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Isolation and identification of bacteria from exposed and sliced *Allium cepa* (onions)

Abstract

Background and objective: Onions (*Allium cepa*) are the one of the most important commercial condiments grown and consumed globally. They, are perennials that can be used as a medicine and food. Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs, and sore throat. It is also rich in proteins, carbohydrates, sodium, potassium, and phosphorus. During storage, some losses occur due to sprouting, drying and rotting that can be caused by some microorganisms particularly fungi. The black mould disease caused by *Aspergillus niger* is an issue of severe concern in onion production worldwide. This study was aimed at isolating and identifying bacteria on sliced onions *(Allium cepa*) exposed to air for three days.

Materials and methods: Three Onion samples were collected from the General Market Tombia, Yenagoa, Bayelsa. The respective onion samples were aseptically peeled, sliced and exposed one hour , after which, 1g of onion was weighed and the extract was serially diluted. Following serial dilution, it was inoculated on nutrient agar and MacConkey agar separately and incubated at 370C for the next 24 hours. The same protocol was followed after peeling, slicing an exposing the onions for 48hrs and 72hrs. Gram staining and biochemical tests (catalase test, coagulase test, blood agar, mannitol salt agar, and bile solubility test) was carried out for each isolates for proper identification.

Results: Sixteen bacterial isolates were obtained out of which streptococci was found to be the dominant species. Gram staining and biochemical tests have further revealed the hidden insights for cohesive identification. The study confirmed the ten(10) isolates of Coagulase Negative Streptococci that accounted to (63%), four (4) isolates *Staphylococcus aureus* accounting to(25%), one (1) isolate of *Streptococcus pneumoniae* and *Streptococcus pyogenes* accounting to (6%). All were gram positive; 12 were gram positive cocci and 4 was gram positive bacilli. This could be because gram-positive bacteria are more tolerant to dry conditions than gram-negative bacteria, whose growth could be reduced when a surface is drying. The highest total colony count after 24, 48 and 72 hours incubation 1.24×10^{10}cfu/ml, 3.22×10^{10}cfu/ml and 3.31 x 10^{11}cfu/ml respectively.

Conclusion: Findings from this study revealed that some pathogenic gram positive bacteria-can be isolated from onions when peeled and left exposed on kitchen cabinets or other containers. Thus the practice of slicing, onions to air and keeping for days before usage is not hygienic and should be avoided.

Keywords: *Allium cepa*, coagulase negative streptococci, *Staphylococcus aureus*, *gram positive*

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Introduction

Onion is one of the oldest cultivated crops and considered as a food of exceptional value for flavoring and seasoning. The green stems and leaves are hollow and can reach 3 ft. (1m) in height. The plants bear small flowers that are usually white or purple. The leaves of the plant are long, linear, hollow, and cylindrical. Thickening of the leaf bases forms a bulb, when the plant reaches a certain stage of growth.[1]

Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs, and sore throat. It is rich in proteins, carbohydrates, sodium, potassium, and phosphorus. It is among the oldest cultivated plants used both as a food and medicine .[2,3] Onions are rich in an ample variety of secondary metabolites such as terpenoids, flavonoids, tannins, and alkaloids which are known to exhibit antimicrobial activities.[4]

Chemicals have been traditionally used to protect food from pathogens and spoilage organisms, but in recent times there has been an increase in consumer interest in developing onion which contain a low level or free of chemical preservatives.[5] Bulb rots which is a common cause of onion loss during storage are caused by microorganisms particularly fungi. The black mould disease caused by *Aspergillus Niger* is a limiting factor in onion production worldwide.[6] *Aspergillus Niger* can survive on onion crops as a soil saprophyte or on bulbs in field or storage and is ubiquitous in nature. It invades the bulbs of onions in field or during storage due to the production of various enzymes or toxins.[7] *Aspergillus Niger* infection in onion accounts cause 30-80% loss or spoilage of onion bulbs.[8]

Most vegetables are consumed raw to retain the natural taste and heat labile nutrients. However, these raw vegetables shown to harbor pathogenic bacteria, which is of a serious health concern.[9,10] Consumption of raw vegetables and fruits without washing increases the risk of disease transmission.[11-14] Vegetables like cabbage can become contaminated with pathogenic micro-organisms whilst growing in fields, or during harvesting, postharvest handling, processing and distribution or even at home.[15-18]

Contamination may be during handling, transportation, storage or due to unhealthy surroundings. Fresh vegetables with huge loads of microbial contaminants may begin to spoil if not sold. However,

adequate cleaning processes can reduce the microbial load.[19] Several studies have revealed the role of microorganisms in spoiling fruits and vegetables which could be due to poor pre-harvest and post-harvest practices.[20] Irrigating with poor quality water and the use of raw animal manure as fertilizer can increase the possibilities of the vegetables spoilage. Some common isolates includes *Listeria monocytogenes, Salmonella* and *Escherichia coli* have been found on various produce including lettuce, cabbage and tomatoes, while *Pseudomonas sp*., *Staphylococcus sp. and Erwinia sp.,* were identified on onions.[21]

Materials and methods

Study area

This study was carried out in Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa state.

