Int.J.Curr.Microbiol.App.Sci (2022) *11*(08): 8-16

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 08 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1108.002

Exploration of Microbes in Different Spoiled Fruits and Vegetables

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Keywords

Microbes, spoilage, Fruits, Vegetables, Bacteria, Fungi

Article Info

Received:
05 July 2022
Accepted:
04 August 2022
Available Online:
10 August 2022

A B S T R A C T

Fruits and vegetables are very significant and have high dietary and nutritional qualities. Ingesting of fruit and vegetable products has histrionically augmented by more than 40 % during the former few decades. India is the fourth major producer of fruits and second foremost producer of vegetables in the world. Most vegetables and fruits have a derisory life and are further vulnerable to spoilage. Besides enzyme degradation, rancidity and oxidation being the roots of spoilage, the other main reason being the spoilage caused by microorganisms such as bacteria, fungi and Yeasts. This is making 30 % of the produce go into rejection. Reduction in the losses of vegetables and fruits leads to various positive outcomes which can be achieved by the assessment of spoilage of causing microorganisms and their preventive measures. The present research study aimed to explore the microbes in different spoiled fruits and vegetables. *Bacillus* sp. (18 %) was found to be the predominant species inhabiting most of the vegetables and fruits and *Penicillium* sp. (35 %) being the predominant species in case of fungi. The utmost prerequisite is the identification of the comprehensive deteriorative microorganisms for diverse kinds of fresh fruits and vegetables stored under exclusive packaging situations. In arrears to the rising awareness, there is also an inevitability for research of spoilage arrays and micro flora of vegetables and fruits commodity packaged with innovative and evolving to safeguard care while preserving the sensory, nutritional properties and to avoid rejections.

Introduction

Consumption of vegetables and fruits has been on a mounting demand over the past decade as they have now turned out to be an indispensable part of our daily diet (Jana *et al.,* 2021). Vegetables are significant sources of sustenance to human beings (Kaur *et al.,* 2017) specifically vitamins, and could aid as an important ingredient in enhancing health and proper diets. The fresh food products like fruits and vegetables have become to momentously influence human food in opinion of their nutritional value and health benefits (Jhee *et al.,* 2019). Daily consumption of 400 g of fruits and vegetables conspicuously averts incidence of some chronic diseases such as heart diseases, high blood pressure, cancer, diabetes, and overweightness (Wallece *et al.,* 2020; Ziaei *et al.,* 2020; Barth *et al.,* 2009).

Many fruits and vegetables extant nearly ideal conditions for the endurance and growth of many types of microorganisms. Microbial food spoilage is a universal issue that results in the wastage of food and customer discontent (Snyder and Worobo, 2018).

According to FAO, a noteworthy portion of the total fruits and vegetables produced in the world are wasted each year due to spoilage (Jeswani *et al.*, 2021). Produce spoilage has wide-ranging socio-economic insinuations as it directly connects with food shortages, food wastage, and hunger in few parts of the world, water stress, uncalled-for biodiversity loss and augmented greenhouse gas emissions (Hammond, 2015).

Some spoilage microbes are proficient in colonizing and forming lesions on healthy, intact plant tissue. Fungi in particular produce a copiousness of extracellular pectinases and hemicellulases that are imperative factors for fungal spoilage (Miedes and Lorences, 2004).

More than 40% of harvested fruits and vegetables for consumption are condemned through isolation of some pathogenic microbes associated with spoilage by microorganisms. Enzymes are responsible for degradation in some vegetables while chemical reactions such as rancidity and oxidation destroy others (Owais *et al.*, 2018).

Since the fruits are reaped locally, there are always discolorations and cuts due to the baskets used in transporting the fruits (Doyle and Sperber, 2009). The fruits are displayed on benches and in baskets in markets until they are sold, thereby exposing them to further microbial infection (Mritunjay and Kumar, 2015).

