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Isolation and identification of spoilage microorganisms from different varieties of tomatoes

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Abstract
Tomatoes is one of the perishable fruits grown in large quantity in Nigeria and the world over. Six different varieties that are mostly grown in Nigeria was studied for the microorganisms responsible for their spoilage using standard microbiological methods. Different media was used to help identify the bacteria and fungi and determine their load. The results showed that cherry tomatoes have the highest bacterial load of 6.9×10³ cfu/g, while the grape and beske tomatoes both have the least bacterial loads of 2.6×10² cfu/g respectively. Beef tomato had the second highest bacterial load of 5.5×10³ cfu/g, while this is closely followed by plum tomato. In all, a total of six (6) bacteria were isolated from the different species of tomatoes used for this assay. They are Enterobacter aerogenes, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis. A total of nine (9) fungi were isolated from the tomato samples and they are Aspergillus Niger, Rhizopus stolonifer, Mucor mucedo, Aspergillus flavus, Fusarium oxysporium, Penicillium oxalicum, Pithomyces sp, Penicillium notatum and Rhizopus oligosporus. The results obtained has shown that spoilt tomatoes habour pathogenic bacteria and fungi which can produce different types of mycotoxins that are toxic to man’s health. Therefore, tomatoes should be preserved properly and spoilt tomatoes should never be consumed. Also, spoilt tomatoes should be disposed properly in order not to endanger the public. Tomatoes to be eaten raw should be washed properly with clean water to avoid consumption of these bacteria and fungi as well as their toxins.

Keywords: tomato, spoilage, bacteria; fungi; microbial load

1. Introduction
In botany, a fruit is a part of a flowering plant that derives from specific tissues of the flower, one or more ovaries, and in some cases accessory tissues. Fruits are the means by which these plants disseminate seeds. A fruit can be defined as the ripened ovary of a flower with or without associated parts. Fruits can be classified into two broad categories; juicy fruits such as tomato, lemon, orange, lime, tangerine and pumelo and pulpy fruits such as mango, pineapple, avocado, pear, guava, pawpaw, sour SOP and banana. (Akalia and Gupta, 2016). Fruits are important in human diets due to their contributions of vital nutrients, most especially vitamin C, they are very low in fats and proteins but high in sugar as they contain large amount of glucose, fructose and sucrose. Other constituents such as organic acids, phenolic substances, volatile substances and minerals may be present and these play an important role in the chemical reactions which occur during processing and storage. In addition, most fruits are often consumed fresh due to their cherished flavour/palatability and they contribute immensely to nutrients intake since no nutrients intake loss are recorded as a result of cooking as in other cooked staple foods. Tomato is a juicy, red fruit eaten raw mostly in salad or cooked as a vegetable. Tomatoes are often considered a vegetable, though in actuality they are a citrus fruit. Tomatoes are an incredibly versatile food. They are delicious eaten raw, in salads or on sandwiches, and take on a wonderful sweetness when cooked. Their high acid content makes them a perfect food for canning. Tomatoes are such an important part of the American diet that it’s hard to believe that they were once considered toxic. It wasn’t until the mid1800’s that they became a staple food in the U.S. (Beuchart, 2008).

One medium whole tomato contains around 22 calories, 0 grams of fat, 5 grams of carbohydrates, 1 gram of dietary fiber, 1 gram of protein and 6 milligrams of sodium. It also provides 40 percent of the recommended daily allowance of vitamin C, 20 percent of the RDA of vitamin A, 2 percent of the RDA of iron, and 1 percent of the RDA of calcium. Here are some of the health benefits of tomatoes (Freeman and Reimers, 2011) [11].

1. Ward off Cancer
Numerous studies have concluded that the more tomatoes people eat the lower their risks of certain cancers, especially lung, stomach and prostate cancers. A substance called lycopene, which is responsible for tomatoes red color, is thought to be the reason for this cancer protective effect. Processed tomatoes contain even more lycopene than raw ones. The process of cooking breaks down the cell walls, helping to release the lycopene. Eating tomatoes with a little bit of fat, such as olive oil, helps lycopene to be better absorbed by the body (Freeman and Reimers, 2011) [10].