Sample collection

Three Onion samples were collected from the General Market Tombia, Yenagoa, Bayelsa state. The collected samples include: one white onion and two purple onions.

Sample isolation of bacteria

The respective Onion samples were peeled, sliced and exposed aseptically for one hour, after which, 1g of the onion was weighed and transferred into l0ml of distilled water and mixed. A serial dilution was done to obtain up to 1 in 10 dilution (10^{-10}) , then 0.1ml of 10^{-7}, 10^{-8}, 10^{-9} dilutions were inoculated on a nutrient agar and MacConkey agar separately and with the aid of spreader; to obtain an even distribution of the microorganisms on the agar plates, incubated at 37^{0}C for the next 24 hours. The same protocol was followed after peeling, slicing an exposing the onions to air for 48hrs and 72hrs.

Identification of bacteria

Colonial morhology, gram staining and biochemical test

After 24hours incubation, the colonies were identified based on their morphological appearance on the plate. The selected colonies were then sub-cultured onto a nutrient agar and MacConkey agar plates and nutrient agar slants,slope, followed by Gram staining.[22]

Biochemical tests such as catalase test, coagulase and bile solubility test was carried out for each isolates for proper identification. Isolates were also cultured ion blood agar (and incubated at 37°C for 24hours, anaerobically) and mannitol salt agar to aid in the identification.[22]

Blood agar was prepared according to the manufacturer's instructions aseptically. The isolates were streaked with a sterile wire loop on the blood agar and incubated at 37^{o}C for 24hours, anaerobically.

Results

Table 1 shows the colonial morphology of the isolates on nutrient agar and MacConkey agar and the gram reactions. Out of the 16 isolates, all were gram positive; 12 were gram positive cocci and 4 was gram positive bacilli. The isolates colony colour and size (mm) were noted for the colonial morphology.

Table 2 shows the total colony counts and their varying dilution factors. The total colony count from Plate A (1.0E-09 dilution) was 1.24×10^{10}cfu/ml, (1.0E-08 dilution) had 1.24×10^{10} cfu/ml, and (1.0E-07 dilution) had 3.64×10^{10}cfu/ml. while Plate B (1.0E-09 dilution) had 2.22×10^{10}cfu/ml, (1.0E-08 dilution) had 2.78×10^{10}cfu/ml, and (1.0E-07dilution) had 3.22×10^{10}cfu/ml. Plate C (1.0E-09 dilution) had 4.00×10^{10}cfu/ml, (1.0E-8 dilution) had 3.31 x 10^{11}cfu/ml, and (1.0E-07 dilution) had 3.31×10^{11}cfu/ml.

Table 1 Gram staining reaction and colony colour/size

Isolate	Gram reaction	Colony colour	Size of colony
A1	Gram positive cocci	Cream	0.5
A2	Gram positive cocci	Light pink	0.25
A3	Gram positive cocci	Cream	0.35
A4	Gram positive cocci	Cream	0.05
A5	Gram positive cocci	Pink	0.2
A6	Gram positive cocci	Cream	0.15
A7	Gram positive bacilli	Cream	0.5
B8	Gram positive cocci	Light pink	0.2
B9	Gram positive bacilli	Cream	0.25
B10	Gram positive bacilli	Cream	0.2
B11	Gram positive cocci	Light pink	0.2
B12	Gram positive bacilli	Cream	0.2
C13	Gram positive cocci	Cream	0.2
C14	Gram positive cocci	Pink	0.1
C15	Gram positive cocci	Cream	0.1
C16	Gram positive cocci	Pink	0.2

KEY: **A, B** and **C** indicate isolate incubated for 24, 48 and 72hrs respectively.

Table 2 Total colony count

Nutrient agar plate	Dilution factor		
	1.0E-09	1.0E-08	1.0E-07
A	1.24×10^{10}cfu/ml	1.24×10^{10}cfu/ml	3.64×10^{10}cfu/ml
B	2.22×10^{10}cfu/ml	2.78×10^{10}cfu/ml	3.22×10^{10}cfu/ml
C	4.00×10^{10}cfu/ml	3.31×10^{11}cfu/ml	3.31×10^{11}cfu/ml

KEY: **A**, B and C indicates incubation for 24, 48 and 72hrs respectively.

Table 3 shows the biochemical test reaction of each isolates using catalase test, coagulase test, blood agar, mannitol salt agar, and bile solubility test to identify the various isolates. Isolates A1, A2, A3, A4, A5, B8, B9, B11, C14 and C16 are suspected to be Coagulase negative staphylococci. Isolates B10, B12, C13 and C15 are suspected to be *Staphylococcus aureus*, while isolates A6 and A7 are suspected to be *Streptococcus pyogenes* and *Streptococcus pneumoniae* respectively.

