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First report on *Ovatospora brasiliensis* from fresh water dried shrimp and prawn in Bangladesh

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Abstract

The current study was aim to isolate and indentify fungus associated with dried prawn and shrimp. *Ovatospora brasiliensis*, was identified through morphological characterization based on mycelium, conidia, colony features as well as molecular characterization. Polymerase chain reaction is remarkably effective at identifying species by genetic fingerprinting. The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) of *Ovatospora brasiliensis* was amplified with the universal primers ITS4 and ITS5. Amplification of genomic DNA of *Ovatospora brasiliensis* length was 556bp. The clustering of the molecular data further revealed that the sequences represented fungal taxa at a cutoff of 99% molecular identity. These facts suggest the existence of enormous resources for molecular identification of fungal species based on ITS sequences. To the best of our knowledge, the experimental result concluded that dry shrimps contaminated with fungus caused by *Ovatospora brasiliensis* is first report in Bangladesh.

Keywords: *Ovatospora brasiliensis*, dried prawn-shrimp, fungus morphology, molecular identification

1. Introduction

Dried prawn and shrimp is an important ingredient of food in certain parts of the world. Fungal growth on dried prawn and shrimps occurs frequently especially on those that come from humid regions ^[1]. The fungal organism, *Aspergillus niger* is a major cause of health problems and they accelerate the deterioration process of the prawn and shrimp ^[2]. In order to ensure that prawn and shrimp remains in good condition and is contaminant free, proper handling and delivery to the consumer is a crucial element in ensuring quality of the final product ^[3]. Spoilage is a metabolic process that causes food to be undesirable or unacceptable for human consumption due to changes in sensory nutritional characteristics ^[4].

Fungal morphology, such as spore-producing structures are very important to identification ^[5]. Morphologically ascospores of *Ovatospora brasiliensis* are brown when mature, broadly ovate, bilaterally flattened, rounded at one end, with an apical germ pore at another attenuate or apiculate end, asexual morph unknown ^[6]. The laborious nature of microscopic analysis and identification is the driving force behind developing such methods as molecular verification of identified morphotypes ^[7]. DNA barcoding based on the nucleotide sequence information of a target gene region can be highly efficient, potentially enabling the rapid and accurate identification of fungal specimens. DNA barcoding is a method of identifying unknown samples by means of known classifications. Generally, short nucleotide sequences in one to three loci are used as DNA barcoding markers to catalog and identify a taxonomic group. The potential importance of DNA barcoding in mycology and ecology is enormous ^[8].

Various molecular genetics tools have been introduced for the verification of fungi, such as restriction fragment length polymorphism (RFLP), RAPD, and small subunit ribosomal DNA and ITS sequence analyses. Recent molecular phylogenetic studies have demonstrated that the ITS region of genomic DNA is very useful for assessing phylogenetic relationships at lower taxonomic levels ^[9, 10, 11]. There is no record of fungal inoculums- *Ovatospora brasiliensis* associated with dried fish and also dried prawn and shrimps from Bangladesh. Therefore, consider all of the above facts the present study deal with the accurate identification on the basis of morphological and molecular characterization of associated fungus of dried prawn and shrimp.

2. Materials and Methods

Fresh water dried prawn and shrimps were collected from dry fish whole sale market at Savar, Dhaka with aseptic condition using sterile polyethylene bags. Fungi isolation, identification and pure culture experiments were conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, Jahangirnagar University. Fungus was isolated through tissue planting method. Infected parts of dried prawn and shrimp were cut into small pieces about 0.5cm in length in such a manner so as to include both fungal infected and non-infected tissues in piece. Then sterilization was done using NaOCl (0.5%) solution for 5 minutes and rinsed with distilled water several times. Four pieces of samples were placed into potato dextrose agar (PDA) medium and were incubated under 12/12 hours dark and light condition at 25 ± 2 °C for 10 days. Mycelial growth of growing fungus colony was transferred to fresh PDA plates as well as PDA slants to obtain a pure culture. The pure culture of the isolated fungus was identified microscopically using standard methods [7].

The fungus, *Ovatospora brasiliensis* was molecularly characterized with the help of commercial service provided by Invent Technology, Dhaka, Bangladesh. Fungus genomic DNA samples were extracted using the Maxwell Cell Kit (AS1030, Promega, USA). The primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAA AGTCG TAACAAGG-3') were used for the PCR reaction [12]. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 25- μ l reaction mixture having a LA Taq (TAKARA BIO INC, Japan). The thermal cycle was performed with activation of Taq polymerase at 94 °C for 1 minute, 35 cycles of 94 °C for 30 Sec, 55 °C for 30 Sec, and 72 °C for 5 minutes each were performed, finishing with a 10-minute step at 72 °C. The Maxwell® 16 DNA Purification Kits was used to purify the amplification products (Promega, USA). The purified PCR products were sequenced by using two primers in First BASE Laboratories SdnBhd (Malaysia). Amplified PCR products were electrophoresed on 1.5% agarose gel in $1 \times$ TAE buffer for 1 hr at 100V with a 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% μ g/mL). The stained gels were visualized and photographed using a UV transilluminator.