2. Prevent DNA Damage
Tomatoes are high in important antioxidants such as vitamin C and Vitamin A. These vitamins work to fend of DNA damage from free radicals. Consequently, tomatoes may help to ward off age related diseases such as atherosclerosis and diabetes (De Rover, 2018).

3. Reduce the Risk of Heart Disease
Tomatoes contain important nutrients, such as niacin, folate and vitamin B6 that have associated with the reduction of heart disease risk. One study found that women who ate 7 to 10 servings of tomato products per week had a 29 percent lower risk of cardiovascular disease than women who
consumed less than a serving and a half of tomato products each week. Results were even more impressive when the women ate oil-rich tomato products (DE Rover, 2018).

4. Protect Against Thrombosis
Another study showed that drinking 8 ounces of tomato juice daily reduced platelet aggregation significantly, among study subjects. Those drinking a placebo showed no benefit. It’s important to drink low-sodium tomato juice if you are trying to protect against thrombosis (blood clots in the blood vessel), as high sodium levels can cause negative effects for this type of disease (Mariga 2012) [17].

5. Ward off Inflammation
A double blind study found that drinking a glass of tomato juice a day can reduce blood levels of TNF-alpha by 34 percent. TNF-alpha causes inflammation. High levels have been found in individuals with most chronic, degenerative diseases such as heart disease, cancer, osteoporosis and Alzheimer’s (Freeman and Reimers, 2011) [16].

Generally, tomatoes are well known for the following health benefits:
- Tomatoes helps in managing blood pressure; Tomato can help in the management of high blood pressure.
- Tomatoes helps to maintain a healthy hair; Tomatoes are good for the hair; the vitamin A in tomato helps in making the hair strong and shiny. Tomatoes are good for the skin; the high level of lycopene in tomato makes the skin to look tender, shiny and smooth. And sometimes, it can also be used as a facial cleanser.
- Tomatoes helps to prevent cancer. The outstanding thing about tomatoes is its ability to prevent cancers. Lycopene is a natural antioxidant that works effectively to slow the growth of cancerous cells so therefore, cooked tomatoes produce even more lycopene which can aid to fight against cancers.
- Tomatoes repairs cigarettes damages; this amazing fruit contain coumaric acid and chlorogenic acid that work to protect the body from carcinogens that are produced from cigarette smoke. So if you know someone who is a heavy smoker; you might want to recommend this fruits to him/her. Tomatoes keeps the kidney in shape; Tomatoes helps to keep the kidney health; thus helps in preventing kidney stone. Tomato is good for the eyes and perfect checker for sugar level; The Vitamin A found in tomatoes is a perfect remedy for improving vision and also prevent night blindness. Tomato can also help diabetes patient to keep their blood sugar levels under better control. Tomato provides essential antioxidants for the body; the Vitamin A and Vitamin C in tomatoes serve as antioxidants and this is because these vitamins and beta-carotene work as antioxidants to neutralize harmful free radicals in the blood.
- Tomatoes help to maintain strong bones; Tomatoes contain a considerable amount of calcium and Vitamin K. These nutrients are usually known for maintaining strong bones (Freeman and Reimers, 2011) [16]. There are several varieties of tomatoes grown in Nigeria. They are quite numerous in types. It is always advisable to make sure that you choose tomatoes plants based on your requirements or needs and/or preferences. The type of soil and the region can determine productivity of commercial tomatoes production, that is why, you are permitted to experiment your choice of tomatoes varieties before you commence your tomatoes production business. Yes, tomatoes come different varieties and types. Below are the descriptions of some of these common varieties and types of tomatoes according to Baiyewu et al, (2017) [8].

