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Isolation and Molecular Identification of *Xylaria adscendens* and *Acremonium zeae* from Exotic Vegetables in Aberdeen

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Abstract

In an experiment involving the isolation and molecular identification of fungi associated with exotic vegetables in an Aberdeen shop, *Xylaria adscendens* and *Acremonium zeae* were isolated and identified from scotch bonnet chilli (*Capsicum chinense*) and baby corn (*Zea mays*) imported from Uganda and Kenya respectvely. Isolates were identified using colony and morphological characters on Potato Dextrose Agar and PCR analysis. The DNA of the fungi were extracted using a QiagenDNeasy Plant Mini Kit and PCR products were purified with a QIAquick PCR Purification Kit after electrophoresis. PCR amplifications were run using the primer pair ITS1/ITS4. DNA sequences were compared to published sequences in GenBank using BLASTn. *Xylaria adscendens* and *Acremonium zeae* have both been reported as edophytes of many important crops plants with potential to serve as biocontrol agents against some pathogenic fungi.

Introduction

Xylaria adscendens and *Acremonium zeae* are reported fungal endophytes of many plants with potential for use as biocontrol agents against some important plant pathogenic bacteria and fungi. *Xylaria adscendens* belongs to the Phylum Ascomycota, Class: Sordariomycetes, Order: Xylariales and family: Xylariaceae (Hawksw, 1973). *Xylaria* Hill ex Schrank is one of the most speciose genera in Xylariaceae, an ascomycete family characterized by stromatic, perithecium-bearing ascomata. This genus has a cosmopolitan distribution (Rogers *et al*. 2002b), with most of the species occurring in tropical and subtropical regions (Lodge *et al*. 2008; Fournier *et al*. 2011). Most species of *Xylaria* play important functional roles in terrestrial ecosystems as saprotrophs on wood and plant debris and on dung and termite nests (Rogers 1979). However, some species are reported as weak or strong phytopathogens (Vannini *et al*. 1996) and others were discovered to be endophytes of many tree species (Whalley 1996; Læssøe 1999; Crozier *et al*. 2006; Thomas *et al*. 2008; U'Ren *et al*. 2009; Vega *et al*. 2010). *Xylaria* are visible during sexual sporulation, forming relatively large, macroscopic stromata, or 'fruiting' structures (Bayman *et al.* 1998, Davis & Shaw 2008). *Acremonium zeae* on the other hand is one of the most prevalent fungal colonists of preharvest corn and produces symptomless infections of corn seeds and has been isolated from the stalks of mature plants 9 Fisher *et al*., 1992; King, 1981; Reddy and Holbert, 1924; Sumner, 1968).

Acremonium zeae has been the subject of recent investigations because of its production of pyrrocidine antibiotics and its potential to serve as a biocontrol agent against mycotoxin producing fungi (Wicklow, 2005). The observation by Harris (1936) that, when cultured on artificial media, *A. zeae* ''grew most vigorously on medium containing xylan isolated from maize cobs'' suggested that *A. zeae* might be a source of hemicellulolytic enzymes uniquely adapted for the utilization of maize cell wall components. *Acremonium zeae* produces two lactam-containing antibiotics, named pyrrocidine A (PA) and B (PB), with PA exhibiting greater inhibitory activity against *Fusarium verticillioides* and other fungi (Gao *et al*., 2016). *Acremonium zeae* has been characterized as a protective endophyte of maize and displays antifungal activity against other fungi. Pyrrocidines A and B were discovered to be the metabolites accounting for this activity. Pyrrocidine A also showed potent activity against major stalk and ear rot pathogens of maize, including *Fusarium graminearum*, *Nigrospora oryzae*, *Stenocarpella* (*Diplodia*) *maydis*, and *Rhizoctonia zeae* while also exhibiting potent activity against *Clavibacter michiganense* subsp. Nebraskense, the causal agent of Goss's bacterial wilt of maize(Poling *et al*., 2008).

