

MAJOR COMPONENT FOR THE ISOLATION AND IDENTIFICATION OF
MICROORGANISMS FOUND IN TOMATO SOIL AT AKWATA GARRIKI

ABSTRACT

*This study was conducted to isolate and identify microorganisms from swamp water samples from the Akwata market in Enugu, where tomatoes were sold. Three wetland water samples, one each at the entrance, middle and end of the Akwata market were collected and analyzed for the presence of bacteria and fungi. The microorganisms were isolated by culturing on Nutrient, MacConkey and Sabouraud glucose agar plates using standard methods. After the incubation period, the number of unit-forming colonies was counted to determine the average total bacterial count. Bacterial isolates were identified based on colony, microscopic and biochemical reactions. Fungal isolates were identified based on their morphological and microscopic features. Wetland water samples taken at the entrance, middle, and edge of the Akwata market had an average total bacterial count of 1.4×10^4 CFU/mL, 2.0×10^4 CFU/mL, and 1.5×10^4 CFU/mL. Fungal counts were 1.6×10^4 CFU/mL, 3.5×10^4 CFU/mL, and 2.4×10^4 CFU/mL, respectively, in swamp water samples collected at the entrance collected at the middle and end of the Akwata market. Bacterial isolates identified were *Escherichia coli* (27.58%), *Pseudomonas sp.* (7.93%), *Klebsiella spp.* (20.7%) and *Salmonella spp.* (13.79%). The identified fungal isolate was *Aspergillus sp.* (38.88%), *Candida sp.* (16.67%), *Fusarium sp.* (27.78) and *Penicillium sp.* (16.67%). This study showed that bacteria and fungi are associated with the swamp water of the Akwata market where tomatoes were sold. Efforts should therefore be made to improve the quality of the water supplied to the Akwata market and thus guarantee the hygienic and sanitary quality of the tomatoes sold.*

Key words: *Microorganisms, microorganisms isolate, Tomato Soil*

INTRODUCTION

“Challenges of tomatoes due to changes associated to climate conditions, use of pesticides against agricultural pests, inadequate rainfall and microorganisms particularly fungi can make tomatoes unsafe to human consumption. One of the limiting factors that influence tomato economic value is its relatively short shelf life caused by pathogen attack” (Chuku et al., 2018; Garg et al., 2013; Kator et al., 2018).

“Spoilage of tomatoes are those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors” (Zdenka et al. 2017). “These may be changes in taste, smell, appearance or texture of the fruits” (Obeng et al., 2018). “Post-harvest handling weak storage practices, transportation and improper marketing, unhygienic marketing environment are seriously affecting the quality of tomatoes” (Lemma et al., 2014). “Fungi were the source of spoilage of most of the tomato samples accessed than bacteria” (Ghosh, 2019). Dimphna (2016) reported that “fungi affecting tomatoes include *Aspergillus phoenicis*, *Absidia spp.*, *Trichoderma spp.*, *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium moniliformis*, *Aspergillus niger*, *Mucor spp.*, *Rhizopus stolonifer*, *Penicillium spp.*, *Geotrichum spp.* and *Phytophthora spp.*”.

“Fungal spoilage of tomatoes has been recognized as a source of potential health hazard to humans and animals due to production of mycotoxins which cause mycotoxicosis in humans following ingestion or inhalation” (Faith et al., 2015). “Superficial areas of tomatoes infections is not limited to mycotoxin. Since tomatoes contain large amount of fluid, these mycotoxins diffuse rapidly throughout them, contaminating all parts and making the fruits unfit for consumption” (Kutama et al., 2017).

“Soil is a critically important component of the earth's biosphere, functioning not only in the production of food and fiber but also in the maintenance of local, regional, and global environmental quality” (Ugwu et al., 2018). “Increasing human populations, decreasing resources, social instability, and environmental degradation pose serious threats to the natural processes that sustain the global ecosphere and life on earth” (Mujib et al., 2017).

