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Research Article

Preliminary Identification of Bacterial Species Contaminating Okra Fruits in Some Selected Markets in Owo, Nigeria

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Abstract:

In spite of Okra (Abelmoschus esculentus L.) cultivation relevance in the society, management of its post-harvest disease has stand out as major issue to plant pathologists. This study therefore reports findings on the spoilage bacteria associated with Okra fruit during post-harvest storage. Okra sample was purchased from three (3) local markets (Ogwagbe market, Areghona market and Oba market) in Owo local government and taken to the Plant Pathology Laboratory of the Department of Crops Soil and Pest Management, Federal University of Technology, Akure. About 2g of deteriorated Okra fruit sample was sterilized and aseptically transfer into the sterile 20cm^2 beaker. The bacteria isolated were incubated at 37+ 2^{0}c for 24hours in a Nutrient Agar (NA) plate. At the end of incubation period the Colonies forming units from violet bacteria cells were counted and recorded appropriately per gram of each of the deteriorating Okra sample. Pure culture of individual isolates was kept at 40c for biochemical test and isolates were identified. A total of 26 bacteria were isolated and identified from the Okra sample from the 3 local markets on the basis of colony morphology and the staining characteristics. Location A has the highest population of bacteria isolate while location B and C had lowest bacterial population. The highest occurring genera can be found in Micrococcus, Bacillus and Staphylococcus followed by Alcaligenes, Pediococcus, and Erwinia. Alcaligenes odorans and Bacillus pumilus are the most occurring species of bacteria isolated from the market. Total viable counts ranges from 1.20 X 10^{-8} (cfu/g) to 1.72 X 10^{-8} (cfu/g). The highest average coliform forming unit occurs in Location A (1.52 X 10^{-8} (cfu/g)) while the least average coliform forming unit occurred in Location B (1.23 X 10^{-8} (cfu/g)). Alcaligenes odorans Bacillus pumilus and Micrococcus spp. had the highest frequency of occurrence (67 %). The presence of these bacteria on Okra fruit are potential threat to its production most importantly coliforms, pose a serious threat to consumers' health as the bacteria could produce toxins, which are poisonous when consumed. Proper handling methods should be adopted by both farmer and consumers to minimize the bacterial deterioration of okra fruit in the study area.

Key words: Okra; diseases; alcaligenes; bacillus; staphylococcus; viable count

Introduction

Increased food production is imperatively needed for the growing global population which pose enormous risks to ensuring food security. Metabolic processes brought about by microbial actions often result in food spoilage and can make food crops undesirable or unacceptable for human consumption due to deterioration in quality characteristics (Doyle, 2007; Salehin et al., 2023). This spoilage type is frequently associated with vegetable crops which often result in postharvest losses incurred as a result of quantity and/or quality loss due to pathological, physiological and mechanical damages (Oladoye, 2013). In most vegetable crops, including okra, these factors are interconnected since physiological stress and mechanical injury may greatly affect the crops susceptibility to diseases attack (Oladoye, 2013).

Okra (Abelmoschus esculentus L.) also known as ladies' finger, okro, or gumbo, is a popular vegetable that is native to Africa. It is the most important vegetable crop commonly grown in tropical Africa and belongs to the Malvaceae family (Onaebi et al., 2020; Mohamed et al., 2023; Salehin et al., 2023). Because of its great nutritional content and socioeconomic benefits, several African countries including Nigeria considered Okra as a basic food for Her teeming populations. Seeds and

fruits of Okra are edible; the immature fruits offer man nutritional dietary fiber, medicinal and therapeutic products. Okra contains B vitamins, calcium, iron, mineral salts, carbohydrates, protein, fat, iron and antioxidant substances (Sharma et al., 2013; Amadi et al., 2014; Mohamed et al., 2023; Salehin et al., 2023).

In spite of Okra cultivation relevance in the society, management of its post-harvest disease has stand out as major issue to plant pathologists. Okra intense metabolism that is characterized by high respiratory rate and high-water content which is about 90%, makes okra fruit quite perishable after harvesting. These factors, which are the biggest challenge in okra storage, makes its fruit to have an extremely short storage period, mainly in poor storage conditions such as high temperature and low relative humidity, because these conditions accelerate the loss of water and darkening of fruits, thus, denigrating its commercial worth for consumption in nature (Salehin et al., 2023).