- Beefsteak Tomatoes. One of the varieties and types of tomatoes grown in Nigeria is called, beefsteak tomatoes.
- Common Beefsteak Tomatoes. Common varieties and types of beefsteak tomatoes are:-
  - Cherry Tomatoes.
  - Grape Tomatoes.
  - Plum Tomatoes.
  - Campari Tomatoes.
  - Patio Tomatoes.
  - Brandywine Tomatoes.

Materials and Methods
The materials used in this work include conical flask, Petri-dishes, test tubes, McCartney bottles, measuring cylinder, thermometer, sterile container for sample collection, foil paper, ethanol, distilled water, cotton wool, paper tape, agar mediums, tomato samples and slides.

Sample collection
All the tomato samples used (different varieties of tomatoes) were obtained in their fresh forms at Sha-sha market (Akure-Owo road) and taken to the Microbiology laboratory of the Elizade University, Ilara-Mokin, Ondo State. Their barks were washed with sterile distilled water before carrying out further Laboratory analysis on them as counselled by Prescott et al., (2018) [21].

Sterilization of glass wares
All the glass ware used such as conical flask, petridishes, measuring cylinder, McCartney bottles and test tubes were properly cleaned and sterilized using oven at a temperature of 180°C for 2 hours (Fawole and Oso, 2004).

Isolation of bacteria and fungi from tomato samples
The different species / varieties of fresh tomatoes were allowed to stay for a period of 3-5 days when spoilage was noticed.

One gram of the samples were aseptically cut into 9 ml of sterile distilled water and mixed thoroughly before serially diluting to power 3.

One ml of the serially diluted specimen was then used for pour plate technique in enumerating the microbial load of the samples, while streaking method was used alongside the pour plate to isolate the microbial type in the samples as described by Cheesbrough (2014).

Sub culturing
Distinct colonies of bacteria and fungi were picked using sterile inoculating loop these was streaked onto the surface of the prepared nutrient agar plate to obtain pure isolates for further characterization and identification of the organism (Baker et al., 2016).

Preservation of culture
Nutrient agar powder was dissolved in distilled water according to the manufacturer specification to prepare a double strength agar. 10ml of the dissolved agar were dispensed into each bijou bottles and screwed tight for sterilization in an autoclave at 121°C for 15 minutes, after sterilization the agar was allowed to set in a slanting position, with sterile inoculating loop, a loop full of the pure inoculum is streaked on the surface of the slant agar aseptically (Baker et al., 2016).
Preparation of Nutrient broth

Nutrient broth powder was weighed into a conical flask and appropriate water was added according to the manufacturer specification and 10 ml was dispensed into each capped test tube. The test tubes were sterilized using the autoclave at 121°C for 15 minutes. They were allowed to cool and the pure cultures of the isolated organisms were inoculated into the tubes, these were incubated at 37°C for 24 hours (Cheesbrough 2014).

Gram’s reaction

A loopful of the culture was transferred onto the surface of a clean grease free sterile glass slide already containing a loopful of distilled water, it was emulsified, air dried, and heat fixed. The smear was stained with crystal violet solution for 1 minute and rinsed with distilled water. Gram’s iodine solution was added to the smear and left for 1 minute, it was rinsed with distilled water and drained. 70% ethanol was used to decolorize the smear for 30 seconds, rinsed with distilled water and drained. The smear was counterstained with safranin, rinsed with distilled water, drained and allowed to air dry. One to two drop of oil immersion was applied on the slide and these were viewed under X100 objective lens. Gram positive organisms appeared under the microscope as purple while the gram negative organisms appeared red. Morphology of the organisms was determined with cocci appearing circular and bacilli having rod shape (Prescott et al., 2018) (21).

Catalase test

A loopful of the culture was placed on a clean grease free slide containing a drop of 3 % hydrogen peroxide these was mixed together, presence of effervescences on the slide indicate positive result and absence indicate negative result (Prescott et al., 2018) (21).