“Constitute of microorganism an important source of biodiversity in soils and are an integral part of terrestrial ecosystems” (Etebu et al., 2013). “They contribute to major biological functions such as nutrient and gas cycling, biogeochemical processes and the decomposition and transformation of organic matter” (Fitzpatrick et al., 2020). “Microbial population in soil counts for a huge mass of organic matter on earth” (Obieze et al., 2019). “Importance of microorganism in maintaining human habitat on earth is now beyond a question of discussion. These microorganisms are of very diverse that includes bacteria, archaea, yeast, fungi, algae, and protozoa” (Lemma et al., 2014).

“Microorganisms can live in environment along with human and in extreme conditions such as hot springs, miles deep in the ocean, inside rocks and in extreme cold temperature” (Chuku et al., 2018).

Although the number of microorganisms varies in different places, it has been indicated that mass of carbon from these microorganisms could be trillions of tons.

“The numbers and species of microbes in soil is depended on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and other environmental conditions” (Ibrahim et al., 2019). “Such waste contains organic or biodegradable waste are in large part. Microbes might be responsible for tomato spoilage, as well cause infection to tomato handlers and to identify microbes present in the soil using general microbiological methods Poor waste dumpsites management could create adverse environmental impacts such as wind-blown litter, attraction of rodent and pollutant for example leachates that can contaminate underground soil bed or aquifer” (Dimphna, 2016).

“The microorganisms found in waste dump sites obtain their nutritional requirements from the waste constituents, thus aiding the detoxification of complex organic molecules” (Garg et al., 2013).

Akwata is a specialised market where tomatoes, peppers and other vegetables for cooking can be bought in bulk in Enugu Nigeria. It is currently located at Garriki Awkwunanaw, Enugu South Local Government Area of Enugu State, Nigeria

MATERIALS AND METHOD

Media Used: The media used in carrying out this research work include MacConkey agar, Nutrient agar (NA) and Sabouraud dextrose agar (SDA).

Reagents: Gram stain reagents, lacto phenol cotton blue stain reagents, chloramphenicol. ethanol. Andrade indicator, Peptone water, crystal violet, Safranin, lugol's iodine, normal saline, ethanol, Kovac's reagent, hydrogen peroxide and distilled water.

Other Materials: swampy water samples from Akwata market in Garriki.

Sources of Materials

The materials (swampy water) used for this study were obtained from Akwata market in Garriki Enugu state where tomatoes are sold. All reagents, media, chemicals and equipment used for this study were supplied by the Laboratory of the Department of Applied

Methods

Sterilization of Materials

All glassware such as test tubes, beakers and conical flasks used in this research were washed with detergent, rinsed with water, air dried and sterilized in the hot air oven at 100°C for 60 minutes. Each of the material was wrapped with aluminium foil before sterilization.

Sample Collection

Swampy water samples were collected from the entrance, middle and end of Akwata market in Garriki Enugu state using soil auger to a depth of 2-Sem, the samples were labelled, stored sterile polyethylene bag and transported to the laboratory within 24 hours of collection for microbial enumeration.

Preparation of Media

Preparation of Nutrient agar

The medium was prepared according to the specification of the manufacturer. The Nutrient agar powder (5.6g) was dissolved in 200ml of distilled water, the medium was sterilized in the autoclave at 121°C and 15p.s.i. for 15 min and cooled to 45°C. The medium was dispensed in about 20ml amount into each sterile petri dishes, allowed to solidify and later dried the surface at 30°C in a hot air oven to get rid of moisture prior to inoculation.

Preparation of MacConkey Agar This was prepared according to its specification by the manufacturer by dissolving 7.7g in 200ml of distilled water and sterilized in the autoclave at 121°C and 15p.s.i. for 15 min and cooled to 45°C. The medium was dispensed in about 20ml amount into each sterile petri dish allowed to solidify and later dried the surface at 30°C in a hot air oven to get rid of moisture prior to inoculation.

