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Isolation and Identification of Lactic Acid Bacteria on Vegetables (Okro)

Dr. Nwagbara Evaristus Chinedu
IMO STATE UNIVERSITY

Abstract- ***Okra (Abelmoschus esculentus) is an important vegetable crop grown worldwide including India. Okra is attacked by various microorganisms like fungi, bacteria, viruses in the field or contaminate during harvesting, processing and packing or transportation. The frequency of damage by various diseases varies greatly with commodity, processing, growing conditions and the way of handling. The post-harvest diseases in transit and storage lead to waste of labor, time, field and money. This study examines the isolation and identification of lactic acid bacteria on vegetables (Okro). The result gotten from the study shows that, the total heterotrophic plate count from the vegetable (Okro) sample, identified lactic acid bacteria which ranged from 4.0 x 104 in sample A to 6.0 x 104 in sample B respectively. The high counts could be associated to the fact that okro is a product that can be consumed raw with no heat treatment to reduce microbial load. It is therefore recommended that special care however, has to be taken in its preparation of okro as contaminants could be introduced through the preparation and education of food handlers and the general public on food safety measures will be encouraged.***

I. INTRODUCTION

Vegetables are considered as leafy out-growth of plants or plant shoots used as food. These include those plants or plant parts used in different ways such as in soup or served integral as part of main meal (Wagner, 2016). They can also be regarded as the edible components of plants, which include leaves, stalks, roots, tubers, bulbs, flowers and seeds. They are important protective foods and are highly beneficial for the maintenance of health and prevention of diseases. They offer the most rapid and lowest cost method of providing adequate supplies of vitamins, minerals, and fibers. These ingredients are essential for proper function of the body.

Okra (*Abelmoschus esculentus L.*) of family Malvaceae is an important vegetable crop grown in many tropical, subtropical and warmer temperate areas. Okra is an erect, coarse, robust annual herb in which flowers are borne singly in the leaf axile on peduncles. The plant has malvaceous floral organization with 8-10 very narrow, hairy, bracteoles forming an epicalyx. The leaves are, leathery or rough, large, alternate, cordate divided into 3-7 lobes with notched or toothed margins (Parker, 207). The most common disease afflicting the okra plant is verticillium wilt, often causing a yellowing and wilting of the leaves. Other diseases include powdery mildew in dry tropical regions, leaf spots, yellow mosaic and root-knot nematodes. The fungi were Penicillium, Penicillium oxalicum, Penicillium palitans, Rhizopus stolonifer, Rhizopus oligosporus, Rhizopus oryzae, *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus japonicus*, *Aspergillus clavatus*, Mucor hiemalis, Mucor racemosus, Alternaria herbarum and Alternaria triticina. Resistance to yellow mosaic virus in *A. esculentus* was transferred through a cross with *Abelmoschus manihot* and resulted in a new variety called *Parbhani kranti*. Raw okra contains 90% water, 2% protein, 7% carbohydrates and negligible fat. In a 100 gram reference amount, raw okra is a rich source (20% or more of the Daily Value, DV) of dietary fiber, vitamin C, and vitamin K, with moderate contents of thiamin, folate and magnesium. Bast fibre from the stem of the plant has industrial uses such as the reinforcement of polymer composites. Hence, the aim is to determine the isolation and identification of lactic acid bacteria from vegetables (Okro).

II. LITERATURE REVIEW

2.1.1 *Okro Fruit Rot and Pod Spots*

The disease is caused by various fungal species. *Rhizopus stolonifer* caused browning and softening of the pods which at high relative humidity covered by mycelium and vegetables bodies of the fungus. *Curvularia oryzae* was isolated from vegetables of

okra collected in markets. Dry vegetables rot disease is caused by *Aspergillus flavus* (Agbodaze et al., 2019). Seventy-eight vegetables samples of okra from different sites were collected from various districts of Rajasthan. Eleven major fungal genera (*Aspergillus flavus, A. niger, A. nidulens, A. fumigates, Alternaria alternate, Curvularia lunata, Rhizopus nigricans, Cladosporium oxysporum, Penicillium chrysogenum, P. citrinum, Stachybotrus atra, Chaetomium globosum, C. murorum, Rhizoctonia bataticola*), and 4 bacterial genera (*Actinomycetes* sp*., Erwinia caratovora, Xanthomonas campestiris pv. campestris, X. c. pv. malvacearum, Pseudomonas syringae pv. syringae*) were found associated with post-harvested diseases or spoilage of okra vegetables in the study. It was concluded that fungal pathogens cause the damage at high temperature, low relative humidity with poor aeration.

