Molecular Characterization of Post Harvest Fungus Associated with Spoilt Broccoli

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Broccoli (Brassica oleracea var. italica) is a nutritional vegetable that looks like a small tree. Despite the fact that it is extensively loaded with arrays of vitamins, minerals, fiber and antioxidants, it has been observed that it has a short lifespan of not more than 2-5 days due to post-harvest deterioration. A study was conducted to isolate and identify the common fungal pathogens causing post-harvest deterioration of broccoli crown. Diseased broccoli crowns were collected from Ogunabali Fruit Garden Market in D-Line, Port Harcourt Local Government Area of Rivers State. Fungal isolates were collected and morphologically identified. The DNA of the most common fungal isolate, BC-3B was molecularly characterized using Internal Transcribed Spacer 4 and 5 (ITS-4 and 5) molecular markers. The morphological studies revealed that the BC-3B isolate was an Aspergillus niger. The BC-3B isolate DNA sequence was aligned using Basic Local Alignment Search Tool for Nucleotide (BLASTN) 2.8.0 version of National Center for Biotechnology Information (NCBI) database. The molecular weight of the DNA of the isolates was over 600base pairs. Based on sequence similarity, it was observed that the broccoli isolate BC-3B was 93% identical to Aspergillus niger. From the above results, these findings showed that Aspergillus niger is the causal fungal pathogen of post-harvest rot of broccoli. Phylogenetic tree was constructed to access the relationship between the isolates obtained from this study. This study has provided
information on some of the fungal organisms found in broccoli. It is anticipated that this result will provide information for disease control approach for alleviating the post-harvest losses of broccoli caused by Aspergillus niger and provide a foundation for further study of possible harm of consuming diseased broccoli.

Keywords: Brassica oleracea; Aspergillus niger; RBCL marker and post-harvest disease.

1. INTRODUCTION

Broccoli belongs to the family of the Brassicas [1] and its scientific name is Brassica oleracea var. italica. It has a short and fleshy stem with curled dark green leaves with several heads of well-developed green-coloured floral buds [2]. Broccoli is similar to cauliflower and some authors consider them to be the same variety. The consumption of broccoli, is on the increase because it is a healthy food with multiple culinary uses. It is low in calories, has a pleasant taste and rich in mineral and vitamins.

Broccoli, is highly perishable and there are several problems that damage the crown during storage caused by certain fungi and bacteria. These pathogens cause rots and weaken its quality, making them unsuitable for sale or consumption. This study is aimed at isolating and identifying fungal pathogens associated with post-harvest diseases of broccoli (brassica oleracea) crown, using molecular method.

2. MATERIALS AND METHODS

2.1 Source of Plant Material

Diseased broccoli crowns were collected from Ogunabali Fruit Garden Market in D-Line, Port Harcourt Local Government Area of Rivers State.

2.2 Isolation of fungi from broccoli (brassica oleracea) using Blotter Method

Standard blotter method was used to isolate fungi pathogens associated with brassica oleracea [3]. The Whatman’s 9cm filter paper, distilled water and Petri dishes used for the work were sterilized for 15mins, at a temperature of 121°C. The Petri-dishes were lined with 3 layers of the sterilized filter paper, which were soaked with distilled water. The diseased broccoli crowns were surface sterilized for 2 minutes using 70% ethanol, plated and incubated for 7 days at temperature of 25 ± 2°C at the Pathology/Mycology laboratory of the department of Plant Science, University of Port Harcourt, Rivers State, Nigeria. The brassica oleracea isolate was coded (BC-3B).

2.3 Morphological and Microscopic Characterization and Identification

The mycelium of the fungal isolate BC-3B was cultured on Potato Dextrose Agar medium at room temperature for 7 days. The morphological identification of isolate BC-3B was conducted visually by observing the mycelium and comparing with the pictorial guide suggested by Snowdon [4]. Colonies were compared for their diameters, overall colors, colors of conidia, reverse colors, texture, zonation and sporulation. After which, the isolate was subjected to microscopic analysis for its characterization and identification using an electron binocular microscope at X40.

2.4 Molecular Characterization using the Internal Transcribed Spacer (ITS) Marker and Identification

The Genomic DNA of the isolate BC-3B was extracted following the protocol of QuickDNA™Fungal/Bacterial MiniPrepKit (Zymo Research Group, California, USA) as described by the manufacturer, with modifications at the Regional Center for Biotechnology and Bioreources (RCBB), University of Port Harcourt, Rivers State, Nigeria. The BC-3B isolate DNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280nm to that of 260nm. The quality of the gDNA of the isolate BC-3B was further quantified using the Agarose gel electrophoresis performed according to the modified method of SaghaiMaroof et al [5]. The DNA sample of the BC-3B isolate shipped to the International Institute of Tropical Agriculture (IITA) Bioscience
Center, Ibadan, Nigeria for amplification and sequencing. The primers used to amplify fragments of the nuclear ribosomal DNA (rDNA) of the BC-3B isolate were the Internal Transcribed Spacer 4 (ITS4) with the sequence TCCTCCGCTTTATTGATATGC and ITS5 with the sequence GGAAGTAAAAGTCGTAACAGGG. The amplicons were sequenced using the capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with an extension .ab1. The sequence was analysed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database.

