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Research Article
Fungal Presence in Cosmetic Facial Powder

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Abstract

Background and Objective: Facial powders are products that are used to cleanse and beautify the face. It is used by many men and women and often misused or stored incorrectly, leading to contamination by fungi. Therefore, this study examined the use of molecular characterization to identify the species of fungi that grows on facial powder. **Materials and Methods:** To directly investigate the species of fungi that grow on facial powders, fungal samples were taken from some popular brands of face powder and cultured in potato dextrose agar (PDA). The established fungal isolates were subjected to molecular analyses and identification protocols. The DNA of the most common fungal isolates, FP-2 and FP-3 molecular markers such as internal transcribed spacer 4 and 5 (ITS-4 and 5) was used to molecularly characterise the DNA of the most common fungal isolates, FP-2 and FP-3. Basic Local Alignment Search Tool for Nucleotide (BLASTN) version 2.8.0 of the National Center for Biotechnology Information (NCBI) database was also used to align the DNA isolates. **Results:** The molecular weight of the DNA of the isolates was 587 base pairs for *Neurospora crassa* and 600 base pairs for *Aspergillus flavus*. Based on sequence similarity, it was observed that the facial powder isolate FP-2 was 99.83% identical to *Neurospora crassa* and FP-3 was 99.48% identical to *Aspergillus flavus*. **Conclusion:** These findings showed that *Neurospora crassa* and *Aspergillus flavus* are some of the causal fungal pathogens on contaminated facial powder. *Aspergillus flavus* is known to cause tissue damage and the development of skin lesions. *Neurospora* species are incapable of causing disease but can stimulate latent pathogens to cause disease.

Key words: *Aspergillus flavus*, cosmetics, microbial contamination, *Neurospora crassa*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently, there is an increase in the use of cosmetic powder as the use of face powder has contributed to beauty values throughout history[1]. Cosmetic facial powders are used by many people as a tool for beautification and are often stored inappropriately thereby encouraging fungi growth and contaminations. A lot of people use contaminated powder without knowing the health implications of the fungi species associated with cosmetic powders. Studies so far in some parts of the world have found some contaminants in cosmetic powders. According to de Oliveira *et al.*[2] cosmetic products showed contamination by filamentous fungi such as *Penicillium*, *Rhizopus* and *Scopulariopsis*.

Inaccurate identification of these organisms can lead to a misrepresentation of community structure. Therefore, it is fundamental for fungi pathogens associated with contaminated facial powder to be properly identified using molecular characterization because there are various fungi species in the environment and the old-fashioned identification method is not suitable for taxonomic purposes. The current study aimed at revealing some common fungal organisms associated with contaminated facial powder.

MATERIALS AND METHODS

Source of powder: Facial powder (plate 1) from a popular brand in Nigeria was obtained in March, 2020 from Port Harcourt Local Government Area in Rivers State.

Study area: This study was carried out at the Mycology/Pathology Laboratory of the Department of Plant Science and Biotechnology where the organism was isolated and the Regional Center for Biotechnology and Biofuel Research Laboratory at the University of Port Harcourt, where the DNA was extracted. Purification and sequencing of the PCR products were carried out at the International Institute of Tropical Agriculture (IITA) Ibadan from March, 2020 to August, 2021.

Isolation and identification of fungi: In a laminar flow chamber, a sterile inoculating loop was used to pick up individual colonies found growing on the surface of a facial powder and then placed in the centre of sterilized Petri dishes containing potato dextrose agar medium. Each Petri dish was sealed with masking tape, labelled with dates and placed upside down to prevent contamination from any moisture that may be present. The Petri dishes were incubated at room temperature (25+2°C) for 7 days to allow the fungal organisms to spread on the PDA surface. A total of 15 Petri dishes were used for the fungus. The cultures were stored at 4°C before DNA extraction. The unidentified fungal organisms were coded FP-2 and FP-3.

Morphological and microscopic characterization and identification: The morphological identification of isolates FP-2 and FP-3 were conducted by visually observing the mycelium and compared with CBS Laboratory Manual Series Food and Indoor Fungi by Samson *et al.*[3]. The diameters, colour of the conidia, the reverse colour of the organism in the Petri dishes, texture, zonation and sporulation of the colonies were compared. Using an electron binocular microscope at ×40, the isolates were analyzed microscopically, characterized and identified.

Molecular characterization using the internal transcribed spacer (ITS) marker and identification: The genomic DNA of the isolates FP-2 and FP-3 were extracted following the protocol of Zymo Quick-DNA™ Fungal /Bacterial Mini-Prep Kit as described by the manufacturer, with modifications at the Regional Center for Biotechnology and Bioresources (RCBB), University of Port Harcourt, Rivers State, Nigeria.

