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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Identification of Vegetable spoilage Microorganisms: Bacteria and fungi associated with postharvest spoilage in carrot

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ABSTRACT
Microorganisms responsible for spoilage in carrots not only affect the quality, but also the safety of the consumer of the product. Hence the present study is aimed to isolate and identify the microorganisms involved in spoilage of carrot. Samples were collected from carrot processing industry at Nilgris and identified by PCR amplification and 16s rDNA sequencing method. Primers fD1and rP2 were used for PCR amplification of conserved region of 16S rRNA. For fungal identification, primers ITSl and ITS4 were used to amplifier RNA gene region. PCR products were sequenced and compared by BLAST analysis in NCBI sequence data bank. Selected isolates were identified at genus and species level. The results indicated that the bacteria belongs to the genus Erwiniasp, Enterobacter cloaceae and Klebsiella pneumoniae, while the fungi predominantly belongs to the genus Aspergillus.
Keywords: *Carrot, Erwinia, Enterobacter, Klebsiella and Aspergillus.*

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INTRODUCTION

Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption [2]. Bacterial spoilage first softens the tissues as pectins are degraded and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol [13]. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue [16].

Spoilage of vegetables especially carrot are not only problem in India but a worldwide one. It is observed to be more prevalent in developing country than in developed nations.Soft rot by bacteria and post harvest storage fungi has been considered a common issue and causes huge damage to carrot, thus making carrot unfit for consumption. The quantum of post harvest losses in fresh carrot is estimated to be around 20 to 30 %. Both biological and physical damages during the harvest, transportation, higher moisture content and soft tissue content makes the carrot more susceptible to spoilage by microorganisms.

Carrots have a good physiological storability and it is not easily infected by microbes causing storage diseases; they can be stored for a period of 6–8 months without loss of quality under optimal storage conditions at temperature 0°C and relative humidity 98% [18]. However, carrot is sensitive to wilting if not protected from water loss. In commercial refrigerated stores, storage diseases are mainly caused by pathogenic fungi, leading to major loss.

Finlayson *et al.*(6) studied the infections caused by *Sclerotinia sclerotiorum* in carrots. *Mycelium* and *ascospores* were inoculated in to the foliage and crown of carrots. The setup was examined in green house chamber. The carrots treated with mycelium in foliage showed more damage than the *mycelium* treated on the roots. *Mycelium* infection is more common in carrots when compared to the *ascospore* treated carrots.

Ghosh *et al.* [8] evaluated the microbial quality of carrots which are used for preparing freshly squeezed street vended carrot juice in India. Samples were collected at different points in distribution chain and analyzed for total aerobes, *Staphylococcus* , *fecalcoliforms*, *Salmonella* and *Shigella* population. Total aerobes and *Staphylococcus aureus* were found positive in samples. The count increased at proceeding

chain of distribution mainly due to poor handling practices followed by street vendors.

Goodliffe and Heale [9]experimented on the incipient infections caused by *Botrytis cinerea* in stored carrots. In the study, 1200 carrot roots were harvested and stored for 40days under refrigerated condition. The most commonly found pathogens limiting the storage life of carrots were *Botrytis cinerea, Sclerotinia sclerotiorum* and *Centrosporsa cerina*.The study proved most of the primary infections has occurred on the crown, then the base of the petioles before appearing on the roots. *Botrytis cinerea* caused infection during the storage period rather than in field. Fungus was found to occur usually when roots were stored in wet condition.

Three pathogenic fungi, namely *Mycocentrospora acerina (Hartig) Deighton*, *Botrytis cinerea Pers* .and *Sclerotinia sclerotiorum* (Lib.) deBary, are considered to be the most harmful disease causing microbes throughout the production of carrot [7]. From the investigation of Mukula [12], the pathogens responsible for the decay during storage were *Sclerotinia sclerotiorum, Botrytis cinerea ,Stemphylium radicum* and *Fusarium avenaceum*, with the first two fungi accounting for 77% of thetotal decay. Control of spoilage in vegetables like carrot always remains challenge for researchers.

Hence the present investigation is carried out to study the presence of various bacteria and mold responsible for the post harvest decay and deterioration of economically important vegetable, carrot.

