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Identification and characterization of Aspergillus species of fruit rot fungi using microscopy, FT-IR, Raman and UV–Vis spectroscopy

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Highlights

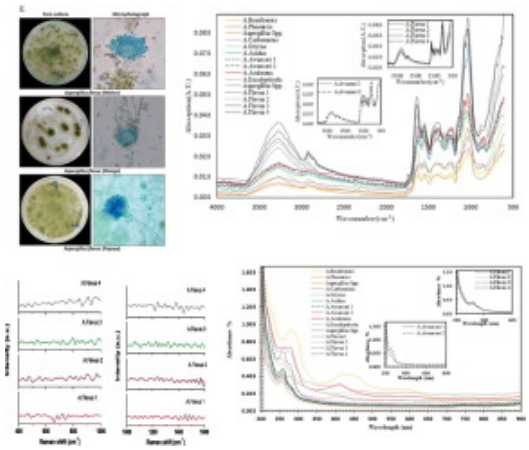
- *Aspergillus* species have been cultured, isolated, differentiate and identify using morphology, FTIR, Raman and UV-vis spectrophotometry.
- The identical species have similar FTIR, Raman and UV-Vis spectra, whereas it is different for different species.
- Used methods are complementary in the presentation of biochemical components information about the types of Aspergillus and their classification.
- These methods are reliable methods for rapid and accurate identification and discrimination between different species.

Abstract

During the investigation of fungal isolation from fruit, the major genera were *Aspergillus*, *Penicillium*, *cladosporium*, *Alternaria*, *fusarium*, *Colletotrichum* were found. Among them *Aspergillus* (15 species)

was found major dominant on different fruits. Fifteen different *Aspergillus* species *viz. Aspergillus brasiliensis*, *Aspergillus phoenicis*, *Aspergillus carbonarius*, four *Aspergillus flavus*, *Aspergillus* a*cidus*, *two Aspergillus awamori*, *Aspergillus aculeatus*, *Aspergillus eucalypticola*, *Aspergillus oryzae* and two *Aspergillus* Spp. have been differentiate and identify using morphology (microscopic technique), Fourier Transforms Infrared spectroscopy (FTIR), Raman Spectroscopy (RS) and UV–visible spectrophotometry (UV–vis). The fungal mass in powder form was used in present study. In FTIR the finger print region is important for the characterization of Aspergillus because this region is unique and contains peaks indicating the presence of DNA. From the results were found Fourier transform infrared (FTIR) technique and Raman spectroscopy a useful tool, sensitive, fast, economical, accurate, not require sample preparation and successfully used to identify fungi.

Graphical abstract



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Introduction

Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to maintain healthy life to human mankind [1]. However, despite all these tremendous benefits of fruits to human well-being, pathogenic attacks threatened the shelf-life of fruits and degrade their economic value. Fruits are mainly exposed to microbial contamination through contact with soil, dust and water and by poor handling or during postharvest processing. This makes them to harbor a wide range of microorganisms [2,3] especially fungi. India achieved a record horticultural production of 314.67 million metric tons in 2018–19 [4]. Storage is the most important cause of post-harvest losses for all types of food in India. Losses due to postharvest disease may occur at any time during postharvest handling, from harvest to consumption. When estimating postharvest disease losses, it is important to consider reductions in fruit quantity and quality, as some diseases may not render produce unsaleable yet still reduce