Henz et al. (2007) and Salehin et al. (2023) reported some endophytic bacteria isolated from okra plants. The bacteria include: Pseudomonas, Azospirillum, Bacillus, Serratia, and Enterobacter. Rot microorganisms can be introduced to the crop during crop growth in the field, during harvesting and postharvest handling, or during storage and circulation. Therefore, early intervention procedures during crop development and harvesting through the use of good agricultural practices will provide vivid reductions in yield loss due to spoilage at all subsequent steps (Oladoye, 2013; Mohamed et al., 2023). There has been dearth of information on Okra spoilage caused by bacteria that led to Okra scarcity in the country. Therefore, there is need for assessing the bacterial associated with postharvest rot Okra. This study therefore reports findings on the spoilage bacteria associated with Okra pod during post-harvest storage.

Materials and Methods

Sample Collection and Study Area

Okra sample was purchased from three (3) local markets (Ogwagbe market, Areghona market and Oba market) in Owo local government Ondo state, Nigeria. The samples were brought to the Plant Pathology Laboratory of the Department of Crops Soil and Pest Management, Federal University of Technology, Akure. About 20gram of the sample was wrapped in sterile Nylon and allow to undergo deterioration over a period of 5-7days in the laboratory.

Preparation of Media

The Nutrient agar (NA) media was prepared in accordance with the method of Muhammad et al. (2018).

Sample preparation/isolation of Bacterial

Okra sample was washed for about 10mins with running tap water and dried off with a sterile filter paper. 2g of deteriorated Okra fruit sample was sterilized with 70% ethanol for 1min, followed by 3% Sodium hypochlorite for 3–4 mins and aseptically transfer into the sterile $20cm^2$ beaker. 9ml of sterile distill water was added gently to the sample in the beaker and mixed thoroughly with glass rod to from a homogeneous suspension or Aliquot. 1ml of the aliquot of the sample was pipetted into sterile test tubes containing 9ml of sterile distill water. Each suspension was serially diluted in another 8 set of Test tubes each to dilution ratio 10^{-8} about 0.1ml portion of the sample aliquot from the 6th and 7th dilution factor was aseptically pipetted independently into different sterile Petri dishes and thoroughly mixed with 20ml of the cool molten Agar media. The plate was gently swirled for even spread of the sample aliquots with media allowed set and incubated at optimum temperature. The bacteria isolated were incubated at $37+ 2^0$c for 24hours in a Nutrient Agar (NA) plate. At the end of incubation period the Colonies forming units from violet bacteria cells were counted and recorded appropriately per gram of each of the deteriorating Okra sample. Subculture were subsequently carried out to obtained pure culture of each isolate for further studies. Pure culture of individual isolates was kept at 40c for biochemical test (Muhammad et al., 2018; Abiodun and Abisola, 2021).

Identification of bacterial isolates by cultural and bio-chemical test

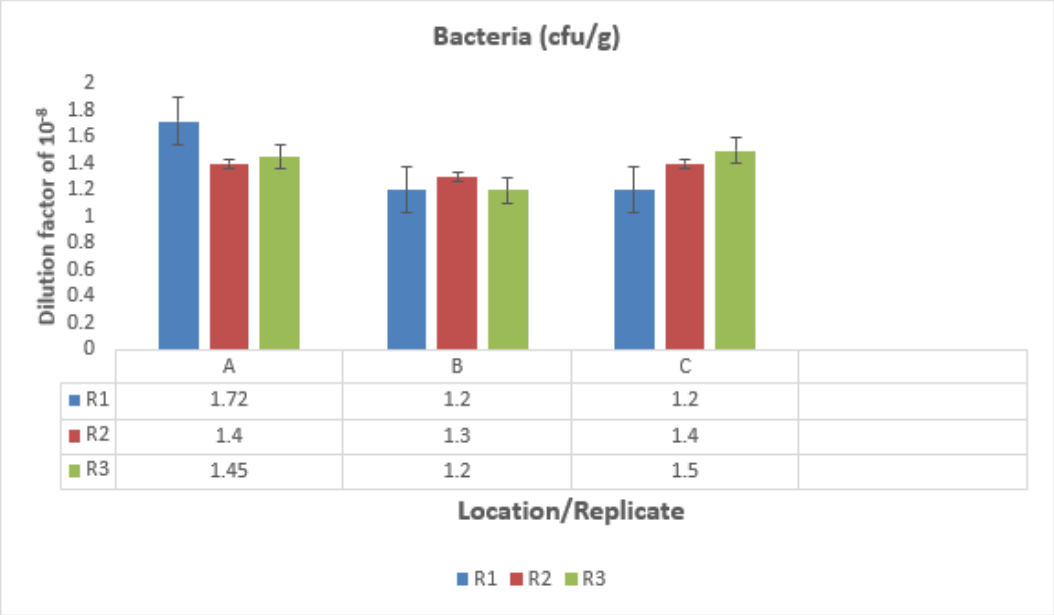
Preliminary identification: Colonial characteristics of the pure bacterial isolated were examined on the solid Agar surface after 18-24 hrs of incubation. These characteristics includes: color, shape, opacity, translucency, elevation, edges and surface texture. Cultural characteristics of the bacterial isolated were recorded while the bio-chemical test (such as Gram staining, Catalase, Motility test, Spore staining, Fermentation of sugars (carbohydrates), Indole Production, Methyl Red Test, Starch Hydrolysis, oxygen-relation and Oxidase tests) were carried out for the identification according using the methods described by Bergey and Holt, (2000) and Sherman (2005).