Motility test

The hanging drop method is used for the demonstrating the motility of microorganism due to flagella. A little Vaseline was placed around the edge of the hollow in a clean cavity slide, a loopful of broth culture of the test organism was placed in the centre of a clean grease free cavity slide covered with cover slip in such a way that the drop is in the center of the cavity, the cavity slide was carefully inverted so that the drop of culture lies in a hanging position and the preparation was observed under immediately under the microscope, the movement of bacteria in different directions indicates a positive which shows the bacteria is motile (Baker et al., 2016).

Indole production test

Some bacteria are capable of hydrolyzing the amino acid tryptophan and one of the products is indole. The ability of a microbe to carry out this reaction can be used for biochemical characterization. The tryptone in the culture medium supplies the tryptophan. Two different tubes of 1 percent tryptone broth were inoculated with the bacterium and incubated at 35°C for 18 hours, another tube was left un-inoculated to serve as control was added and was shaken gently as well. The test tube was well returned to the rack and allowed to stand for 20 minutes. This is to permit the reagent to rise to the top. A red color at the reagent layer indicates indole production (Baker et al., 2016).

Coagulase test

A drop of distilled water each was placed on two clean grease free slides, an inoculum of test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma (plasma was collected from Elizade University health centre) was added to one of the suspension and was mixed gently and was observed for clumping after 10 seconds. No plasma was added to the other suspension, as it was used as control to differentiate any granular appearance of the bacteria from true coagulase clumping. The presence of clumping within ten seconds indicates a coagulase positive reaction while the absence of clump indicates a negative coagulase reaction. Clumps or precipitate in the mixture indicates a positive coagulase test. This shows that the bacteria produces coagulase enzyme (Baker et al., 2016).

Fermentation of sugar

Different types of sugar were used such as lactose, mannitol, arabinose, maltose, glucose, and fructose. 1g of each sugar was weighed into different conical flask and labeled accordingly into each flask; 1g of peptone was added and made up to 100 ml with distilled water. 0.01g of phenol red was added as an indicator. About 5 ml each of the 100 ml sugar solution was dispensed into different test tubes with Durham tubes inserted into each in an inverted form. The tubes were labeled appropriately with their mouth plugged with cotton wool and sterilized in an autoclave for 15 minutes at 121°C. These tubes were allowed to cool down before inoculation. Bacterial isolates were inoculated into the sugar solution inside the test tubes and incubated at 37°C for 72 hours. After incubation, the tubes were observed for acid production by a change in color from red to yellow, the change tubes were compare with the control to ascertain any color change, tubes were also examined for accumulation of space in the inverted Durham tubes. The presence of space indicates the production of gas as a result of utilization of the sugar by the inoculated organism which brought about color change (Cowan, 2018).

Methyl red test

Each of the bacterium was aseptically inoculated into appropriately labeled and sterile nutrient broth medium, using a sterile inculcating loop, the tubes were incubated at 37°C for 24 hours, after incubation, 5 drops of methyl red indicator was added to the culture broth. The tubes were then examined for color change, the formation of red color is a positive test while the formation of yellow color is a negative test (Baker et al., 2016).

Citrate utilization test

The coliform bacteria may be differentiated by their ability to utilized citrate as a sole carbon source. Simmon’s citrate medium was prepared and poured into McCartney bottles and sterilized. These were slant and allowed to cool before inoculation with the test organism, incubated at 37°C for 2-5 days. The bottles were examined for color change from green to deep blue (Baker et al., 2016).

Spore staining test

A heat fixed smear of isolate on a slide was flooded with 5% aqueous malachite green and steamed on a water bath for 5 minutes to enhance the penetration of the dye for the
results were then washed under tap and counter stained with 0.5% aqueous safranine for 20 seconds. It was then rinsed with water, air dried and then viewed using the oil immersion objective microscope (Baker et al., 2016).

Statistical analysis of result
Result obtain will be subjected to descriptive one way analyses of variance, SPSS version 21 Microsoft windows 8.1 and Duncan multiple range test will be used as follow up test (Omoya and Momoh, 2019).