Acremonium zeae is not recognized as causing ear, kernel or storage rots of maize (White, 1999) and there have been no reports that *A. zeae* isolates from maize produce any metabolites toxic to animals or plants (Dillon *et al*., 2018)**.** *Acremonium zeae* was found to be antagonistic to kernel-rotting and mycotoxin-producing fungi *Aspergillus flavus* Link and *Fusarium verticillioides* (Sacc.) Nirenb. in cultural tests for antagonism (Wicklow *et al*., 1980, 2005). The fungus also limited *Aspergillus flavus* colonization and aflatoxin contamination of intact grains removed from ears produced in an environmental chamber and wound-inoculated in the milk stage with both *Aspergillus flavus* and *A. zeae* (Wicklow *et al*., 1988). Chemical studies of the organic extract from maize kernel fermentations of *A. zeae*, which displayed significant antifungal activity against *Aspergillus flavus* and *F. verticillioides* in conventional paper disc assays, revealed that the metabolites accounting for this activity were two polyketide – amino acid derived antibiotics pyrrocidines A and B (Wicklow *et al*., 2005). Pyrrocidine A also exhibits potent in vitro activity against major stalk and ear rot pathogens of maize including *Stenocarpella maydis* (Berk.) B. Sutton and *Fusarium graminearum* Schwabe (Wicklow and Poling 2006). *Acremonium zeae* was characterized as a "protective endophyte" of maize that might best defend against pathogen attack at the more vulnerable seed and seedling stage (Wicklow *et al*. 2005). This work reports another detection of *Xylaria adscendens* and *Acremonium zeae* from exotic scotch bonnet chilli and baby corn in Aberdeen.

II. Materials and Methods

A. Sterilization of Equipment

The glassware used were washed in detergent, rinsed with tap water and dried with paper towel. They were then either rapped or covered aluminum foil paper before being autoclaved. Scalpels were sterilised by dipping in 70% alcohol for 5 minutes and then passing through a flame. Inoculating chamber were sterilised by scrubbing with 70% alcohol. All isolations and inoculations were carried out in the sterile inoculating chamber. The working surfaces of laminar flow cabinets were disinfected with 70% ethanol before working on them.

B. Isolation of Fungi

The isolation of fungi were carried out from scotch bonnet chilli and baby corn purchased from a shop in Aberdeen. The labels on the vegetables indicated that the scotch bonnet chilli was imported from Uganda, while the baby corn was imported from Kenya. Isolation from tissues were done by surface sterilizing 5 pieces of tissue in 3% NaOCl for 3 minutes and rinsing in 2 changes of sterile distilled water before placing on 1/4 strength Potato Dextrose Agar (PDA) medium. They were then incubated at 25°C for two to three days after which they were examined for colony growth. Subcultures were made on new 1/4 strength PDA to obtain pure isolates. Single spore/hyphal tip isolation on water Agar were carried out to further purified cultures. The spores/hyphal tips were transfered to new full strength PDA plates and incubated at 25°C in the incubator for 7 days. The purified cultures were then stored in the cold room for subsequent colony and molecular analyses.

C. Identification of Isolates

Morphological identification was carried out by observing colony features on full strength PDA. Mycelia structures were examined and photographed with a research light microscope by interference contrast microscopy. Morphological features with reference to standard literatures were used for the preliminary identification of the isolates. Molecular identification was carried out by DNA exraction and PCR analysis.

1) DNA Extractions / PCR Analysis

Isolates were grown on a sterile cellophanes placed on full strength PDA for 7 days after which fungal mycelia were scraped washed into a sterile conical flasks with sterile distilled water The DNA of fungal isolates were extracted using a QiagenDNeasy Plant Mini Kit and PCR products were purified with a QIAquick PCR Purification Kit after electrophoresis. The PCR amplification was done using the ITS1 and ITS4 primers designed to amplify the internal transcribed spacer (ITS) region. DNA sequences were compared to published sequences in GenBank by blasting the sequences at NCBI database using BLASTn.

Quick DNA extraction was carried out with a modified method from Modified method from Collado-Romero *et al.*, 2006. The samples were sent to 'Source BioScience' company, Scotland, for sequencing. The software used to analyse them was CLC Main Workbench.