Grey mold (Botrytis cinerea) reported from Romania on okra which on high relative humidity grows profusely. The symptom appeared as whitening of the stem and pods which gradually attacked all aerial organs. Sclerotia were formed on the stem in the autumn. Choanophora pod rot (*Choanophora cucurbitarum*) reported from Rajasthan produces water-soaked areas on green pods which later turned brownish black symptoms. Seed-borne pathogen *Cladosporium cucurbitarum* with *Rhizoctona* sp. caused vegetables rot of Hibiscus esculentus (Amoah, 2018). The *C. cucurbitarum* isolate was pathogenic to nondetached flowers and wounded vegetables but not unwounded vegetables and seedlings. Both were pathogenic to non-detached wounded or unwounded green vegetables.

2.1.2 *Seed-Borne Fungi*

The seeds of okra are found to be associated with a large number of fungal species among them species of *Aspergillus, Alternaria, botrytis, Curvularia, Dreschlera, Chaetomium, Fusarium, Penicillium, Rhizoctonia bataticola, Colletotrichum, Macrophomian phascolina, Choanophora cucurbitarum, Ascochyta belmoschi, Rhizopus* reported as major fungi. Five homeopathic drugs were tested against various fungal species and found effective. These drugs increased percent seed germination, root-shoot length of okra and inhibition of 22 fungi associated with seeds by Thuja, citric acid and sulfur 200 drugs. Agrawal and Singh (2004) studied the efficacy of latex of some plants and *found Calotropis procera, Datura innoxia*, *Michalia champaca* and Ricinus communis effective at 30% concentration against seed-borne fungi viz., *Aspergillus flavus, A. fumigates, A. niger, Cercospora sp., Fusarium* sp*, Rhizoctonia bataticola, Curvularia* lunata and Penicillium sp. that were eliminated completely.

The crop is attacked by various microorganism or pests that are responsible for its poor quality and low yield approx 20-30% every year in most okra-growing areas of the country. Crop suffers from a number of phytopathogenic fungal and bacterial species causing severe losses, reduces yield and market value of okra seeds. Annotated list of Seed-borne Diseases has listed Ascochyta blight, pod spots (*Ascochyta abelmoschi*), stem and capsule disease (*Botrytis* sp.), anthracnose (*Colletotrichum dematium*), wilt (*Fusarium oxysporum f.* sp. *vansifectum* and *Fusarium solani*), charcoal and root rot (*Macrophomina phaseolina and Rhizoctonia solani*), bacterial blight (*Pseudomonas syringae*), okra leaf curl virus and yellow vein mosaic virus as seed-borne diseases. About 32 fungal species on okra seeds from various districts of Rajasthan viz. *Actinomycetes, Arthrobotrys supberba, Aspergillus fumigates, Cladosporium oxysporum, Drechslera* sp., *Fusarium moniliforme, Stachyobotrys* sp., *Verticillium alboatrum* and 3 bacterial species namely *Ralstonia solanacearum*. Out of these 10 fungal species were found dominant associated on okra. This microflora severely affecting seeds germination and cause many seedling abnormalities like failure or delayed seeds germination, bacterial oozing, rotting, collapse of hypocotyls and cotyledonary leaves that resulting seedling mortality. High incidence of 5 seed-borne fungal diseases was reported from Pakistan from the field. The treatment of seeds with of Vitavex-200 found effective to control these diseases and reduce the incidence. Major seed-borne diseases of okra in Bangladesh are seed rot, seedling blight, die back, anthracnose, stem rot, die back, seed rot, germination failure or seed discoloration, seed rot and seedling blight.

2.1.3 *Fungal Diseases*

Fungi are mostly filamentous microorganisms commonly known as molds. In nature, they often

appear thread- like, cottony, or as yeast-like scum. Many fungal species can cause vegetables decay in okro. Fungi are generally more difficult to eradicate than bacteria, because fungal cells are much larger and produce spores that are highly resistant to drying and other environment stresses. The major postharvest diseases caused by fungi are Sour rot, Rhizopus rot, Buckeye rot and Black mold rot. Descriptions of these diseases have been well articulated by various workers (Chuku *et al.*, 2018). The characterization and identification of some bacteria isolate from okro include *Bacillus subtilis*, *Bacillus cereus*, *Bacillus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis* and *Staphylococcus aureus*.

III. MATERIALS AND METHODS

3.1 *Study Area*

The study shall be carried out at Owerri, Imo State which is located South Eastern Nigeria lies between Latitude $5^0$10 and $5^0$51 North and Longitude $6^0$35 and $7^0$28 with a population of about 215, 038.

3.2 *Sample Collection*

Ten (10) samples of vegetables (Okro) were purchased from different vendors in Owerri Imo State. The sample was collected in sterile specimen containers and was transported in cold pack to the laboratory for analysis within 30 to 45 minutes of collection.

