JX681086.1)*	2/30 (6.67%)
Penicillium crustosum (MT582770.1)	2/30 (6.67%)
Lophiostoma macrostomum (EU552140.1)*	2/30 (6.67%)
Pleosporales – uncultured (HG995691.1)	2/30 (6.67%)
Cladosporium sp. (MH047202.1)	1/30 (3.33%)
Monochaetia sp. (KC311518.1)	1/30 (3.33%)
Pestalotiopsis maculiformans (EF451801.1)	1/30 (3.33%)
Diplodia corticola (KF500478.1)*	1/30 (3.33%)
Neomassarina thailandica (NR154244.1)	1/30 (3.33%)
Chaetomium globosum (MN602645.1)	1/30 (3.33%)
Alternaria infectoria (MK828116.1)*	1/30 (3.33%)
Fungal sp. (MG925035.1)	1/30 (3.33%)
Clones from washing control	
Fungal endophyte (KR016091.1)*	7/28 (25.00%)
Uncultured fungus (LR993876.1)	4/28 (14.29%)
Didymella pinodella (MW784722.1)*	3/28 (10.71%)
Seiridium marginatum (KT949916.1)*	2/28 (7.14%)
Vishniacozyma sp. (MN450784.1)	2/28 (7.14%)
Coniothyrium palmicola (JX681086.1)*	1/28 (3.57%)
Lophiostoma macrostomum (EU552140.1)*	1/28 (3.57%)
Alternaria infectoria (MK828116.1)*	1/28 (3.57%)
Lophiostoma compressum (MW759263.1)	1/28 (3.57%)
Penicillium commune (GQ458026.1)*	1/28 (3.57%)
Diplodia tsugae (MH858473.1)	1/28 (3.57%)
Diplodia corticola (KF500478.1)*	1/28 (3.57%)
Dothideomycetes sp. (JQ760421.1)	1/28 (3.57%)
Uncultured fungus (KY687847.1)	1/28 (3.57%)
Tilletiopsis sp. (MN901708.1)**	1/28 (3.57%)
Clones from environmental control	
Tilletiopsis sp. (MN901708.1)**	9/28 (32.15%)
Basidiomycota – uncultured (EU490099.1)	6/28 (21.43%)
Lecanicillium saksenae (AB360363.1)	6/28 (21.43%)
Tilletiopsis lilacina (KP322984.1)	2/28 (7.14%)
Paraisaria heteropoda (AB027373.1)	2/28 (7.14%)
Malassezia globosa (KM370119.1)	1/28 (3.57%)
Trametes hirsuta (CP019375.1)	1/28 (3.57%)
Uncultured fungus (GU053834.1)	1/28 (3.57%)

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