Results
The results of the bacterial load of the different species of tomatoes used for this assay is shown in table 1. Cherry tomatoes have the highest bacterial load of 6.9×10^4 cfu/g, while the grape and beske tomatoes both have the least bacterial loads of 2.6×10^4 cfu/g respectively. Beef tomato had the second highest bacterial load of 5.5×10^4 cfu/g, while this is closely followed by tomato with plum tomato. In all, a total of six (6) bacteria were isolated from the different species of tomatoes used for this assay. They are Enterobacter aerogenes, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis. Of these six bacteria, all the six were found in the cherry tomato, while the five were isolated from the beef tomato. The five bacteria are Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis. The other four tomato species all had four bacteria isolated from them varying different types in the above not found in them.

The fungal isolation from these tomatoes also followed the same pattern of result obtained for the bacterial isolation. The beef tomato had the highest fungal load of 5.0×10^3 spf/g, while grape tomato had the least fungal load 1.0×10^3 spf/g. This is shown in table 2. A total of nine (9) fungi were isolated from the tomato samples and they are Aspergillus Niger, Rhizopus stolonifer, Mucor mucedo, Aspergillus flavus, Fusarium oxysporium, Penicillium oxalicum, Pithomyces sp, Penicillium notatum and Rhizopus oligosporus.

Table 1: Bacteria load and types isolated from different varieties of tomato samples

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Sample identity</th>
<th>Bacterial load (cfu/g)</th>
<th>Bacterial type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cherry tomato</td>
<td>6.9x10^4</td>
<td>Enterobacter aerogenes, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis</td>
</tr>
<tr>
<td>2</td>
<td>Grape tomato</td>
<td>2.6x10^4</td>
<td>Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter aerogenes and Escherichia coli</td>
</tr>
<tr>
<td>3</td>
<td>Beef tomato</td>
<td>5.5x10^4</td>
<td>Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis</td>
</tr>
<tr>
<td>4</td>
<td>Beefsteak tomato</td>
<td>2.9x10^4</td>
<td>Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus</td>
</tr>
<tr>
<td>5</td>
<td>Beske tomato</td>
<td>2.6x10^4</td>
<td>Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus</td>
</tr>
<tr>
<td>6</td>
<td>Plum tomato</td>
<td>3.2x10^4</td>
<td>Pseudomonas aeruginosa, Enterobacter aerogenes, Bacillus subtilis and Escherichia coli</td>
</tr>
</tbody>
</table>

Table 2: Fungal load and types isolated from different varieties of tomato samples

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Sample identity</th>
<th>Fungal load (spf/g)</th>
<th>Fungal type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cherry tomato</td>
<td>3.0x10^3</td>
<td>Aspergillus niger, Rhizopus stolonifer, Mucor mucedo, Aspergillus flavus</td>
</tr>
<tr>
<td>2</td>
<td>Grape tomato</td>
<td>1.0x10^3</td>
<td>Fusarium oxysporium, Aspergillus niger, Rhizopus stolonifer</td>
</tr>
<tr>
<td>3</td>
<td>Beef tomato</td>
<td>5.0x10^3</td>
<td>Penicillium oxalicum, Fusarium oxysporium, Aspergillus niger, Aspergillus flavus</td>
</tr>
<tr>
<td>4</td>
<td>Beefsteak tomato</td>
<td>2.0x10^3</td>
<td>Pithomyces sp, Penicillium notatum, Rhizopus oligosporus, Aspergillus niger</td>
</tr>
<tr>
<td>5</td>
<td>Beske tomato</td>
<td>2.6x10^3</td>
<td>Mucor mucedo, Fusarium oxysporium, Aspergillus niger</td>
</tr>
<tr>
<td>6</td>
<td>Plum tomato</td>
<td>3.2x10^3</td>
<td>Rhizopus stolonifer, Fusarium oxysporium, Aspergillus niger</td>
</tr>
</tbody>
</table>

Table 3a: Morphological characteristics of bacterial isolates from different varieties of tomato