Preparation of Sabouraud Dextrose Agar

A total of 13g of the powder of Sabouraud Dextrose Agar was suspended in 200ml of distilled water. The medium was made to dissolve by heating while stirring. Thereafter, autoclaved at 121°C at 15psi for 15minutes. The sterilized medium was allowed to cool down to about 45°C. Then, antibacterial agents - chloramphenicol (1 ml) was added and mixed thoroughly. Then the medium was poured into sterile petri dishes 15-20 ml aliquot. The medium was allowed to solidity on the plates and were thereafter, used.

Enumeration of Microorganisms from the Swampy water Samples

One milliliter (1 ml) of each swampy water sample was measured into a conical flask and 9ml of distilled water was mixed with the sample. This was placed in a laboratory shaker (S150) for 3 hours to homogenize the solution and this served as the stock solution. Ten-fold serial dilution of all the homogenized mixture was carried out using sterile distilled water as diluent.

Six test tubes containing 9ml of distilled water was used for the serial dilution.

Spread plate technique was used for inoculation. About one milliliter (1 ml) aliquot of diluted samples (10^{-3} and 10^{-4}) were dropped on Nutrient agar, MacConkey agar, Sabouraud Dextrose agar plates and spread on the surface of the media with a sterile swab stick. Nutrient agar and MacConkey agar plates were incubated at 37°C for 24 hours and the cultures were examined periodically for bacterial growth. The Sabouraud Dextrose agar plates were incubated at 28°C for 2-10 days and the cultures were examined periodically for fungal growth. Colony morphologies were recorded and purified by repeated sub-culturing on freshly prepared Nutrient agar, MacConkey agar and Sabouraud Dextrose agar plates and later subculture on sterile Agar slants to obtain pure colonies for characterization, identification purposes and serving as stock culture also.

Identification of Bacteria Isolates

Gram Staining Procedure

A smear of each colony to be stained was made and heat fixed by carefully passing it through a Bunsen burner three times. The slides were flooded with crystal violet for 60 sec and washed off with water, then each smear again was flooded with iodine solution for 1-minute and washed off with water. Thereafter, the slide was tilted and decolorized with acetone until the solvent draining from the slide appeared colorless and was immediately washed with water. It was counterstained with safranin for 30 sec and washed off with water. The slides were plotted and air dried and observed under oil immersion objectives lens (X100).

Biochemical Analysis to Identify the Bacterial Isolates

Catalase Test (Slide Method)

A drop of hydrogen peroxide was made on one side of clean microscopic slide and a drop of water on the other side as the control. A colony was then collected with a sterile applicator

stick and smeared on the slide containing hydrogen peroxide and was also done for the control. The presence of bubbles within 10 sec which indicated positive result was observed and recorded.

Oxidase Test (Filter Paper Spot Test)

Using the Whatman Number one filter paper method, a strip of filter paper was soaked with a little freshly made 1% of oxidase reagent (NNNN-Tetramethyl-phenylene Diaminedihydrochloride). A distinct colony was then collected with a sterile applicator stick and rubbed on the filter paper that was already containing the reagent. A positive reaction which indicated a deep-purple colouration was observed within 10-60 sec.

Congulase Test (Slide Coagulase Test)

The slide test was performed by preparing a suspension of bacterial cells (a colony of bacteria) mixed into a drop of human plasma on the microscopic slide. The presence of clumping of blood as precipitates on the slide was observed for a positive result.

Indole Test

Peptone water (5ml) was dispensed into test tubes and sterilized at 121°C/15psi for 15 mins. A small amount of pure culture was inoculated into the broth and incubated at 37°C for 24hours after which 3 drops of Kovac's reagent were added directly on the side of the tube while being added (for a more accurate result, Formation of a green coloration which indicates positive result was observed and recorded.

Methyl Red Test

Cultures were inoculated into MR-Vp medium and incubated at 37°C for 2 days. A total of 3 drops of methyl red indicator was then added; a bright red colouration indicated a positive test while a yellow colouration indicated a negative reaction.

Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment five sugars with production of acid and as Peptone powder (15g) was dissolved in 100ml of distilled water with 0.5g of bromothymol blue. Durham tubes were inserted inversely into all the test tubes. Then 9ml of the mixed peptone medium was added to each of the test tubes and sterilized in an autoclave 121°C/15psi for 15 mins. The sugars were prepared by adding 0.5g of the sugars to 5ml of sterile distilled water. The sugar was purified using membrane filter to filter the dissolved sugar solutions. Thereafter, 1 ml of each sterile sugar solution was added into the 9ml of sterile peptone water to form sugar fermentation medium. Subsequently, a loopful of each isolate

was inoculated into the fermentation medium and incubated at 37°C for 24 h. Presence of acid (yellow coloration) and gas (bubbles in Durham tubes) was observed and recorded.

Morphological Identification of Fungi

Morphological identification of fungi was done based on the appearance on the plates: colour pigmentation, mycelia growth, texture, structure, size and shape.

Microscopic Identification of Fungi

The lactophenol cotton blue staining wet mount preparation was used for staining and observing fungal isolates.

Microscopy of Isolates Using Lactophenol Cotton Blue Stain

Two drops of lactophenol cotton blue stain was placed on a clean grease free microscopic slide. Inoculating wire needle was flamed by holding it upright in the hottest part of the Bunsen flame, just above the blue cone until the whole length of the wire glow red hot. Then cooled, and then placed in a fungal culture. Small amount of culture was removed, taking some precautions to avoid dropping the culture on the table. The fungal colonies picked was placed on a clean glass slide containing the two drops of the lactophenol cotton blue stain. The neck of the slide was flamed and the cap replaced. The materials were then pressed out very gently with inoculating needle. Then a clean cover slip was placed over the slide and then pressed firmly but gently with the thumb of the finger to remove excess stain. The excess stain was blot dry using blotting paper. The preparation was allowed to air dry before sealing the edges of the cover slip with colorless nail polish. The preparation was examined using a low power objective lens, X10 magnification then X40 magnification for proper viewing and identification.

RESULTS

Three (3) swampy water samples, one each from the entrance, middle and end of Akwata market where tomatoes are sold were collected and investigated for the presence of bacteria and fungi. From the studies, bacterial and fungal species were prevalent in all the swampy water samples studied. The mean total bacterial counts from the soil samples studied were 1.4×10^4 cfu/ml, 2.0×10^4 cfu/ml, and 1.5×10^4 cfu/mL for samples collected from the entrance, middle and end of Akwata market where tomatoes are sold, respectively while mean total fungal count were 1.6×10^4 cfu/ml, 3.5×10^4 cfu/ml, and 2.4×10^4 cfu/ml for samples collected from the entrance, middle and end of Akwata market where tomatoes are sold, respectively (Table 1). The colony characteristics of the bacteria isolates identified along with their

biochemical, Gram reaction and microscopic examination were recorded in Table 2. The morphological and microscopic characteristics of fungal isolates were recorded in Table 3.

The bacterial isolates identified were *Escherichia coli* (8 - 27.58%), *Pseudomonas sp.* (11-37.93%), *Klebsiella sp.* (6 - 20.7%) and *Salmonella sp.* (4 - 13.79%). The fungal isolates identified were *Aspergillus sp.* (7 - 38.88%), *Candida sp.* (3 -16.67%), *Fusarium sp.* (5 - 27.78%) and *Penicillium sp.* (3 - 16.67%) (Table 4).

Table.1: Mean Microbial Count of the isolates

Samples	Mean of total bacterial counts (cfu/mL)	Mean of total fungal counts (cfu/mL)
A	1.4×10^4	1.6×10^4
B	2.0×10^4	3.5×10^4
C	1.5×10^4	2.4×10^4

A= soil samples from the entrance of Akwata market.

B=soil sample from the middle of Akwata market.

C= soil samples from the end of Akwata market.