III. Results and Discussion

Macroscopic features of *Xylaria adscendens* colony on PDA and mycelia characteristic were characterised under a microscope and conformed morphologically most closely to the genus *Xylaria*. Morphological and molecular analyses identified isolates as *Xylaria adscendens* and *Acremonium zeae*. At the early stage of growth, *X. adscendens* myceliom was cottony-white and gradually showed pronounced zonation after 2 weeks of growth. There after the mycelium became grayish white in appearance. *Acremonium zeae* growth rate was moderately rapid following incubation at 25°C on PDA. The texture of the colony was compact, flat, and occasionally raised in the center. It was glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed. The color of the colony was white or pale pink on the surface. The reverse side was either uncolored or a pink to rose colored due to pigment production. *Acremonium* spp. possessed hyaline, septate hyphae which were typically very fine and narrow. The phialides were not very visible under the microscope even at high magnification. However numerous single and multicellular conidia were observed (Fig. 1).

Dillon *et al*. (2018) similarly reported a species of *Xylaria* to possess colony with cotton -white mycelium at 5 days incubation, which later showed pronounced zonation with finely plumose margin at 15 days. Colony eventualy showed Whitish-gray stromata after 35 days of incubation. Some workers have also reported the growth rate of *Acremonium* colonies to be moderately rapid, following incubation at 25°C on potato glucose agar. The texture of the colony has been observed to be compact, flat, and occasionally raised in the center. The colony was also glabrous, velvety, and membrane-like at beginning. Powdery texture may also be observed. By aging, the surface of the colony became cottony due to the overgrowth of loose hyphae. The color of the colony is white or pale pink on the surface. The reverse side was observed to be either uncolored or a pink to rose colored due to pigment production (Collier *et al*., 1998; Larone, 1995; St-Germain and Summerbell, 1996). Other workers have also reported *Acremonium* spp. to possessed hyaline, septate hyphae which were typically very fine and narrow. Single or multicellular conidia were also reported (Collier *et al*., 1998; Larone, 1995; St-Germain and Summerbell, 1996).

The BLAST search revealed 99% and 100% identity to *X. adscendens* and *A. zeae* in GenBank respectively. *Xylaria adscendens* had 5% frequency of isolation from scotch bonnet chilli imported from Uganda, while *Acremonium zeae* had isolation frequencies of 60% on baby corn from Kenya.

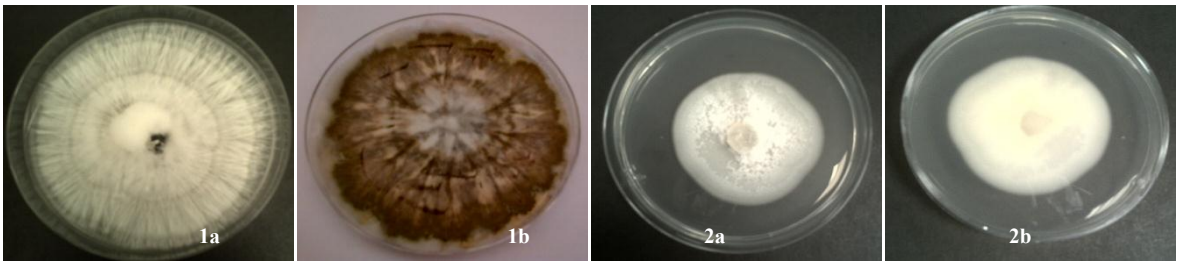

Fig. 1 Colony morphology on of *Xylaria adscendens* (1) and *Acremonium zea* (2) on PDA, 1a. 14 days after incubation showing cottony-yellowish white mycelium with zonation; 1b. greyish white colony after 25 days incubation; 2a. Front; 2b. Back

Conclusion

The use of chemicals for pest and disease control can be drastically reduced if integrated disease management that is based on the use of endophytes is practised. The widespread use of the chemical fungicides has become a subject of the research concern due to their harmful effect on non-target organisms as well as their possible carcinogenicity. The use of fungal endophytes as biocontrol agents is gaining popularity as important alternative to chemicals in crop protection against many diseases. Endophytic fungi play an important role in protecting their host from attack by phytopathogens; in addition to several other benefits (Maheshwari 2011; Rana *et al*., 2016a, b, 2017; Verma *et al*., 2015b, c, 2016a, b). This work reports another detection of endophytic *Xylaria adscendens* and *Acremonium zeae* from exotic scotch bonnet chilli and baby corn in Aberdeen. Further studies should be carried out to established their biological control potentials or pathogenicity.

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