Nanodrop 2000 c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) was used to measure the DNA quantities and concentrations of pure cultures of the facial powder purity is measured at 280 nm to that of 260 nm as a ratio of absorbance.

The modified method of Ikechi-Nwogu and Amos[4] was used to perform the Agarose gel electrophoresis. International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria, amplified and sequenced the DNA samples of the isolates. Internal Transcribed Spacer 4 (ITS4) with the sequence TCCTCCGCTTATTGATATGS and ITS5 with the sequence GGAAGTAAAAGTCGTAACAAGG were used to amplify fragments of the nuclear ribosomal DNA (rDNA) of the FP-2 and FP-3 isolates. The capillary electrophoresis sequencer was used to sequence the amplicons and the DNA sequence file was saved in the Bioedit file with an extension.ab1 analysis was carried out on the sequence using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software and Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology Information (NCBI) database, was used to align the sequence.

RESULTS

The result of the fungal isolation was presented in Fig. 1a and b. The unidentified fungal organisms FP-2 and FP-3 were isolated and found to be associated with facial powder. One had a white hair-like mycelial mass which almost overwhelmed the entirety of the culture medium. The growth of which, at a point, changed the colour of the culture medium to a tint of orange.

The other plated culture showed signs that the fungi initially had the white colour of mycelia. After 3 days, the isolates produced dark green conidia, which then dominated colony appearance, occurring as concentric rings. The colonies were plain and flat at the ends but elevated in the middle. It also appeared wrinkled in a nearly cerebriform form. Similarly, a white border encircled the colonies and the diameter ranged between 60-70 mm, with pale undersides. The organisms were identified as *Neurospora crassa* and *Aspergillus flavus*.

DNA quantification: The genomic DNA of the isolates FP-2 and FP-3 associated with facial powder was successfully extracted. The NanoDrop result shown in Table 1 showed the concentrations of the DNA isolates.

DNA amplification: The results of the amplified DNA or PCR band of the isolates FP-2 and FP-3 are presented in Fig. 2a and b. When the amplified DNA was observed under UV light, it showed a band on the gel. The ladder used indicated that the FP-2 and FP-3isolates sequence had over 587 base pairs and 600 base pairs, respectively.

Table 1: Concentration of DNA extracted from fungal isolates of FP-2 and FP-3 associated with facial powder using nanodrop (2000 c) spectrophotometer

Sample ID	Nucleic acid concentration (ng μL^{-1})	Absorbance at 260 (purity)	Absorbance at 280	260/280	260/230
FP-2	43.0	0.860	1.285	1.87	0.28
FP-3	46.9	0.937	0.824	1.83	0.29