Results

The distribution of bacteria isolated from the three (3) different local markets is shown in Table 1. A total of 26 bacteria were isolated and identified from the Okra fruit sample from the 3 local markets on the basis of colony morphology and the staining characteristics. Location A has the highest population of bacteria isolate while location B and C had lowest bacterial population. Bacteria isolated are Micrococcus varians, Alcaligenes odorans, Proteus vulgaris, Staphylococcus saprophyticus, Streptococcus spp, Lactobacillus brevis, Bacillus cereus, Pediococcus spp., Corynebacterium sp., Bacillus pumilus, Erwinia carotovora, Enterobacter aerogenes, Ralstonia solanacearum, Pediococcus cerevisiae, Bacillus pumilus, Micrococcus spp., Staphylococcus hominis, Aerococcus bacteremia, Pseudomonas aeruginosa, Xanthomonas spp., Erwinia herbicola, Bacillus subtilis, Leuconostoc mesenteroides, Staphylococcus aureus (Table 1).

The highest occurring genera can be found in Micrococcus, Bacillus and Staphylococcus followed by Alcaligenes, Pediococcus, and Erwinia. Alcaligenes odorans and Bacillus pumilus are the most occurring species of bacteria isolated from the market (Table 1). Total viable counts ranges from 1.20 X 10^{-8} (cfu/g) to 1.72 X 10^{-8} (cfu/g). The highest average coliform forming unit occurs in Location A (1.52 X 10^{-8} (cfu/g)) while the least average coliform forming unit occurred in Location B (1.23 X 10-8 (cfu/g)) (Figure 1).

The result shows the frequency of occurrence of the several of bacteria associated with okra fruit spoilage in the study. Alcaligenes odorans Bacillus pumilus and Micrococcus spp. had the highest frequency of occurrence (67 %) while the lowest frequency of occurrence (33 %) occur in Micrococcus varians, Proteus vulgaris, Staphylococcus saprophyticus, Streptococcus spp, Lactobacillus brevis, Bacillus cereus, Pediococcus spp., Corynebacterium sp., Erwinia carotovora, Enterobacter aerogenes, Ralstonia solanacearum, Pediococcus cerevisiae, Bacillus pumilus, Staphylococcus hominis, Aerococcus bacteremia, Pseudomonas aeruginosa, Xanthomonas spp., Erwinia herbicola, Bacillus subtilis, Leuconostoc mesenteroides, Staphylococcus aureus. Percentage of occurrence of the frequency of occurrence with respect to locations of sample collection was presented in Table 2. Ogwagbe market had the highest percentage of fungal loads (38.5 %) and the other two markets (Areghona market and Oba market) had the lowest (30.7 %).



(cfu/g): Coliform forming unit per gram of the sample
10-8: Dilution factor
R: Replicate

Figure 1: Shows the total viable count of Bacteria (cfu/g) present in the samples at dilution factor of 10^{-8}

Location	Bacteria isolated and identified from Okra sample
A	*Micrococcus varians*, *Alcaligenes odorans*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Streptococcus* spp, *Lactobacillus brevis*, *Bacillus cereus*, *Pediococcus* spp., *Corynebacterium* sp., *Bacillus pumilus*
B	*Erwinia carotovora*, *Enterobacter aerogenes*, *Ralstonia solanacearum*, *Pediococcus cerevisiae*, *Bacillus pumilus*, *Micrococcus* spp., *Staphylococcus hominis*, *Aerococcus bacteremia*
C	*Pseudomonas aeruginosa*, *Xanthomonas* spp., *Erwinia herbicola*, *Alcaligenes odorans*, *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Micrococcus* spp., *Staphylococcus aureus*

Lab Ref No **Original Sample Label**
A- Okra from Ogwagbe market Owo
B- Okra from Areghona market Owo
C- Okra from Oba market Owo

Table 1: Isolation and identification of bacterial associated with postharvest rot of Okra samples