DNA sequences were checked by BioEdit and MEGA6. Sequencing data were submitted to the NCBI, and received an accession number (MN886599). Multiple sequence alignments were done using MEGA6. Data was converted from fasta to MEGA format with Clustal W. The models of evolution were determined under the Akaike Information Criterion (AIC). The model selected was Tamura-3 parameter for analysis. Maximum likelihood (ML), Neighbor-joining (NJ), and Maximum parsimony (MP) analysis were done and robustness of the branches were determined with 1000 bootstrap replicates along with max-trees set at 1000. The number of replications was inferred using the stopping criterion. Bootstrap values greater than 60% were accepted [13].

3. Results and Discussions

Ovatospora brasiliensis belong to the family Chaetomiaceae of the ascomycotous fungus isolated from dried prawn and shrimp. Ascomata superficial, pale olivaceous grey to mouse grey in reflected light due to ascomatal hairs, globose or subglobose, ostiolate, 85-135 μ m high, 75-110 μ m diam. Ascomatal wall brown, textura angularis, sometimes mixed with textura intricate in surface view. Terminal hairs undulate to loosely coil with erect or flexuous lower part, conspicuously rough (granulate), greyish sepia to brown, septate, 2-3.5 μ m diam in the undulate or coiled upper portion. Lateral hairs flexuous, tapering and fading towards the tips. Asci fasciculate, cylindrical, sporebearing part 35-45 \times 5-7.5 μ m, stalks 8-18 μ m long, with 8 uniseriate ascospores, evanescent before ascospores become mature. Ascospores olivaceous brown when mature, ovate, bilaterally flattened, with an apical germ pore at the attenuated end. Culture characteristics: Colonies on OA with entire or slightly undulate edge, about 41-47 mm diam in 7 d at 25 °C, usually irregularly concentric because of the concentric formation of ascomata between the floccose rings consisting of pale grey to pale olivaceous grey or honey-coloured aerial mycelium, with pigmented hyphae immersed in medium, and with dark exudates diffusing into the medium, reverse black. Typification of this species awaits obtaining a culture or isolate morphologically consistent with the holotype [6].



Fig 1: Morphological characteristics of *Ovatospora brasiliensis*. A: Vegetative growth of *Ovatospora brasiliensis* on PDA medium, B: Microscopic view of *Ovatospora brasiliensis* (40 X 10x).

The results of a PCR reaction are usually visualized using gel electrophoresis. The size of the fragments in the PCR sample can be determined. DNA fragments of the same length form a band on the gel, which can be seen by eye if the gel is stained with a DNA-binding dye. ITS regions of fungal rDNA are highly variable sequences of great importance in distinguishing fungal species by PCR analysis. The size of the internal transcribed spacer region as measured by gel

electrophoresis of PCR products, amplified by primers ITS4 and ITS5, was 556 bp for *Ovatospora brasiliensis*, but other species of this genus had a shorter ITS region, making this characteristic potentially useful in the identification of *Ovatospora brasiliensis* (Fig. 2). Recent molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) region of genomic DNA is very useful for identification of fungi at lower taxonomic levels [9].

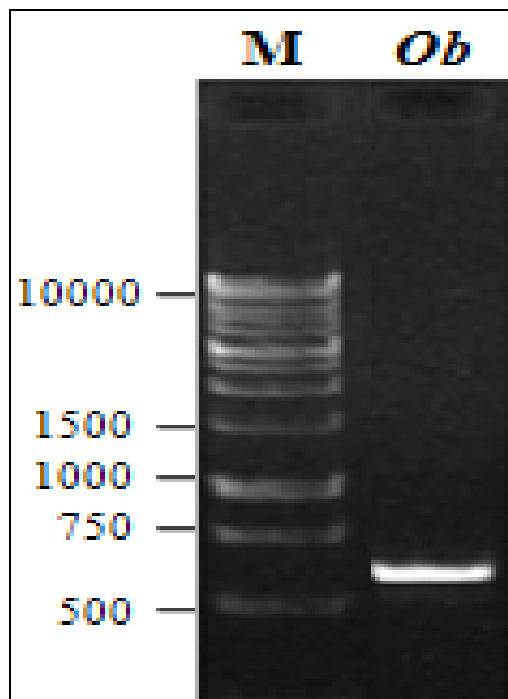


Fig 2: PCR products of the ITS region of *Ovatospora brasiliensis*. M, molecular size marker (1 kb DNA ladder); lane Sb, *Ovatospora brasiliensis*.