MATERIAL AND METHODS

Collection of Raw materials for the study

Freshly harvested carrots (New kuroda variety) were collected from a carrot machinery plant run by farmer at Ooty, Tamil Nadu, India.Samples were collected in sterile polythene bags and transferred to the laboratory and was stored in a refrigerator until mycological analysis.

Isolation of microorganisms from postharvest carrots

The microorganisms responsible for spoilage in carrots were estimated during different days of storage-0,3,5 and 7th day. The naturally present microorganisms responsible for spoilage in carrots were identified using pour plate method. The bacterial populations during storage were enumerated by plating one ml of 10^{-3} in nutrient glucose agar [3]. The fungal population was enumerated at 10^{-5} dilution in Martins Rose Bengal Agar [11]. The plates were incubated at 30^0C for 24 to 48 hrs for bacteria and 4 to 8 days for fungal growth.

Analysis of different microbial groups in postharvest carrots

Subsequent purifications to identify the bacterial and fungal growth on different storage days 0, 3,5 and 7th in carrots was done by streak plate method using Nutrient Agar and Martins Rose Bengal Agar respectively. Furthermore, purification in bacterial and fungal growth was done by streaking the colonies from petri-plates to test tube slants. Six test tube containing each of individual bacterial and fungal colonies were incubated at 30^0C for 3 to 5 days for the microbial growth. Six bacterial and six fungal test tube slants were taken for sequencing analysis.

Identification of microorganisms by PCR amplification and sequencing of rRNA gene

Sequencing of ribosomal RNAgeneis the technique used for phylogenetic placement, identification and diversity analysis of bacteria. Results of this technique are undisputable as it unveils the details of most conserved region of DNA,coding1rRNAgenethatisuniquetoeachof the species found in the world. Primers fD1 and rP2 were used for PCR amplification [17]. The primers used are from a conserved region of 16SrRNA and amplify full-length 16S rDNA. For fungal identification, primers ITSl and ITS4 were used to amplifier RNA gene regions [19].

Sequencing analysis

PCR products were sequenced through single pass analysis from forward and reverse direction. Sequence data was compared with available sequence data by BLAST analysis in NCBI sequence databank. Selected isolates were identified at genus and species level.

RESULT AND DISCUSSION

Identification of spoilage causing bacteria and fungi in carrot

Bacterial pathogens:

Six bacterial isolates which have shown distinguishing morphological characters were subjected to 16srDNA sequencing analysis. Bacterial isolates identified by rDNA sequencing is listed in Table. 1. Bacterial isolates were found to be long *Klebsiella*, *Enterobacter* and *Erwinia*. Among the different isolates identified, *Eriwinia* is a well-known postharvest spoilage bacteria among the root vegetables. *Klebsiella* and *Enterobacter* are ubiquitous in nature, found to harbor soil samples,vegetables etc.These bacterial isolates are opportunistic pathogens to humans.

Figure 1: Bacterial cultures isolated from carrot samples for identification of bacteria.

Table. 1 List of bacterial isolates identified byrDNA sequencing				
S. No.	**Isolate Name**	**Identified organism**	**Significance**	**Reference**
1.	A01	*Klebsiella pneumoniae*	Ubiquitous bacteria. Cause human infection	14
2.	B01	*Enterobacter cloacae*	Ubiquitous bacteria.Opportunistic infections	4
3.	C01	*Klebsiella pneumoniae*	Ubiquitous bacteria. Cause human	14
4.	D01	*Enterobactersp*	Ubiquitous bacteria. Cause human infection	14
5.	E01	*Klebsiella pneumoniae*	Ubiquitous bacteria.Cause human infection	14
6.	F01	*Erwiniasp*	Ubiquitous bacteria.Post-harvest pathogen. Opportunistic infections	4

Fungal pathogens:

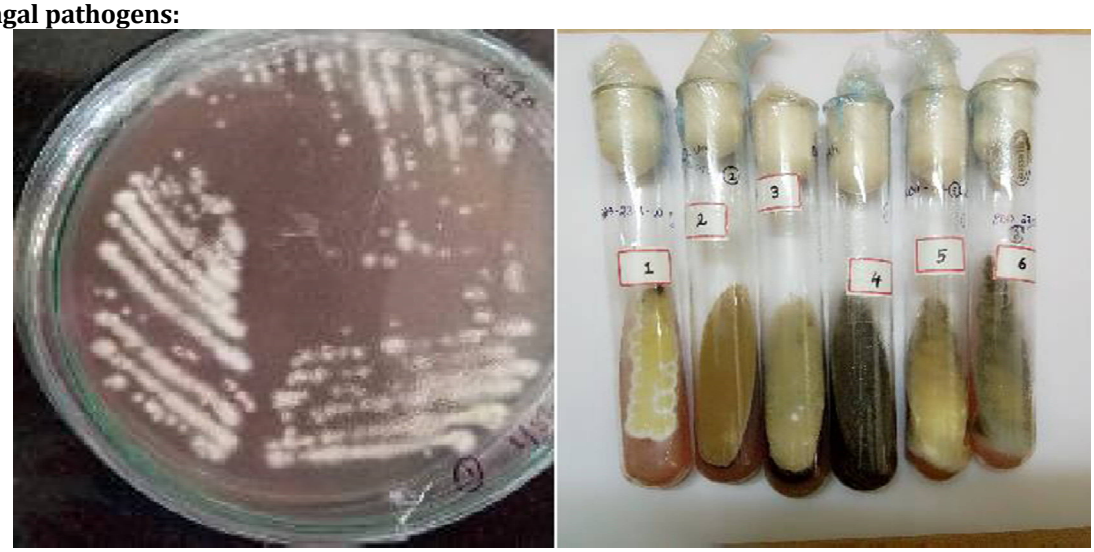

Figure 2: Fungal cultures isolated from carrot samples for identification of fungus.

Isolates of fungi are identified to belong to the genus *Aspergillus*. Different species of *Aspergillus* as described in Table2 were identified from the carrot samples on different days in storage. These fungal isolates are ubiquitous in nature, found in most of the tropical and subtropical soil samples. *Aspergillus* genus causes postharvest rotting in vegetables, produce mycotoxins and cause different diseases in humans.

Table. 2 List of fungal isolates identified by rDNA sequencing				
S. No.	**Isolate**	**Identified organism**	**Significance**	**Reference**
1.	NS1-A01	*Aspergillus ustus*	Soil fungi, causing human skin and nail infection	15
2.	NS1-B01	*Aspergillus unguis*	Soil fungi. Found in Decaying matter.Causing human disease.	5
3.	NS1-C01	*Aspergillus ustus*	Soil fungi, causing human skin and nail infection	15
4.	NS1-D01	*Aspergillus niger*	Soil born fungi. Produce mycotoxins.	1
5.	NS1-E01	*Aspergillus keveii*	Soil borne fungi. Produce mycotoxins.	10
6.	NS1-F01	*Aspergillus keveii*	Soil borne fungi. Produce mycotoxins.	10

CONCLUSION

The twelve strains six each of bacterial and fungal spoilage microorganisms were obtained by isolation and morphological screening of isolates followed by 16s rRNA sequencing method. The microbial pathogens present in the carrot sample were as follows; bacterial pathogens belongs to genera *Erwinia*sp, *Klebsiella pneumonia* and *Enterobacter cloacae.* The dominant fungal pathogen belongs to genera *Aspergillus* and species includes; *Aspergillus unguis*, *Aspergillus niger, Aspergillus keveii and Aspergillus ustus.*

Among the listed microbes, *Aspergillus unguis, Aspergillus ustus, Enterobacter cloacae, Erwiniasp,*and *Klebsiellapneumoniae* are identified as human pathogens.Hence, itis important to reduce the microbial load before packaging. The present work identifies preponderance of pathogenic bacteria and different species of *Aspergillus*, in carrot sample. These organisms and others identified as storage fungi and are mycotoxigenic fungi. Their presence in carrot sample could lead to severe food poisoning.

Hence proper post harvest management especially during storage and handling should be imposed in carrot processing industry to protect carrot from microbial spoilage. To bring into action, the importance of personal hygiene, using sterilized water for washing carrot during pre processing /cleaning equipment, integrated post harvest management practices and storage of carrot at cold temperature should be popularized or awareness should be made to the people involved in carrot processing industry.

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