Fungal Isolates	Ogwagbe market	Areghona market	Oba market	Frequency of Occurrence	% of Occurrence
Micrococcus varians	+	−	−	1	33
Alcaligenes odorans	+	−	+	2	67
Proteus vulgaris	+	−	−	1	33
Staphylococcus saprophyticus	+	−	−	1	33
Streptococcus spp	+	−	−	1	33
Lactobacillus brevis	+	−	−	1	33
Bacillus cereus	+	−	−	1	33
Pediococcus spp.	+	−	−	1	33
Corynebacterium sp.	+	−	−	1	33
Bacillus pumilus	+	+	−	2	67
Erwinia carotovora	−	+	−	1	33
Enterobacter aerogenes	−	+	−	1	33
Ralstonia solanacearum	−	+	−	1	33
Pediococcus cerevisiae	−	+	−	1	33
Micrococcus spp.	−	+	+	2	67
Staphylococcus hominis	−	+	−	1	33
Aerococcus bacteremia	−	+	−	1	33
Pseudomonas aeruginosa	−	−	+	1	33
Xanthomonas spp.	−	−	+	1	33
Erwinia herbicola	−	−	+	1	33
Bacillus subtilis	−	−	+	1	33
Leuconostoc mesenteroides	−	−	+	1	33
Staphylococcus aureus	−	−	+	1	33
Total	10	8	8	26	
% of Occurrence	38.5	30.7	30.7		

+: Present
−: Absent

Table 3: Frequency of Occurrence of Bacteria Isolates Associated with Okra Fruit in the Study Area

Discussion

The colonization of Okra fruits by the invading bacteria is a critical step in the microbial spoilage of crop plant. This study was designed to determine bacteria isolates contaminating for Okra fruits at 3 local market, Owo Nigeria. A total of twenty-six (26) bacteria isolates were isolated which has 17 distinct genera. The number of bacteria isolates identified in this study is higher than that reported by Al-Kahtani et al. (2020) and Salehin et al. (2023). Al-Kahtani et al. (2020), isolated 13 putative bacterial endophytic strains from two medicinal plants growing under the adverse conditions of the Sinai desert; while Salehin et al. (2023), isolated 12 endophytic bacterial strains from the roots of okra plants The bacterial isolates were characterized on the basis of colony morphology, staining characteristics and biochemical test.

The result obtained shows that the bacteria found in spoiled fruits samples include; Micrococcus varians (33 %), Alcaligenes odorans (67 %), Proteus vulgaris (33 %), Staphylococcus saprophyticus (33 %), Streptococcus spp. (33 %), Lactobacillus brevis (33 %), Bacillus cereus (33 %), Pediococcus spp. (33 %), Corynebacterium sp. (33 %), Bacillus pumilus (67 %), Erwinia carotovora (33 %), Enterobacter aerogenes (33 %), Ralstonia solanacearum (33 %), Pediococcus cerevisiae (33 %), Bacillus pumilus (33 %), Micrococcus spp. (67 %), Staphylococcus hominis (33 %), Aerococcus bacteremia (33 %), Pseudomonas aeruginosa (33 %), Xanthomonas spp. (33 %), Erwinia herbicola (33 %), Bacillus subtilis (33 %), Leuconostoc mesenteroides (33 %) and Staphylococcus aureus (33 %).

The higher coliform forming unit observed in Location A (1.52 X 10^{-8} (cfu/g)) may be storage condition and duration of storage before they were sold out to final consumer. The study shows Alcaligenes odorans Bacillus pumilus and Micrococcus spp. had the highest frequenctly occurring species (67 %) and found in almost all the okra fruit samples examined. The finding of this study was in conformity with the report of Doyle, (2007) Zumunta et al., (2020) and Muhammad et al. (2018). Naturally occurring bacteria can be found on fruits surface and vegetables; and cause nearly half of post-harvest rot of fresh crop plants stored at cold temperatures (Doyle, 2007; Otieno et al., 2023). Previous research work have documented several of bacteria species as being phytopathogenic (Doyle, 2007; Muhammad et al., 2018; Otieno et al., 2023). The detection of the bacteria isolates from Okra fruits in this study indicated poor postharvest handling of the Okra fruits which could also be from contamination during planting, harvesting and transporting.

Conclusion

The present study revealed the presence of several bacteria associated with okra fruit in three (3) different market in Owo local government area, Ondo state Nigeria. The presence of these bacteria on Okra fruit are potential threat to its production most importantly coliforms, pose a serious threat to consumers' health as the bacteria could produce toxins, which are poisonous when consumed. Proper handling methods should be adopted by both farmer and consumers to minimize the bacterial deterioration of okra fruit in the study area.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contributions

O.A.O conceived the study, A. O. A. prepared the draft manuscript; A. K. V sourced for literature O. B. O. carried out the laboratory work, all the

authors proofread the draft manuscript and Jointly supervised and proofread the study.

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