According to Mukherjee *et al.*, (2004), the neck and neck of food borne outbreaks caused by spoilt fruits and vegetables has been on a mounting side in recent years, thus, an expedition to isolate and identify these pathogens that causes spoilage should be endorsed as a control measure (Akinyele and Akinkunmi, 2012). Hence, the study on exploration of some microbes associated with spoilt fruits and vegetables obtained from local market in Rythu Bazar, Nizampet, JNTU, Hyderabad.

Materials and Methods

Collection of samples

A total of 25 spoiled fruits and vegetables were randomly collected from the local market, Rythu bazar, JNTU, Hyderabad. The fruits and vegetables were put in 90 % ethanol sterilised polyethene bags and transported to the laboratory for analysis.

Examination of the spoiled food samples

Each type of spoiled fruit and vegetable was examined carefully for the spoilage symptoms such as colour, consistency, rots, odour, any other type of visible symptoms (Table: 1). In our present study we examined Tomato, Carrot, Potato, Cauliflower, Lady's Finger, Lemon, Onion, Mango, Papaya, Sapota and Banana for noticeable spoilage.

Direct microscopic examination of spoiled fruits and vegetables

After examination of the spoiled foods for the symptoms, smears were prepared from the spoiled portions of fruits and vegetables and were examined under the microscope for identification of spoilage causing bacteria and fungi respectively. For the identification of bacteria and fungi, Gram staining and Lacto-phenol blue methods respectively were employed and observed under the microscope at different magnifications.

Isolation of Bacteria

The bacteria were isolated from spoiled fruits by using serial dilution agar plate method (Aneja, 2009). The spoiled fruits were crushed into presterilized mortar and pestle with distilled water to form suspension, which was serially diluted from 10^{-1} to 10^{-5} dilutions. 100 µl of different fruits and

vegetables' suspensions from each dilution was spread over nutrient agar medium (NAM) plates. The NAM was supplemented with amphotericin B (10µg/ml) before pouring to prevent fungal growth and the inoculated Petri plates were incubated at 37 °C for 24 hours for bacterial growth. After incubation the morphologically different colonies of bacteria were isolated and sub-cultured. The bacterial isolates were maintained and stored on NAM slants at 37 °C for further use.

Identification of the Bacterial Isolates

Cultural and Morphological Identification

The bacterial isolates were identified on the basis of morphological and biochemical characteristics according to the Bergey's Manual of Systematic Bacteriology (Clauss and Berkeley, 1986). The cellular morphology of isolated bacteria was studied by Gram staining. The bacterial species were further identified on the basis of endospore, flagellar, capsular staining and motility test.

Biochemical Characterization of Bacterial Isolates

Biochemical characteristics viz. carbohydrate fermentation, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Catalase, Oxidase, Nitrate reductase, Hydrogen sulphide production, Amylase and Gelatin hydrolysis (Cappuccino and Sherman, 2005) were performed.

Isolation and Identification of Fungi

Isolation of fungi from each of the blemished fruit and vegetable was carried out using the method of the technique of Oyeleke and Manga (2008). Spoiled tissues from the rotten fruits and vegetables were cut with a sterile scalpel and placed on the previously prepared Potato dextrose agar (PDA) in Petri dishes and incubated at 28 °C for 3-4 days. The detected fungi were carefully isolated into pure cultures on Potato Dextrose Agar plates. Fugal isolates from plates were prepared into mounts using Lacto-Phenol blue on microscopic slides. These were examined under the microscope for comparison of fungal morphology with descriptions given by Samson and Reenen–Hoekstra (1988).

Graphical Analysis

The data obtained was analysed using MS-Excel-2019.

Results and Discussion

The current study was conducted to isolate and explore different microbes in spoiled fruits and vegetables viz., papaya, mango, banana, sapota, cauliflower, lemon, carrot, tomato, potato, lady's finger sold in the local market, Nizampet, JNTU, Hyderabad. The result showed that the produces are prejudiced by quite a lot of pathogenic microorganisms.

Each spoiled fruit and vegetable were carefully examined for the spoilage symptoms such as color, uniformity, rots, smell and any other type of visible spoilage symptoms. Most of the spoilage symptoms included black spots, lesions, mushiness, slime formation, visible mold growth with unpleasant odour.