The ITS region was amplified using ITS4 and ITS5 primers and sequenced. Phylogenetic tree based on the nucleotide sequences of the ITS regions in thirty two fungal taxa were selected from the NCBI database for phylogenetic analysis. Percent homology of rDNA sequence of ITS region (MN886594) was compared with formerly identified fungi MH865522.1 *Ovatospora brasiliensis*, MH860888.1 *Ovatospora brasiliensis*, NR147679.1 *Ovatospora brasiliensis*, KX976685.1 *Ovatospora brasiliensis*, MH860712.1 *Ovatospora brasiliensis*, MH860313.1 *Ovatospora brasiliensis*, MH864003.1 *Ovatospora brasiliensis* and MH861687.1 *Ovatospora brasiliensis*. In maximum parsimony tree, there are five different cluster were found in the phylogenetic tree (Fig. 3). Reciprocal homologies of the ITS region sequences ranged from 99 to 100%. The sequencing data of the selected NCBI GenBank strain (LT993630.1 *Thielavia antartica*) was used as control strain for the comparative studies on phylogenetic relationships with

the selected strain of *Ovatospora brasiliensis* (MN886594). The results indicated that all the individual species of *Ovatospora brasiliensis* belong to single major cluster. The ITS region is relatively short and can be easily amplified by PCR using universal single primer pairs. Genetic distance exhibited high level of similarity with identical ITS sequences. The size variation was caused by differences in the number of nucleotides, revealing that these strains are clearly distinguishable from each other based on the ecological distribution, substitution, and insertion or deletion polymorphisms of the base position [9]. Alam *et al.* [12] reported that ITS sequences are genetically constant or show little variation within the species, but vary between species in a genus. The genetic diversity detected within groups is probably due to an efficient gene flow and to a high genetic compatibility within the strains tested. Based on molecular evidence, it is clearly indicated that our studied fungus is *Ovatospora brasiliensis* under the family- Chaetomiaceae.