Table 2: Morphological, Microscopic and Biochemical Characteristics of Bacterial Isolates

Isolates	<u>Colonial appearance</u>		<u>Biochemical tests</u>							<u>Sugar fermentation</u>				Possible Organisms	
	Nutrient Agar	MacConkey agar	Gram reaction	Cat	Ox	Coa	Mr	Ind	Glu	Fru			La		
										Mal	Man	AG			
A	Grayish white, smooth, mucoid and medium seized	Pink coloured, smooth, medium sized colonies.	Gram -ve short rod in pairs	+ve	-ve	-ve	+ve	+ve	AG	AG	AG	AG	AG	AG	<i>Escherichia coli</i>

	colonies.														
B	Greenish large, opaque, flat colonies with irregular margins.	Greenish flat and smooth colonies with alligator skin-like appearance	Gram -ve short rod in singles	+ve	+ve	-ve	-ve	-ve	AG	A		A	A	-ve	<i>Pseudomonas sp.</i>
C	White coloured mucoid and large sized colonies	Pale pink coloured mucoid and medium sized colonies	Gram -ve short rod in pairs	+ve	+ve	-ve	-ve	-ve	A	A		A	A	A	<i>Klebsiella sp.</i>
D	Opaque or black-centered colonies	NA	Gram -ve short rod in singles	-ve	-ve	-ve	+ve	-ve	A	A		A	A	-ve	<i>Salmonella sp.</i>

Table 3. Morphological and Microscopic characteristics of Fungi isolates

Isolates	colonial appearance	Microscopic appearance	Possible organisms
A	Upper: black middle with White peripheral and Compact wool Reverse: middle is light Brown with peripheral	Large conidial heads, dark brown becoming radiate and split to columns.	<i>Aspergillus sp</i>
B	Upper: dirty white, flat With raised middle Reserve: colony appeared Deep cream	Ovoid in shape and has large blastoconidia	<i>Candida sp</i>

C	Upper: colony appears White, flat with raised/elevated middle Reverse: Light chocolate	Presence of macrospores and microspores. The conidiophore is borne on the macrospore which is septate, cylindrical and moderately Curved while the microspore is Spread randomly, cylindrical to Oval in shape	<i>Fusarium sp</i>
D	Upper: white elevated Colony Reverse: middle is black With light brown peripheral	Septate hyaline hyphae with simple conidiophores. Phalides are grouped in brush-like clusters at the end of the conidiophores. Conidia is unicellular, round to Ovid, pigmented, smooth walled And in chains.	<i>Penicillium Sp</i>

Table 4: Frequency of isolation of microbial isolates

Bacteria isolates	Number of isolates	Frequency of Occurrence (%)
Bacteria		
<i>Escherichia coli</i>	8	27.58
<i>Pseudomonas sp.</i>	11	37.93
<i>Klebsiella sp</i>	6	20.7
<i>Salmonella sp.</i>	4	13.79
Total (%)	29	100
Fungi		

<i>Aspergillus sp.</i>	7	38.88
<i>Candida sp.</i>	3	16.67
<i>Fusarium sp.</i>	5	27.78
<i>Penicillium sp.</i>	3	16.67
Total	18	100

DISCUSSION

The results presented agreed with the search of Mbajiaku and Enya (2014), who reported and concluded that fungus may be the major organism responsible for the spoilage of tomato fruits due to poor sanitation, overcrowding, poor storage, and unhygienic practises by the fruit handlers.

The bacteria isolates identified from swampy water samples at Akwata market where tomatoes are sold were *Escherichia coli*, *Pseudomonas sp.*, *Klebsiella sp.* and *Salmonella sp.* These isolated organisms agree with Wogu and Ofuase (2014). The presence of these organisms in swampy water from Akwata market where tomatoes are sold is an indication that the soil samples were exposed to faecal contaminated water or organic sewage. From all the swampy water samples obtained, 29 bacterial isolates were seen, of which *Pseudomonas sp.* was the most prevalent at 11 (37.93%), followed by *Escherichia coli* 8 (27.58%), *Klebsiella sp.* 6 (20.7%) and *Salmonella sp.* 4 (13.79%).