The bacterial and fungal colonies from different fruits and vegetables and were given the labels viz., B-1 to B-29 and F1-F8 respectively for the pure isolates. Among them, unique isolates viz., B-1, B-3, B-4, B-6, B-9, B-10, B-14, B-17, B-18, B-19, F-3, F-5, F-6, F-7 were selected for further analysis.

The isolates, B-3, B-4, B-6, B-9, B-17, B-18 were found be positive and B-1, B-10, B-14, B-19 were negative for Gram staining (Figure: 1). The isolates, B17, B18 were found be positive and B-1, B-3, B-4, B-6, B-9, B-10, B-14, B-19 were negative for endospore staining. The isolates, B-1, B-3, B-14 were found be negative and B-4, B-6, B-9, B-10, B-17, B-18, B-19 were positive for Capsular staining. The isolates, B-1, B-3, B-14, B-18, B-19 were found be flagellated and B-4, B-6, B-9, B-10, B-17 were

non-flagellated. All the isolated were found be negative for acid-fast staining.

Biochemical characteristics viz. carbohydrate fermentation, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Catalase, Oxidase, Hydrogen sulphide production, amylase, nitrate reductase and Gelatin hydrolysis revealed the following results (Figure: 2, Table: 3).

From the tables above (Table: 3 and Table: 4), the bacterial isolates B-1, B-3, B-4, B-6, B-8, B-9, B-10, B-14, B-17, B-18 and B-19 were found to be *Serratia, Lactobacillus, Lactococcus, Streptococcus, Staphylococcus, Klebsiella, Proteus, Clostridium* and *Pseudomonas* species respectively.

Ten (10) bacterial species including Staphylococcus, Pseudomonas, Serratia, Lactobacillus, Streptococcus, Bacillus, Clostridium, Proteus, Klebsiella and Lactococcus were isolated. The result was similar to those isolated. The predominant species found in most of the spoiled fruits and vegetables were Bacillus, Staphylococcus, Pseudomonas and clostridium. Bacillus was found to be the most dominant species and Proteus was found to be the least dominant bacterial species causing spoilage. Among fungi, *Penicillium* sp. was found to be the dominant species.

The bacteria and fungi (*Bacillus* sp. and *Aspergillus* sp.) isolated from carrot samples were in mark with those documented. (Ehimemen *et al.,* 2019; Harding *et al.,* 2017; Adebayo-Tayo *et al.,* 2012) in which they characterized the microbial communities associated with vegetables (Erickson, 2010). It was reported that *Aspergillus niger, Aspergillus flavus Rhizopus oryzae, Fusarium equiseti, and Fusarium moniliforme* are some fungi that affect fruits (Iniekong *et al.,* 2015). Ehimemen *et al.,* (2019) has also reported that pathogenic bacteria normally found in the fruits include S*almonella* sp., *Staphylococcus* sp., *Bacillus* sp. and these microbes causes spoilage.

Four (4) fungal species were apparently isolated from this research study viz., Aspergillus sp., Penicillium sp., Rhizopus sp., and Mucor sp. The fungal species were compatibly identified by different researchers (Adebayo-Tayo *et al.,* 2012; Iniekong *et al.,* 2015; Benner *et al.,* 2015) from different vegetables retailed in the market. Most of these fungi isolates associated with vegetables and fruits have shown to reason spoilage (Harding *et al.,* 2017).

Table.1 Morphological Characteristics of spoilage

S. No	Food	Place of Collection	Spoilage Symptoms
1	Tomato	Rythu Bazar, Nizampet	Lesions, White powdery mycelium
2	Carrot	Rythu Bazar, Nizampet	Mold growth with slime around
3	Potato	Rythu Bazar, Nizampet	Dark deepened lesions with foul smell
4	Cauliflower	Rythu Bazar, Nizampet	Mold with unpleasant smell and black spots
5	Lady's Finger	Rythu Bazar, Nizampet	Slime formation with physical damage to the tissue
6	Lemon	Rythu Bazar, Nizampet	Darkened black lesions, unpleasant smell
7	Sapota	Rythu Bazar, Nizampet	Dark brown spots with Molds
8	Mango	Rythu Bazar, Nizampet	Blackened spots with oozing fluid
9	Papaya	Rythu Bazar, Nizampet	Excess softness with dark spots
10	Banana	Rythu Bazar, Nizampet	Small black spots with mushiness