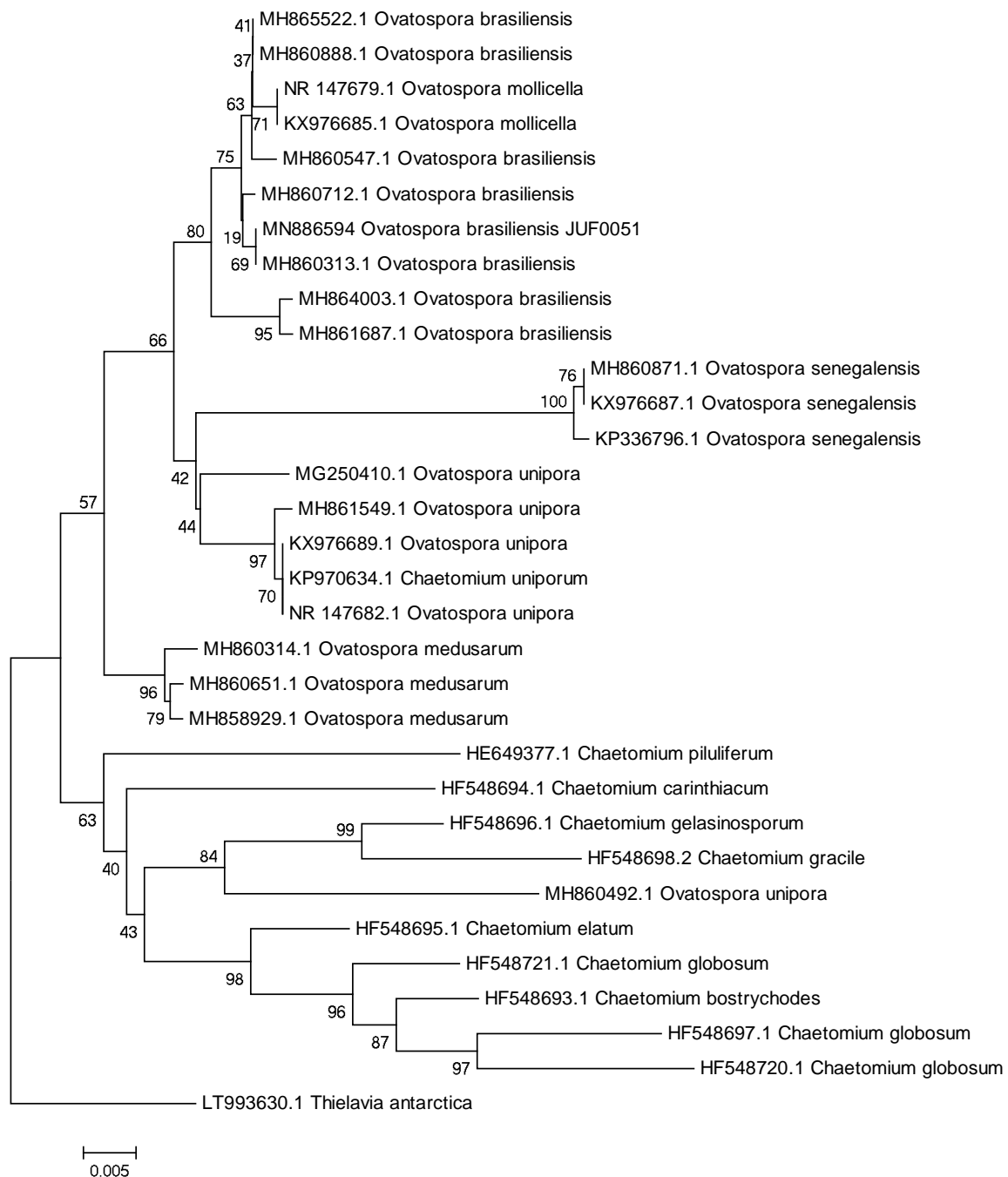


Fig 3: Maximum likelihood tree derived from analysis of ITS sequence dataset of the studied organism with bootstrap value (Bootstrap replication=1000). Our organism (MN886599) marked with 'JUF0051'.

4. References

- Siddique MAM, Mojumder P, Zamal H. Proximate composition of three commercially available marine dry fishes (*Harpodon nehereus*, *Johnius dussumieri* and *Lepturacanthus savala*). American Journal of Food Technology. 2012; 7:429-436.
- Chakrabarti R, Varma PRG. Residual potassium sorbate level effective to control fungi in dried salted fish at tropical ambient temperature. Indian Journal of Fisheries. 2009; 56(2):129-134.
- Ali SSR, Ramachandran M, Chakma SK, Sheriff MA. Proximate composition of commercially important marine fishes and shrimps from the Chennai coast, India. International Journal of Fisheries and Aquatic Studies. 2017; 5(5):113-119.
- Ayotunde EO, Ada FB, Udeh GN, Otu VO. Weevils and fungi infestation of dried fish: A case study of Uyo local government area, Akwa Ibom state, Nigeria. International Journal of Fisheries and Aquatic Studies. 2016; 4(4):359-367.
- Afsary Z, Alam N, Sarker NC, Amin R, Kanon AJ. Morphological characterization of commercially cultivated oyster mushrooms in Bangladesh. Bangladesh Journal of Mushroom. 2013; 7(2):29-36.
- Wang XW, Houbraken J, Groenewald JZ, Meijer M, Andersen B, Nielsen KF *et al.* Diversity and taxonomy of *Chaetomium* and chaetomium-like fungi from indoor environments. Studies in Mycology. 2016; 84:145-224.
- Sikder MM, Mallik MRI, Alam N. Identification and *in vitro* growth characteristics of entomopathogenic fungus-

- Aschersonia* sp. in Bangladesh. *Advances in Zoology and Botany*. 2019; 7(1):11-18.
8. Alam N, Rahman F. Vegetative growth and genetic diversity in different strains of *Pleurotus salmoneastramineus* based on pcr polymorphism. *Bangladesh Journal of Botany*. 2020; 49(1):125-134.
 9. Alam N, Shim MJ, Lee MW, Shin PG, Yoo YB, Lee TS. Phylogenetic relationship in different commercial strains of *Pleurotus nebrodensis* based on ITS sequence and RAPD. *Mycobiology*. 2009; 37(3):183-188.
 10. Alam N, Kim JH, Shim MJ, Lee UY, Lee TS. Mycelial propagation and molecular phylogenetic relationships of commercially cultivated *Agrocybe cylindracea* based on ITS sequences and RAPD. *Mycobiology*. 2010a; 38(2):89-96.
 11. Alam N, Cha YJ, Shim MJ, Lee TS, Lee UY. Cultural conditions for mycelial growth and molecular phylogenetic relationship in different wild strains of *Schizophyllum commune*. *Mycobiology*. 2010b; 38(1):17-25.
 12. Alam N, Lee JS, Lee TS. Mycelial growth conditions and molecular phylogenetic relationships of *Pleurotus ostreatus*. *World Applied Sciences Journal*. 2010; 9(8):928-937.
 13. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*. 2013; 30:2725-2729.