The fungi isolated in this study were *Aspergillus sp.*, *Candida sp.*, *Fusarium sp.* and *Penicillium sp.* A total of 18 fungi species were isolated. Among the four pathogens identified, *Aspergillus sp.* were found to have highest frequency of occurrence 7 (38.88%). This is to say that *Aspergillus sp.* were the major fungal pathogens associated with the spoilage of tomato fruit sold in Akwata market, which might be due to the presence of all the necessary growing factors such as nutrients, pH, temperature etc. required for this pathogen followed by *Fusarium sp.* 5 (27.78%) then *Candida sp.* and *Penicillium sp.* at 3 (16.67%).

This finding is in conformity with that of Akinmusere (2011), Dimphna (2016), Ghosh (2009), Ogundipe et al. (2018), Wogu and Ofuase (2014) among many other researchers whose revealed in their research that the identified bacterial and fungal pathogens mentioned above were responsible with the spoilage of tomato at different part of the world. The result is similar to the work of Akinmusire (2011) but with variation in the frequency of occurrence. They reported that *Aspergillus sp.* had the highest rate of occurrence of 47.27% in the tomato fruits, while in this present study, *Aspergillus sp.* had the highest rate of occurrence of 38.88%. This may be due to the variation in the market, poor practises or unhygienic practises by the fruit handlers. Wogu and Ofuase (2014) isolated *Aspergillus niger* (47.27%), *Aspergillus flavus* (30.1%), *Penicillium notatum* (15.3%), *Fusarium oxysporum* (12.73%) and *Saccharomyces cerevisiae* (3.64%) from spoilt tomato fruits, which is also similar to the organisms isolated from this present study, whereas, there is variation in the frequency of occurrence. These isolated fungi could probably be due to fungal spores usually found in the environment, their spores can be carried on air, and thus can infect exposed tomato fruits, as well as tomato soils.

The implication of microbial contamination and growth on tomato soil causes spoilage of tomatoes decreased sensory appeal and decreased shelf life, leading to loss and wastage of product that have significant economic consequences.

“The prevalence frequency of occurrence of bacteria was higher than that of fungi which demands that appropriate control measures against infection should be employed. Adequate microbiological knowledge and handling practices of tomato and tomato soil would help minimise wastes due to deterioration. It is therefore, important that both the farmers who harvest the fruits into bags for transportation, the marketers, and consumers take necessary precautions in prevention contamination and eating contaminated tomato fruits. This sill however, enhance reduction in the risk of microbial toxins that are deleterious to human health which are produced from these microorganism that have been isolated from tomato soil. Besides causing huge economic losses, some fungal species could produce toxic metabolites in tomatoes, constituting a potential health hazard for humans” (Bashir *et al.*, 2016).

“Additionally, vegetables have often served as vehicles for pathogenic microorganisms and were implicated in many foodborne illness outbreaks. Therefore, in order to slow down

vegetable spoilage and minimize the associated adverse health effect, great cautions should be taken to follow strict hygiene, good storage, transportation and marketing practices” (Onuorah *et al.*, 2015).

CONCLUSION

This study showed that bacteria and fungi are associated with swampy water from Akwata market where tomatoes are sold. The result showed high mean total microbial counts for all the samples tested. The different bacterial and fungal species identified in this study suggest that microbial contamination on swampy water from Akwata market where tomatoes are sold can be a potential risk to consumers. Such contamination can lead to deterioration of tomatoes, food poisoning and foodborne illnesses. As a result, efforts should be made to discourage purchasing spoilt tomatoes from Akwata market in Enugu as they can predispose vendors and public to infection. It was also revealed that mechanical injuries such as bruises or cuts that occur during harvesting, post-harvesting and packaging could provide infection sites for spoilage pathogens from the swampy water. These infection sites can therefore be greatly reduced and brought to a minimal by proper storage and handling of the tomatoes. The major organisms associated with the spoilage of tomato fruits may be due to poor sanitation, poor packaging and storage, and unhygienic practices by the tomato handlers.

Recommendations

1. Tomatoes must be thoroughly washed with clean water and properly cooked before consumption.
2. Proper cleaning and sanitation of warehouses and disinfection of packaging containers.
3. Proper handling of the tomatoes during harvest should be done to prevent bruises and scars or other mechanical injuries.
4. Implement a monitor plan to improve the quality of water supplied to the Akwata market.

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