Table.2 Cultural and Morphological Characterization of Bacterial Isolates

S. No	Microscopical Test	Isolates									
		B-1	B-3	B-4	B-6	B-9	B-10	B-14	B-17	B-18	B-19
1	Growth medium	NAM	NAM	NAM	NAM	NAM	NAM	NAM	NAM	NAM	NAM
2	Colony Morphology	Red, round	Round smooth colony	Round and oval	Circular, elevated colonies	Round, convex	Large mucoid white colonies	Opaque round colonies	Large irregular	Circular, opaque white colonies	Green large opaque colonies
3	Gram Staining	_	+	+	+	+	_	_	+	+	_
4	Endospore Staining	_	_	_	_	_	_	_	+	+	_
5	Capsular	_	_	+	+	+	+	_	+	+	+
6	Flagellar	+	+	_	_	_	_	+	_	+	+
7	Acid Fast Staining	_	_	_	_	_	_	_	_	_	_

Table.3 Biochemical Characterization of the bacterial isolates.

S. No	Biochemical Test	B-1	B-3	B-4	B-6	B-9	B-10	B-14	B-17	B-18	B-19
1	Indole Test	+	_	__	_	_	+	_	_	_	_
2	Methyl Red Test	+	_	_	+	+	+	+	_	_	_
3	Voges -Proskauer Test	_	_	_	_	+	_	_	_	+	_
4	Citrate utilization Test	+	_	_	+	+	+	+	+	+	+
5	Amylase Test	_	+	+	+	+	_	+	+	+	+
6	Gelatin Hydrolysis Test	+	_	_	_	+	+	+	+	_	+
7	Catalase Test	+	_	_	_	+	+	+	_	+	+
8	Hydrogen sulfide test	_	_	+	_	_	_	+	+	_	_
9	Nitrate reductase test	+	_	+	_	+	+	+	_	+	+
10	Oxidase test	_	_	_	+	_	+	_	_	_	+
11	Carbohydrate fermentation test Sugars										
	a) Lactose	_	+	+	+	+	_	_	_	_	_
	b) Sucrose	+	+	+	+	+	_	+	_	+	__
	c) Dextrose	_	+	+	+	+	_	+	+	+	_