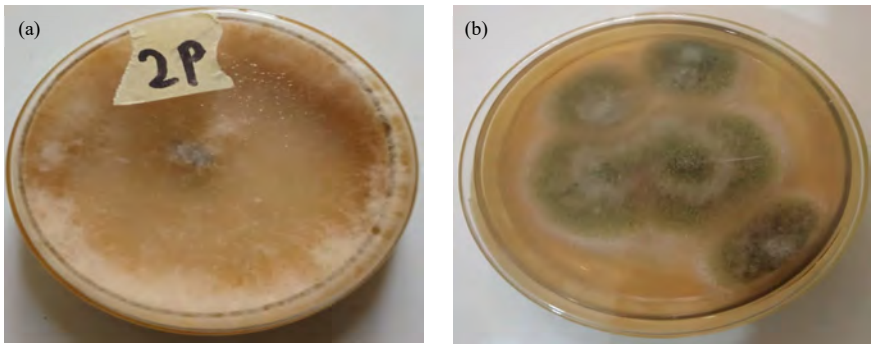

Fig. 1(a-b): Pure culture of fungus isolated from facial powder

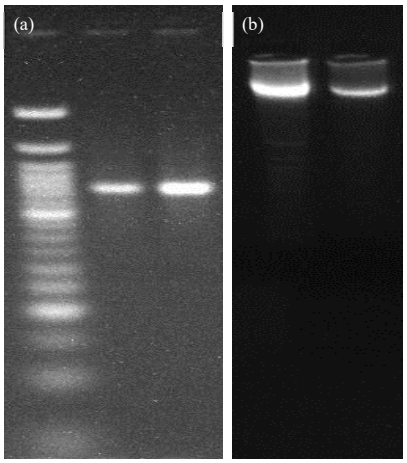

Fig. 2(a-b): Amplified PCR product generated from FP-2 and FP-3 isolates

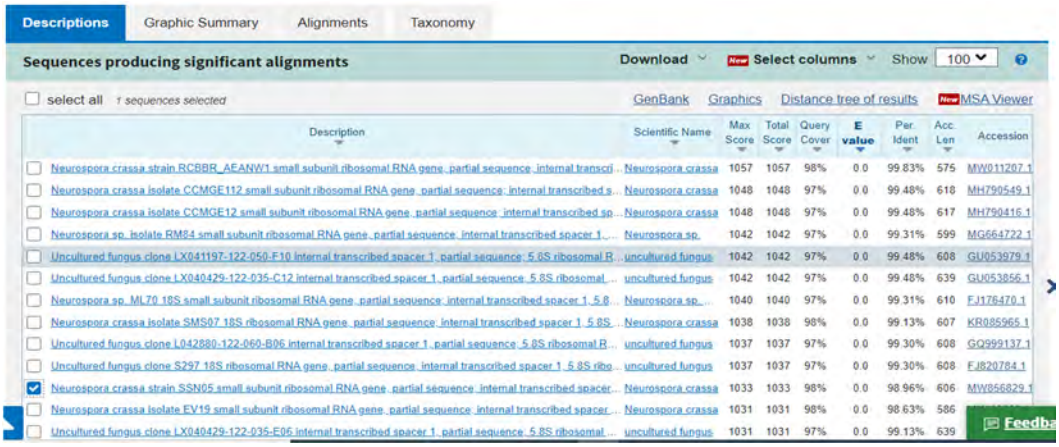

Fig. 3: Sequence alignments of FP-2 isolate sequence with NCBI database sequences

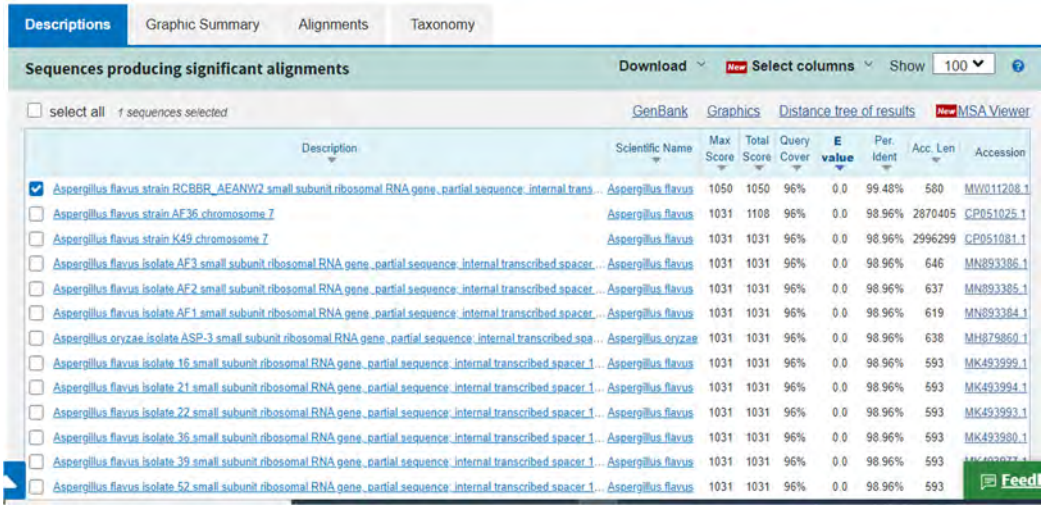

Fig. 4: Sequence alignments of FP-3 isolate sequence with NCBI database sequences

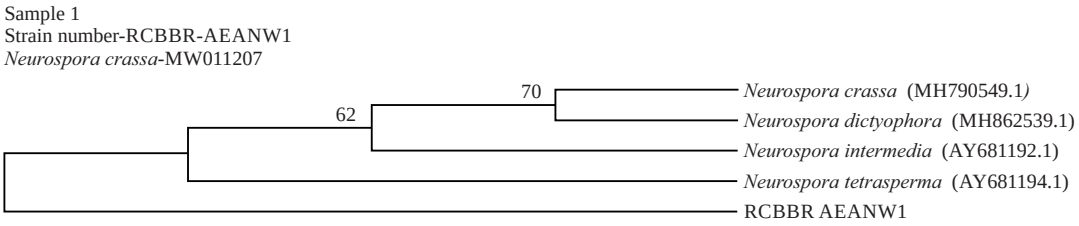

Fig. 5: Phylogenetic tree of sample 1

Fungal isolate identification: The results for the genotypic identification of the fungal isolate FP-2 and FP-3 isolates are presented in Fig. 3 and 4. The isolate sequences aligned with 100 sequences deposited in the composite biological database of the National Center Biotechnology Information (NCBI). From the results, the FP-2 isolate sequence was 99.83% identical to *Neurospora crassa* (red arrow) and FP-3 was 99.48% identical to *Aspergillus flavus* (blue arrows).

Phylogenetic analysis: The relationship between the isolates from this study and other fungal isolates in the NCBI database is shown in the phylogenetic trees constructed in Fig. 5 and 6.

