Isolation of Natural Colorant Producing *Aspergillus niger* from Soil and Extraction of Pigment

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ABSTRACT

This study was conducted to isolate colorant-producing *Aspergillus niger* from the soil for its potential use to extract natural colorant for food production. A total of 14 soil samples were collected from Madhupur National Park at Madhupur Upazila under Mymensingh district. The *Aspergillus niger* was isolated and identified from the soil samples by following conventional mycological methods, followed by confirmatory identification by a polymerase chain reaction using specific oligonucleotide primers. For pigment production, a mass culture of *A. niger* was done in Sabouraud Dextrose Broth in shaking conditions for seven days. The biomass was subjected to extraction of the pigments following ethanol-based extraction methods. The extracted colorant was then concentrated using a rotary evaporator to obtain the pigments. An *in vivo* experiment was done with mice to assess the toxicity of the pigments. The extracted pigments were used to make cookies and lemon juice. *A. niger* could be isolated from three samples. The yield of pigment from *A. niger* was 0.75% (w/v). This is the first attempt to use *A. niger* isolated from soil samples for successful food production in Bangladesh. The fungal pigments can be used in the emerging fields of food and textile industries in Bangladesh.

INTRODUCTION

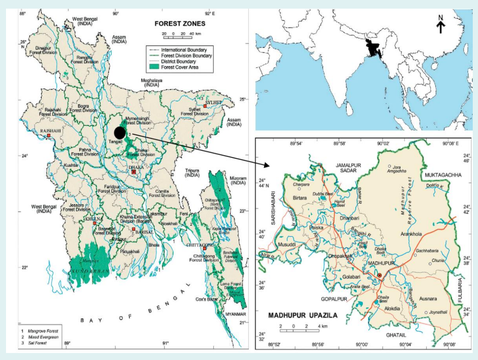
- *Aspergillus niger*, a brown rot fungi is one of the most common species of the genus *Aspergillus* and also a good source of producing food grade color as well as for industrial use.
- Demand of natural color increasing globally over synthetic color due to the detrimental effects of synthetic color on both human, animal, and environment.
- Safe, healthy, and eco-friendly to use due to their non-toxic, non-carcinogenic and biodegradable nature.

OBJECTIVES

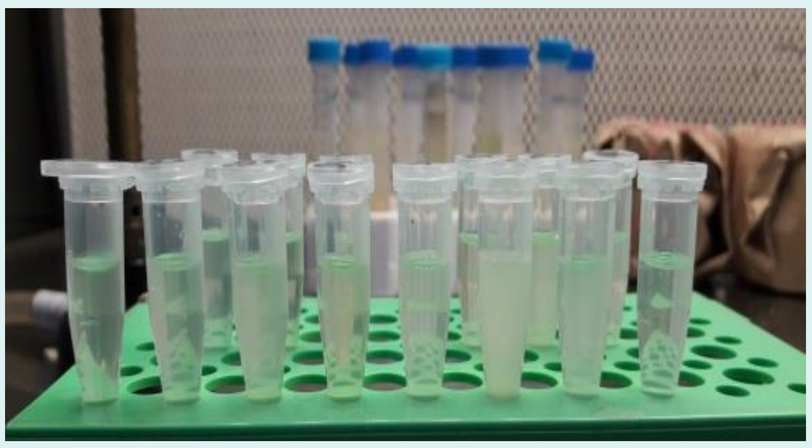
- To isolate natural colorant producing *Aspergillus niger* from soil sample
- To extract natural colorant for food production from isolated fungi

METHODOLOGY

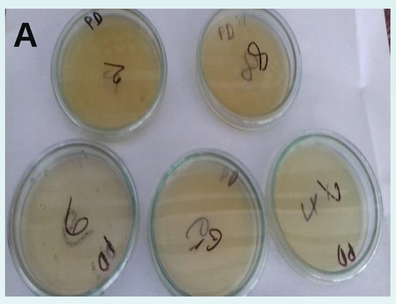
Sample Collection and Location:
Soil from Madhupur National Park at Madhupur Upazila of Mymensingh district

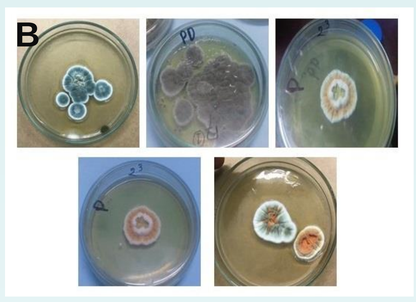


Preparation of soil sample



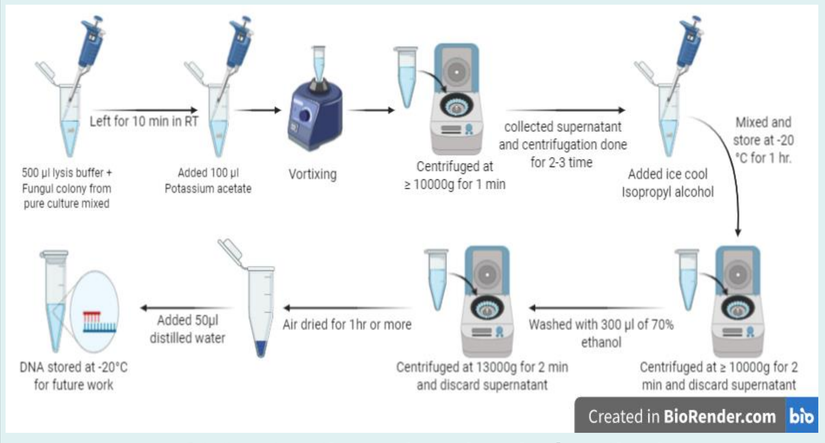
Prepared inoculum with antibiotic treatment (Gentacin 5%, 50 mg/mL)





(A) Culture on PDA media, (B) Growth of different fungi on media, (C) Submerged culture on Sabouraud Dextrose Broth

DNA extraction



DNA extraction using chemical method

PCR and gel electrophoresis

Primers used to detect *Aspergillus* genes and spp.