Fig.1 Microscopical observation of bacterial and fungal isolates

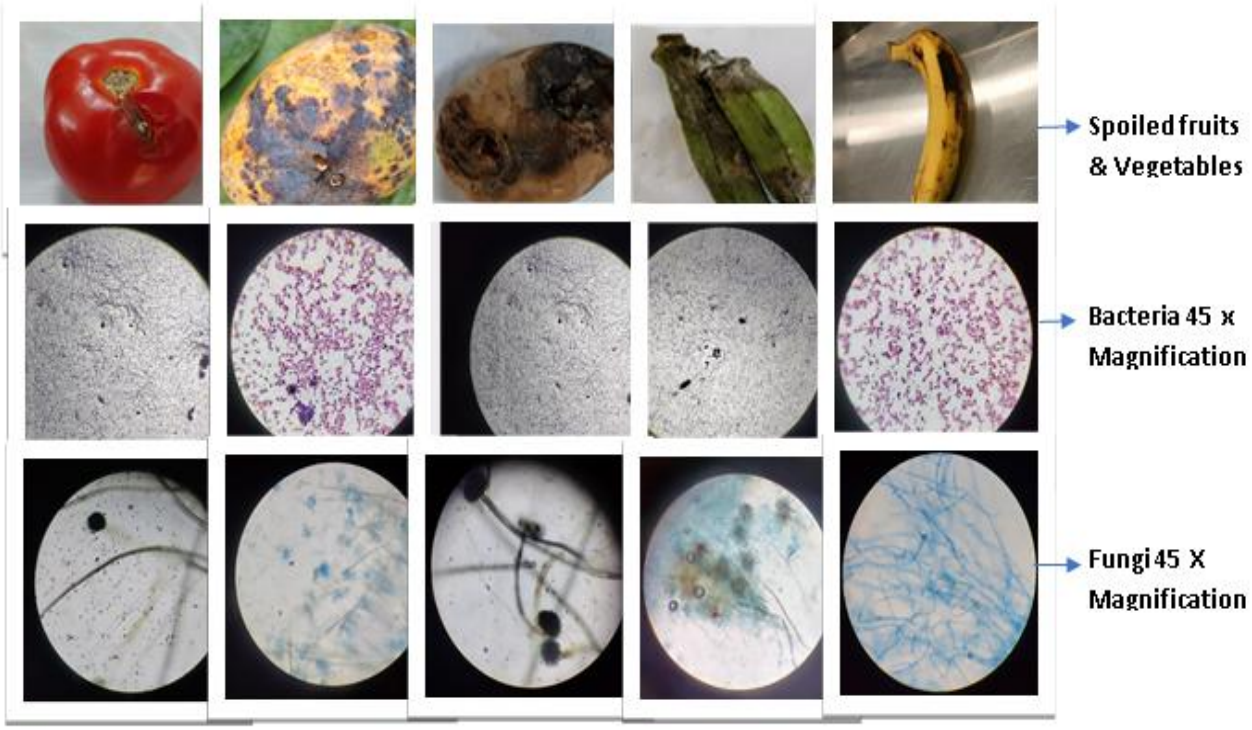


Fig.2 Biochemical tests of the bacterial isolates

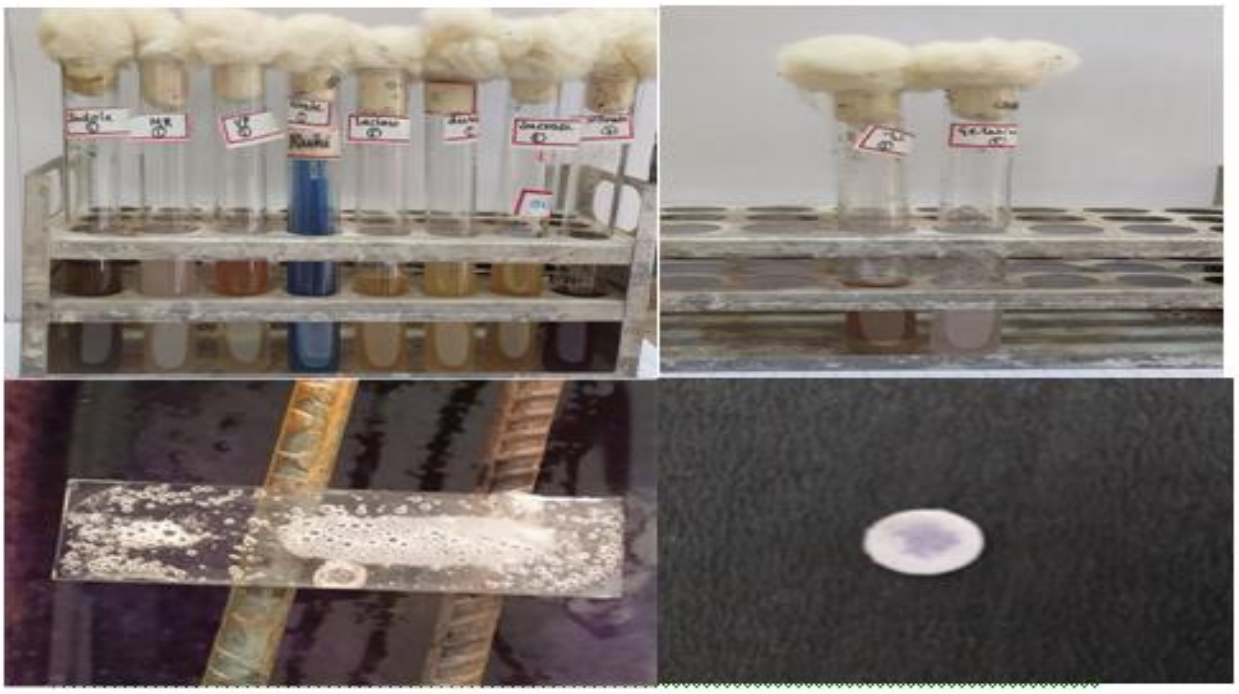
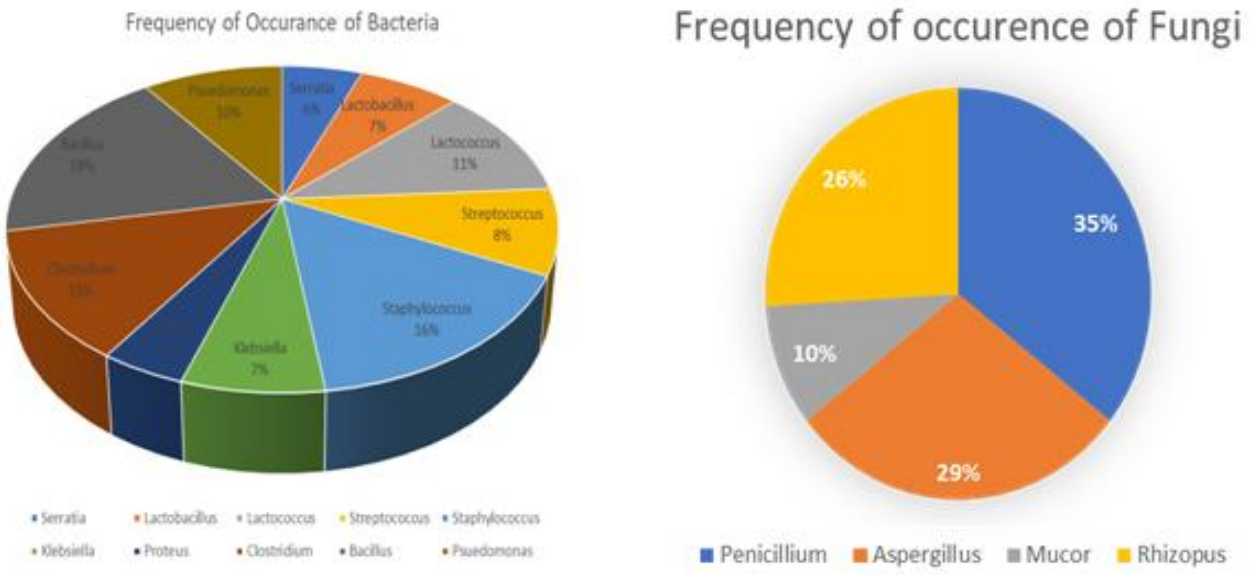


Fig.3 Frequency of occurance of Bacteria and Fungi in spoiled fruits and Vegetables



The frequency of the occurrence of the bacteria and fungi were given in the figure below (Figure: 3). This study revealed that spoiled fruits and vegetables have a plethora of bacteria and fungi which cause spoilage and are also morbific to human health. There is, therefore, need to safeguard that care is taken in handling, washing and processing fruits and vegetables before consumption so as to prevent food spoilage that valour led to infections and food-borne diseases caused by fungi and bacteria. Safety practices associated with the commodity should therefore not be limited to external washing only. There is the additional need of heating vegetables to eliminate microbes both externally and internally before consumption. It is also expedient to control food spoilage microorganism in order to reduce economic loss due to food spoilage.

Acknowledgement

The authors are grateful to Mr. M.N. Raju, Chairman, MNR Degree & PG College, Mr. M. Prasad, Director, MNR Degree & PG College, Kukatpally, Hyderabad.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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How to cite this article:

Ashwitha, K., Steffi D'Souza, Ritu Sarda, K. Krishnaveni, B. Likhitha, B. Thabitha Grace and Ritvik, P. 2022. Exploration of Microbes in Different Spoiled Fruits and Vegetables. *Int.J.Curr.Microbiol.App.Sci.* 11(08): 8-16. doi: https://doi.org/10.20546/ijcmas.2022.1108.002