3.3 *Methods*

Pieces of the vegetables (Okro) from each sample was removed and mashed in a sterile laboratory type mortar and pestle into a paste. Ten percent of the stock solution was prepared by weighing 10g into 100ml of sterile buffered saline, properly shaken and sieved before a twofold dilution was performed. Serial dilution was carried out from the stock solution to obtain 1:10 dilution from 6 test tubes, given a dilution of 10-1, 10-2, 10-3, 10-4, 10-5 and 10-6 respectively. Pour plate was conducted by obtaining 1ml of an aliquot from each of the test tubes and pouring into sterile petri-dishes. It was allowed to settle and 15ml of liquid nutrient agar (total plate counts) and MacConkey agar (total coliforms counts) cooled at 45°C was poured and incubated at 37°C for 24hrs. The plate viable counts were conducted as described by Chessbrough. (2019). Microorganisms growing on solid surfaces medium tend to form colonies with distinctive morphologies. The variation in bacterial colony morphology can be observed with the naked eye. This morphological variation includes shape, odour, elevation surface on agar media. Specific biochemical test was carried out to distinctively identify the isolates. The biochemical tests include gram staining, indole, catalase, coagulase, motility citrate and sugar fermentation of the method of Kamil. (2018)

IV. RESULTS

Table 1: Suspected Bacteria Isolates and its total heterotrophic plate count from the Vegetable (Okro) samples.

Sample	Suspected Bacte isolates	Cfu/ml	Dilution Cfu/ml
A	Lactic Acid Bacte	60	6.0×10^4
B	Lactic Acid Bacte	40	4.0×10^4

Table 2: Morphological Characterization of Isolated Organisms

Isolated	Colors of the Colony Observed	Shape	Edge	Elevation	Surface	Probably organism
A	Golden yellow	Spherical	Entire	Raised	Smooth	Lactic acid bacteria
B	Milky colonies	Irregular round	Undulate	Flat	Smooth	Lactic acid bacteria

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Table 3: Biochemical Test of Bacterial Isolates gotten from Vegetable (Okro) samples

Cat	Oxi	Coag	Ind	MR	VP	Cit	Ure	Suc	Lac	Mann	Isolates
+	-	-	-	-	+	+	-	-	-	+	Lactic Acid Bacteria
+	+	-	-	+	-	+	+	-	-	+	Lactic Acid Bacteria
+	-	-	-	+	-	+	-	-	-	-	Lactic Acid Bacteria

KEY:
-- NEGATIVE, + POSITIVE
Cat, catalase; Coag, coagulase; Oxi, oxidase;
Ind, indole; Mr, methyl red; Vp, voges proskeur,
Ure, urease production; suc, sucrose;
Mal, maltose; Lac, lactose; Mann, mannitol;

V. DISCUSSION

This study examines the isolation and identification of lactic acid bacteria on vegetables (Okro). The result gotten from the study shows that, the total heterotrophic plate count from the vegetable (Okro) sample, identified lactic acid bacteria which ranged from 4.0×10^4 in sample A to 6.0×10^4 in sample B respectively. The high counts could be associated to the fact that okro is a product that can be consumed raw with no heat treatment to reduce microbial load.
The data obtained in this study has demonstrated the activities of pathogenic bacteria associated with ready to eat vegetables (Okro) and confirmed previous microbiological report on other ready to eat foods (Ezeronye, 2017). The total bacterial count of screened food samples implies extreme contamination and potential health risk of these food samples. The high incidence of bacterial contamination encountered in this study were mainly due to the largely unhygienic nature of the food preparations and services areas of foods are good indicators of the state of environment in which they are prepared or served. Majority of the food centers are located beside waste disposal points and duty roads. Furthermore, lack of running water, sewage disposal infrastructure, inappropriate storage conditions and the presentation of these foods in the open encouraged multiple contaminations. The predominant bacterial pathogens isolated in this study include *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and various origins are an indication of poor hygienic practices.

VI. CONCLUSION

Fresh vegetables have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms. However, this protection may be eliminated and vegetables may become contaminated during their growing in fields or during harvesting, post-harvest handling and distribution. The diverse ingredients as may be added to Okro in its preparation will determine the chemical composition of the product and to a large extent the microbial flora because the different ingredients contribute immensely to the general microbial load as may be presented. This work has for the first time presented a scientific fact on the microbiological and chemical quality of Okro; it has not presented the diverse variations in the quality of Okro based on different ingredients composition. This will serve as bases for research into this product and thus building of a compendium on the composition of this all-important African dish. Okro as seen from this report is a nutritious food, consumed fresh, without preservatives and thus it is recommended for all age groups. Vegetables (Okro) as seen from this report is a nutritious food, fit for all age groups. Special care however, has to be taken in its preparation as contaminants could be introduced through the preparation.

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