3. RESULTS

The result of the fungal isolation is presented in Plates 1a and b. The isolates developed a black spore surrounded by white spore that was visible to the eyes. From the photomicrograph, the isolate was identified as an Aspergillus spp. This organism was white at first but turns dark black after a few days. The conidia of A. niger, are brown to black in color, with smooth conidiophores and conidia. The edges of the colonies appear pale yellow and produce circular gaps. The reverse of the Petri dish, appears white to yellow in color.

3.1 Molecular Characterization using the Internal Transcribed Spacer (ITS) Marker and Identification

The genomic DNA of the isolates BC-3B of broccoli was successfully mined. The NanoDrop result showed that the concentration of the DNA of the isolates was 238.5 ng/µl. The 260nm/280nm readings are 1.86 and 2.56 (ng/µl) respectively, and then, the 260nm/230nm readings are 1.52 and 4.77 (ng/µl) respectively.

However, to reduce the cost of sequencing, the isolate BC-3B with the highest DNA concentration was selected. The result of the amplified DNA PCR band of the isolate BC-3B is presented in Fig. 2. The amplified DNA showed a band on gel when observed under UV light. From the result, the ladder used indicated that the BC-3B isolate sequence had 600 base pairs.

The result of the BC-3B isolate sequence alignment is presented in Fig. 1. The result indicated that BC-3B isolate sequence aligned with 100 sequences deposited in the composite biological database of National Center Biotechnology Information (NCBI). It indicated that the BC-3B isolate sequence was 93% identical to Aspergillus niger. These findings showed that isolate BC-3B is an Aspergillus spp.

4. DISCUSSION

This study was carried out to use morphological and molecular methods to identify fungi isolated from broccoli crown. Molecular techniques have proven to be more reliable than traditional methods as it allows for the comparison of DNA sequence information between known and unknown fungal species. Traditional methods commonly used for identification of fungi have led to the misrepresentation of many isolates. The molecular techniques employed for identification of fungi in this study led to the successful characterization of a fungus isolated from broccoli. The fungus obtained from this study belongs to the division Ascomycota, class Eurotiomycetes, order Eurotiales and family Trichocomaceae. This study revealed the identity of the isolated fungal organism to be Aspergillus niger. The results of another study conducted by Campbell [6] also revealed that Aspergillus species are among the most numerous and most abundant of the saprophytic moulds, occurring on a wide variety of substrates, wherever vegetation decomposes.

This result is in agreement with the result of Tournas [7], who stated that vegetables are often damaged by a variety of fungi and examples of these fungi that cause spoilage of fresh vegetables are Aspergillus, Botrytis cinerea, various species of the genera Alternaria, Cladosporium, Colletotrichum, Phomopsis, Fusarium, Penicillium, Phoma, Phytophthora, Pythium and Rhizopus spp., Botrytis cinerea, Ceratocystis fimbriata, Rhizoctonia solani, Sclerotinia sclerotiorum, and some mildews.

The result is also similar to that of Singh et al [8] which confirms Aspergillus spp as one of the fungi associated with Brassica oleracea spoilage.

This finding is also in conformity with previous works of Mailafia et al. [9] which reported that A. niger causes black mold in certain fruits and vegetables.
Besides causing huge economic losses, Aspergillus species are producers of mycotoxins. These mycotoxins are secondary metabolites which are harmful to both animals and humans. The genus Aspergillus are used in oriental food fermentation, and as host for heterologous genes expression [10]. They produce aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ ochratoxins and other not only an agricultural issue but also a major mycotoxins $B_1$, $B_2$, $G_1$, and $G_2$ [11]. Aflatoxins are global public health issues like liver cancer.

Fig. 1. Amplified PCR Product generated from fungal DNA isolates of broccoli

Fig. 2. The Sequence Alignments of BC-3B Isolate with NCBI Database Sequences
The use of molecular techniques provides accurate identification of microorganisms unlike the cultural techniques which is based on the use of morphological and microscopic characteristics of the organisms. Many scientists in this part of the world still dwell on the use of cultural techniques in the identification of microorganisms and this can be misleading. The economic importance of the organisms isolated and identified in this study have been highlighted and this will give plant pathologists the insights required to proffer preventive and control measures towards reducing crop loss as a result of these pathogens.

5. CONCLUSIONS

The identity of causal pathogen of post-harvest deterioration of broccoli was studied using both morphological and molecular techniques. The findings showed that *Aspergillus niger* is one of the fungal pathogens causing post-harvest rot of broccoli. The fungus is among the saprophytic moulds associated with decomposition of post-harvest vegetables. It is anticipated that this result will provide information for disease management and control approaches for alleviating the post-harvest losses of broccoli and provide a foundation for further study of possible harm of consuming diseased broccoli.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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