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>Pigmentation/color</th>
<th>Shape</th>
<th>Edge</th>
<th>Optical characterized</th>
<th>Consistency</th>
<th>Colony surface</th>
<th>Spore formation</th>
<th>Gram’s reaction</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White</td>
<td>Circular</td>
<td>Entire</td>
<td>Translucent</td>
<td>Butyrous</td>
<td>Smooth</td>
<td>Negative</td>
<td>-ve rod</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>White</td>
<td>Irregular</td>
<td>Lobate</td>
<td>Translucent</td>
<td>Viscid</td>
<td>Smooth</td>
<td>Negative</td>
<td>-ve rod</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Milky white</td>
<td>Circular</td>
<td>Entire</td>
<td>Opaque</td>
<td>Butyrous</td>
<td>Smooth</td>
<td>Positive</td>
<td>+ve rod</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Green</td>
<td>Circular</td>
<td>Entire</td>
<td>Translucent</td>
<td>Butyrous</td>
<td>Smooth</td>
<td>Negative</td>
<td>-ve rods</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>White</td>
<td>Irregular</td>
<td>Entire</td>
<td>Translucent</td>
<td>Swamy</td>
<td>Smooth</td>
<td>Negative</td>
<td>-ve rod</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3b: Biochemical characteristics of bacterial isolates from different varieties of tomato

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>Cat</th>
<th>Oxi</th>
<th>Ind</th>
<th>H2S</th>
<th>Nit red</th>
<th>Ure</th>
<th>Lact</th>
<th>Fruc</th>
<th>Malt</th>
<th>Gala</th>
<th>Glu</th>
<th>Arab</th>
<th>Raf</th>
<th>Man</th>
<th>MR</th>
<th>VP</th>
<th>Identified organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Enterobacter</td>
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<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Escherichia coli</td>
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<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Proteus vulgaris</td>
</tr>
</tbody>
</table>
Table 4: Identified fungal isolates characteristics

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Cultural Characteristics</th>
<th>Arrangement of Spores</th>
<th>Probable Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White base with black conidiophores</td>
<td>Conidia heads radiate, conidiophores stripes smooth wall. Conidia are 1-celled and vesicles globose</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>2</td>
<td>White base with yellowish green color in appearance</td>
<td>Conidia heads radiate and typically vesicles globose, surface contain many flask shaped phialides and chains of conidia. Hyphae septate, no collumella</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>3</td>
<td>White base with brown conidiophores which makes it brown in color</td>
<td>An upright conidiophores that terminate in a clarate swelling, bearing phialides at the apex. Conidia are 1-celled and vesicles globose. Hyphae septate, non-collumella</td>
<td>Phytomyces sp</td>
</tr>
<tr>
<td>4</td>
<td>White cotton like mycelia turning dirty with development of block spores.</td>
<td>Non-septate hyphae and coenocytic thin sporangiophores. Sporangium has well developed collumella which is umbrella-like in form. Spores are of various shapes but generally oval.</td>
<td>Rhizopus stolonifer</td>
</tr>
<tr>
<td>5</td>
<td>Fluffy white in appearance which grows rapidly</td>
<td>Mycelium extensive, with some tinge of yellow, conidiophores variable, slender and simple.</td>
<td>Fusarium oxysporium</td>
</tr>
<tr>
<td>6</td>
<td>Yellow base with black conidiophores</td>
<td>Conidia heads radiate, conidiophores stripes smooth wall. Conidia are 1-celled and vesicles globose</td>
<td>Mucor mucedo</td>
</tr>
<tr>
<td>7</td>
<td>Blue base with yellowish green color in appearance</td>
<td>Conidia heads radiate and typically vesicles globose, surface contain many flask shaped phialides and chains of conidia. Hyphae septate, no collumella</td>
<td>Penicillium notatum</td>
</tr>
<tr>
<td>8</td>
<td>Green base with brown conidiophores which makes it brown in color</td>
<td>An upright conidiophores that terminate in a clarate swelling, bearing phialides at the apex. Conidia are 1-celled and vesicles globose. Hyphae septate, non-collumella</td>
<td>Penicillium oxalicum</td>
</tr>
<tr>
<td>9</td>
<td>Yellow-green base with yellowish green color in appearance</td>
<td>Conidia heads radiate and typically vesicles globose, surface contain many flask shaped phialides and chains of conidia. Hyphae septate, no collumella</td>
<td>Rhizopus oligosporus</td>
</tr>
</tbody>
</table>