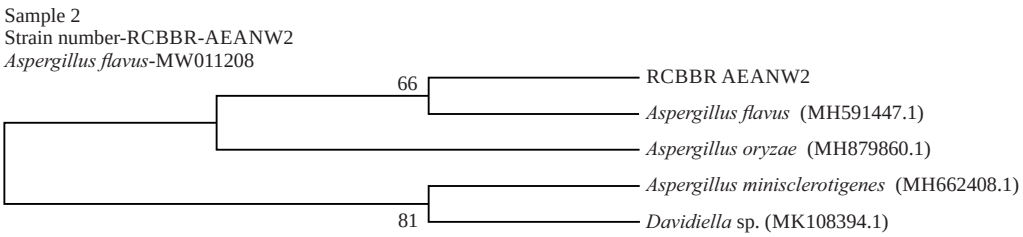

Fig. 6: Phylogenetic tree of sample 2

The phylogenetic analysis, shows that *Neurospora crassa* and *Aspergillus flavus* are closely related to the fungal isolates obtained from facial powder.

DISCUSSION

Cosmetic powders are at risk of fungal contamination. These contaminations may take place during production, storage or come from the raw materials used, poor personal hygiene of consumers, poor sanitary conditions of the environment and equipment. This makes the cosmetic powders unfit for facial use.

This study revealed the presence of *Neurospora crassa* and *Aspergillus flavus* as pathogens associated with cosmetic facial powder. Some filamentous fungal species have been reported to be present in fungi in powder and semi-aqueous makeup commonly used in Brazil and they are *Penicillium* sp., *Rhizopus* sp. and *Scopulariopsis* sp[2]. According to Dadashi and Dehghanzadeh[5], about 19.2% of in-use cosmetics were contaminated by fungus and yeast. Omorodion *et al.*[6] in their work, identified *Aspergillus* spp., *Rhizopus* spp., *Candida* spp., 18%, *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp., fungal isolates from the baby and adult powders. The results in this study are comparable to the results found by Elmorsy and Hafez[7], who found 30% of *Aspergillus* spp., in the cosmetic sample.

Neurospora crassa is a filamentous ascomycete and according to Kuo *et al.*[8] *Neurospora crassa* is used as a model for genetic, cellular and biological and chemical research. It does not pose any health or pathogenic threat to humans but *N. crassa* switches to an infective state when its interaction with the host is disturbed.

Aspergillus flavus is a pathogenic mould that produces numerous mycotoxins that can cause severe liver damage, vomiting, abdominal pain, tremors, lung infections, cerebral edemas and eventually death. In the process of applying cosmetic powder, the human could inhale fungal spores and inhalation of *A. flavus* fungi causes *Aspergillosis* infection that attacks the respiratory system[4,9]. In this study, the presence of this pathogen suggests a high risk to users' health.

Aspergillus flavus can attack various tissues of the human body including the skin and cosmetic powders are the vehicles for mycosis. Using contaminated facial powder could lead to the development of skin lesions such as single or multiple red or violet hardened pimples. The study was limited to the isolation of fungi that grow on the used cosmetic powders. From the findings of this work, it is advised that consumers should not share their facial powders or other cosmetics, as most of the cosmetics in use are loaded with fungal contaminants. Some of these contaminants are both invasively and superficially pathogenic. Thus, since each individual has a unique and distinctive skin microfloral, sharing of cosmetic powders could lead to the introduction of a "foreign" flora which will upset the balance of the individual's unique microfloral balance and thus lead to superficial or invasive disease(s).

It is recommendation-worthy that manufacturers of these facial powders strictly use preservatives that have fungicidal properties, as these will help combat the proliferation of fungal contaminants in these facial powders. It is also recommended that more studies be done to determine the possible preservative(s) that can be efficiently used to combat fungal contaminants in the facial powders currently in use in Port Harcourt. It was recommended that the cosmetics industry provide information on makeup handling, storage and expiry dates should be conspicuously displayed.

CONCLUSION

Fungal contamination occurs mostly on used or stale cosmetic powder and the presence of this pathogenic organism pose a potential health risk. *Neurospora crassa* and *Aspergillus flavus* were identified as pathogens associated with cosmetic facial powder. This study will help promote the knowledge of fungal species growing on cosmetic powder and increase the information for facial disease control.

SIGNIFICANCE STATEMENT

Facial powders may be vehicles for facial infection. As a result of this, there was a need to investigate microorganisms such as fungi that are associated with contaminated facial powder. This study enables proper identification of fungi species associated with contaminated facial powder revealing some common fungal organisms associated with contaminated facial powder and the tools used in its application. This will help in enlightening the public on the knowledge of the diseases the fungi cause, their implications on the skin and help the general public especially women maintain adequate hygiene when using cosmetic powder.

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