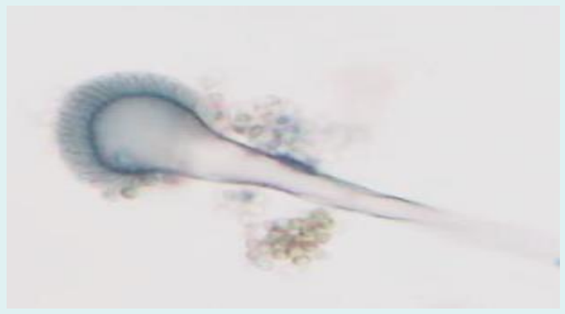
SL No	Oligonucleotide Sequence (5′-3′)	Target Species	Amplicon size (bp)	References
01	ASAP 1: CAGCGAGTACATCACCTTGG ASAP 2: CCATTGTTGAAAGTTTTAACTGATT	*Aspergillus* spp.	521bp	Sugita et al., (2004)
02	ASPU: ACTACCGATTGAATGGCTCG Nilr: ACGCTTTCAGACAGTGTTCG	*Aspergillus niger*	310bp	

In vivo test for toxic analysis

Feeding the extracted color to the mice at different doses.

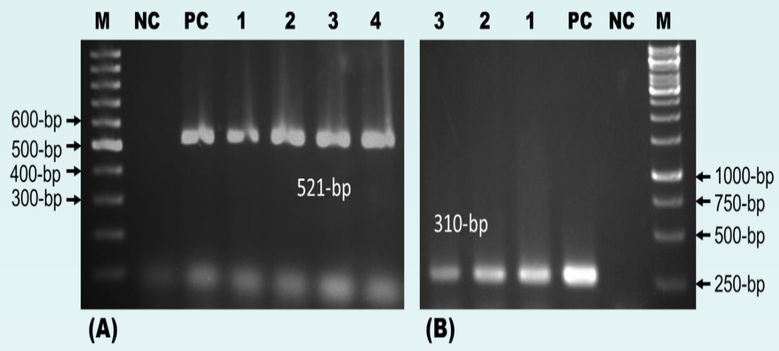
RESULTS

Microscopic morphology of *A. niger*



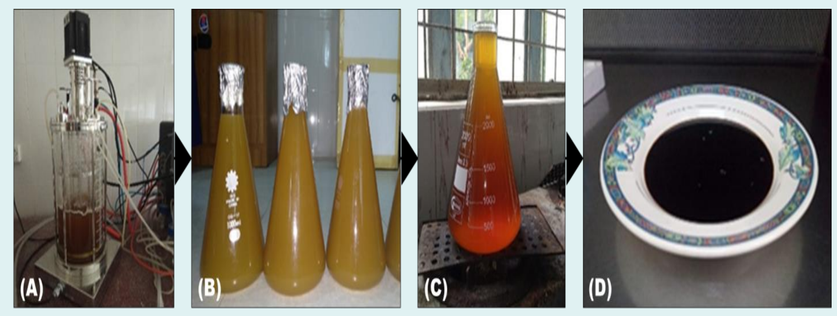
Microscopic morphology of A. niger viewed in 100X. The morphology of *A. niger* showed large, globose, dark brown conidial heads. Conidiophores were smooth-walled, hyaline or turning dark towards the vesicle.

Molecular detection of *A. niger*



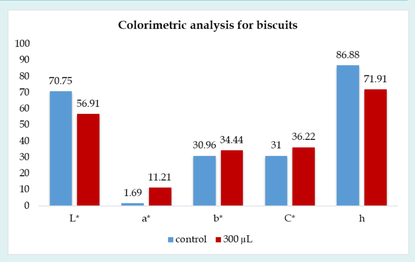
Identification of Aspergillus sp. and Aspergillus niger by polymerase chain reaction. (A) PCR identification of Aspergillus sp by using genus specific primers. Lane M- 100-bp DNA ladder, NC- Negative control, PC: Positive control, and Lane 1-4 test samples. (B) PCR identification of Aspergillus niger by using specific primers. Lane M – 1 Kb DNA ladder, NC- Negative control, PC: Positive control and Lane 1-3 test samples.

Pigment extraction from *A. niger*

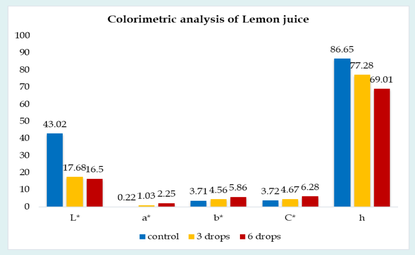


Pigment extraction produced by *A. niger*. (A) Fermentation by Aspergillus niger in the fermenter, (B) Filtrate after fermentation, (C) Heat treatment of the filtrate, (D) Final product found after rotary evaporation.

Quantitative colorimetric analysis of pigments in food products



(A) Biscuit Dough without color, (B) Dough with color



(A) Lemon juice without color, (B) Lemon juice with color

Toxic analysis test

Five different doses was applied during *in vivo* test to the mice for 28 days. Mice were found robust and alive during this period.

CONCLUSION

In the research process, soil was used for obtaining different color producing filamentous fungi. Among them, Aspergillus niger was mainly isolated and brown colored pigment was obtained. In this research, 0.75% (w/v) semi-liquid color was obtained per liter of fermented solution of A. niger which is really appreciable.

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