Discussion

Tomato has long been known as one of the perishable fruits that is classified under berries. Because of the juicy nature of it, it is capable of harbouring an array of microbes; especially during deterioration (Prescott et al., 2018) [21]. Many factors are responsible for this; among which are the fact that the tomato contains all the necessary ingredients that aid the proper growth of microorganisms in general (Nester et al., 2018). From the results obtained in this study, the amount of juice present in a particular type or variety of tomato can be seen to favour the microbial load of bacteria isolated from the different varieties that was used.

The results of the microbial loads from the soil samples showed that the tomato samples contain different bacterial and fungal flora. These bacteria are mostly the non-pathogenic species and few pathogenic bacterial species, while others are opportunistic pathogens. According to Alo et al., (2013) [19], most of the pathogenic bacteria, especially the enteric pathogens are mostly from contaminated soil. The fungal isolates from the tomato samples are mostly fungi that are involved in the production of mycotoxins of different types. For example, Aspergillus flavus produces aflatoxin, a very poisonous lethal toxin in cereals and fruits. (Al-Yemeni and Hashem, 2016) [9], the presence of pathogenic bacteria such as Pseudomonas calls for attention as it poses danger to the health of the indigenes that consumes the tomatoes directly or indirectly. Some of these microorganisms are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, urinary tract infections and others (Nwidu et al., 2018) [10]. They are known as causative agents of many water and food borne diseases and they may indicates that these tomatoes must be properly washed before use such as in preparation of salad and raw consumption. Cherry tomatoes have the highest bacterial load of 6.9×10^7 cfu/g and this result is similar to the result obtained by Ajayi (2013) [1] when he isolated from cherry tomatoes used for fruit salad in Eastern part of Nigeria. The grape and beske tomatoes both have the least bacterial loads in these research. According to Buck et al., (2003), these two species of tomatoes share a lot of characteristics which include their sour taste and high alkaline nature. Wogu and Ofuse (2014) are of the opinion that the beske species of tomato is just the local version of grape tomatoes. Therefore, the similarities in their microbial load and microbial types may be due to their similarity and characteristics.

Ogundipe et al., (2014) [20] describes the bacteria often isolated from tomatoes and more of the enterics and Mariga (2012) [17] initially gave the reason for this to be due to the fact that most of these bacteria accessed tomatoes either from the soil or from the water used to wash the tomatoes after the harvest. The Gram negative bacteria isolated from these different species of tomatoes are all enterobacteriaceae. Therefore, this result is in agreement with the result of these researchers. The fungi isolated are those that have been implicated in different varieties of fruits due to their ability to survive in adverse environmental condition. They are known to be capable of producing different toxins to aid their extracellular production of enzymes to digest their substrate before assimilating the nutrients. Aspergillus flavus and Fusarium oxysporium for instance are known to produce different mycotoxins of class aflatoxins and ochratoxins respectively which poses danger in consumption of spoilt tomatoes. Therefore, based on the results obtained in this research, both Gram positive and Gram negative bacteria as well as different species of fungi are involved in the spoilage of tomatoes. However, the types of these bacteria and fungi are influenced by the type of species of the tomato. Therefore, tomatoes should be kept properly and once spoilage is noticed, the tomato should be discarded properly to avoid ingestion of mycotoxins. The results obtained has shown that spoilt tomatoes harbour pathogenic bacteria and fungi which can produce different types of mycotoxins that are toxic to man’s health. Therefore, tomatoes should be preserved properly and spoilt tomatoes should never be consumed. I therefore recommend that spoilt tomatoes should be disposed properly and never be consumed. Tomatoes to be eaten raw should be washed properly with clean water to avoid consumption of these bacteria and fungi.

Discussion

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