product value. The fungi have importance in nature rotating organic plant materials, important in the environment, food production, pharmacy and industry. Fungi are almost the only organisms that can degrade cellulose and lignin, they are indispensable to ecosystems [5]. Rotting fruits are the main food sources of fungi, containing essential nutrients to grow fungi such as Aspergillus [6]. Aspergillus causes the rotting of different fruits such as oranges, apples, guava, grapes, water melons and papaya, mold grows in different colors, mostly black mold that grows on most fruits. Aspergillus adapts to the physical, chemical and biological environment changes that are rapidly growing and developing. Aspergillus has a variety of genes, many of which grow on the outer layer of damaged fruits. The genus Aspergillus is taxonomic group organisms, it has diverse properties in agriculture, pharmaceutical, industrial and biological characteristics make it cultivation important [7]. Aspergillus are shown in damaged fruits by their infected as decay and rot, they have different colors rot, and the most common is a black rot. Some genus of Aspergillus important for biotechnology applications such as *A. oryzae*. Large amounts of proteins have been produced from Aspergillus oryzae, so it was used in food fermentation in Japan [7,8]. Aspergillus able to produced biologically active chemical compounds such as antibiotics, mycotoxins. Aspergillus flavus is able to produce mycotoxins as cyclopiazonic acid and Aspergillic acid [9]. Aspergillus is using for the biosynthesis of nanoparticles because it has enough amount of enzymes and it cultured on different medium [10]. The aim of this study is to identify Aspergillus species, which collected from infected fruits from different markets in Aurangabad, India by using microscopic technique, FTIR spectroscopy, Raman spectroscopy and UV–Vis spectroscopy. Microscopic examination used for studying insight information related to structures and physiology for fungi [11]. Macroscopic tool is used for identification of Aspergillus species and microscopic characteristics such as conidial color, colony diameter, growth rate, color of conidia and texture [12]. FTIR-ATR spectroscopy has high quality properties such as rapid scanning, extra sensitivity, inexpensive, that have been successfully used to characterize chemical composition of fungi samples [13,14]. It does not take more time to identify and characterize the biological samples such as fungi, and it needs a small quantity of fungi sample about 15 mg of sample in powder form. FTIR spectrum showed high accurate results for the identification and characterization of Aspergillus at the genetic level through the “molecular fingerprint” of each sample. The infrared radiation fall on the sample, some of them are absorbed and some of them carry out, the absorbed radiation energy that interacts with the molecular bonds vibrational and functional groups found in the chemical components of the fungi such as fatty acids, proteins, polysaccharides, carbohydrates, nucleic acids and aromatic compounds, which then showed specific wave numbers to form characteristic spectra for each sample of Aspergillus [15]. Raman spectroscopy has been widely used to study the biochemical composition and its analytical tool for rapid characterization and identification of microorganisms. An advantages of the Raman spectroscopic method is that it is fast, specific, a non-destructive for microbial analysis and an extended analytical method [16]. When we have been used Raman spectroscopic method, we did not need to add chemicals dyes to fungi samples for identify and classify them by that method. Raman spectroscopy is easy to use, its highly molecular specific and it gives wide information about chemical composition content of microorganisms such as fungi [17,18]. Raman spectra of biological molecules has been complex peaks because of fluorescence, therefore the fluorescence peaks has

been removed from spectra by using first derivative spectra [19]. The π–π∗ electronic transition of the amino acids available in protein due to the absorption of ultraviolet radiation [20]. Aspergillus contain aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, they represented of protein and they have ability to absorb ultraviolet radiation [21].

In this study, we used the Aspergillus spores to identify and distinguish eleven different Aspergillus species, four *Aspergillus flavus* and two *Aspergillus awamori* using morphology (MP), FTIR-ATR spectroscopy, Raman spectroscopy (RS) and UV–Vis spectroscopy (UV–Vis). We obtained similar results using those four methods, because each sample has a distinct chemical composition from the other sample through the spore. The advantages of Raman spectra are that for biological samples, it avoids infrared water absorption and it has non-broad spectral bands [22] when compared to IR spectra for same samples. IR spectra and Raman spectra generate specific spectra representing protein, lipids, polysaccharides, carbohydrates and nucleic acids of biological molecules such as Aspergillus species which provide information related to molecular structure and chemical information about fungal mass.

Section snippets

Collection of samples

Infected fruit samples were collected from different marketplaces of Aurangabad, Maharashtra state, India. Infected fruits *viz.* which include pomegranate, orange, papaya, tomato, grapes yellow, melon, mango, guava and lychees were collected in separate polythene bags. They were observed in laboratory. Primarily observations were made by preparing slides of fungal parts presents on the infected leaves and bulbs of onions.…

Media preparation

Potato Dextrose Agar (PDA) Peeled Potato-200g, Dextrose-20g, Agar-20g…

Morphological characterization of Aspergillus species

Fig. 1(A, B, C, D and E) shows the micromorphology and macro morphology of the 15 Aspergillus species growth on potato dextrose agar (PDA) after culturing for 7days at 28±2°C. In this study all Aspergillus species moderate to rapid growth on PDA. ***Aspergillus flavus***, isolates acquired the white color of the mycelia, the color of the colonies is green, reverse side colorless to yellow, soluble pigments, conidial head was greyish green.Conidia size range was between 3.5 and 5μm; globose;…

Conclusion

Aspergillus species of fruit rot fungi have been characterized and identified using FTIR-ATR spectroscopy, Raman spectroscopy, UV–Vis spectroscopy and microscopy. From this study it is found that each species has a special feature and reflects in their FTIR, Raman and UV–Vis spectra. Results showed that spectroscopic methods are consonant with morphological characteristics for identification and characterization of studied species. It is found that four identical *A.Flavus* and two identical …

CRediT authorship contribution statement

All authors listed have made a substantial, direct and intellectual contribution to the study, manuscript preparation and approved it for publication.…

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.…

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