Table 3 Biochemical test reaction of Isolates

Isolate	Catalase	Coagulase	Blood agar	Mannitol Salt Agar	Bile Solubility Test	Result
A1	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
A2	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
A3	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
A4	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
A5	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
A6	-	-	Alpha haemolysis	No growth	-	*S. pyogenes*
A7	+	+	Beta haemolysis	No growth	+	*S. pneumoniae*
B8	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph

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Table Continued...

Isolate	Catalase	Coagulase	Blood agar	Mannitol Salt Agar	Bile Solubility Test	Result
B9	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
B10	+	+	Beta haemolysis	Growth	N/A	*S. aureus*
B11	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
B12	+	+	Beta haemolysis	Growth	N/A	*S. aureus*
C13	+	+	Beta haemolysis	Growth	N/A	*S. aureus*
C14	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph
C15	+	+	Beta haemolysis	Growth	N/A	*S. aureus*
C16	+	-	No haemolysis	Growth	N/A	Coagulase Negative Staph

KEY: +, positive; -, Negative; *S. aureus, Staphylococcus aureus; S. pneumoniae, Streptococcus pneumoniae; S. pyogenes, Streptococcus pyogenes;* N/A, not applicable; coagulase negative staphylococcus

Discussion

A total of 16 isolates were isolated and identified from the exposed and sliced onion samples on the nutrient and MacConkey and all the isolates were found to be gram positive; orgaisms. 12 were gram positive cocci and 4 were gram positive bacilli. The suspected bacterial isolates were ten (10) Coagulase Negative Streptococci (63%), four (4) *Staphylococcus aureus* (25%), one (1) *Streptococcus pneumoniae* (6%), and one (1) *Streptococcus pyogenes* (6%). In a related study by Obajuluwa et al.[23] more gram negative organisms were isolated, out of a total of 46 bacteria isolates which disclosed the following bacterial isolate: *Staphyloccocus aureus* (41.3%), *Escherichia coli* (28.3%), *Salmonella spp* (19.6%), *Pseudomonas aeruginosa* (8.7%) and *Serratia spp* (2.2%). According to Kayode et al.[24] gram positive bacteria isolates from selected vegetables were similar to this present study where all isolates were gram positive; and the bacterial isolates from cabbage include; *Brevibacillus laterosporus*, and *Lactobacillus sp.* Isolates from lettuce include; *Enterococcus faecalis*, *Bacillus licheniformis, Bacillus lentus*, and *Staphylococcus condimenti*, While the isolates from cucumber were found to be; *Bacillus cereus, Staphylococcus aureus*, and *Streptococcus faecalis*. The highest total colony count after 24, 48 and 72 hours incubation was 1.24×10^{10}cfu/ml, 3.22×10^{10}cfu/ml and 3.31 x 10^{11}cfu/ml respectively. In a study on Food-borne outbreaks in Brazil associated with fruits and vegetables, *S. aureus* was responsible for 7 outbreaks of food poisoning (7 outbreaks), *E. coli* (3 outbreaks), *B. cereus* (2 outbreaks), and thermotolerant coliforms (1 outbreaks) (Elias et al.[25] The CDC in the United States of America estimated that, Staphylococcal food poisoning caused >240,000 illnesses leading to >1000 hospitalizations and six deaths per year .Coagulase negative Staphylococci are common colonizers of human skin and have been recurrently considered culture contaminants. They have also been detected as contaminants of food products, sucha as ready-to-eat foods of animal origin, retailing raw chicken meat, and in bulk tank milk or minced meat .Some enterotoxin-producing CoNS strains have been isolated from cases of Staphylococcal food poisoning but with low frequency according to Franca et al.[26]

A 2011 report by the Centers for Disease Control and Prevention (CDC) estimated that 11,217 cases of foodborne Streptococcal illness occur annually in the United States, and food handlers are thought to be a major source of food contamination with Streptococcus Group A as posited by Food and Drug Administration.[27] Gram-positive bacteria are more tolerant to dry conditions than gram-negative bacteria, whose growth could be reduced when a surface is drying.[28]

Conclusion

Findings from this study have validated the potentiallitly of pathogenic bacteria causing unwanted manifestations in sliced and exposed onions, which could lead to bacterial food poisoning as a result of inadequate handling or storage of onions. After ingestion of enterotoxins from *Staphyloccoccus*, a disease called staphylococcal food poisoning (SFP) can occur, due to this usually happens due to improper handling or storage of staphylococcal contaminated foodstuff, such as meats, salads, creams, and dairy products.

After an incubation period of 6–10 hours, headache, nausea, abdominal cramps, vomiting, general weakness and prostration, dizziness and chills, and diarrhea or dysentry can occur.

Foods that have been associated with Streptococcus A contamination include milk (both pasteurized and unpasteurized), ice cream, cream, eggs, cooked seafood, ground ham, potato salad, egg salad, custard, rice pudding, and shrimp salad. In almost all cases, the foods were allowed to stand at room temperature for several hours between the time of preparation and the time of consumption.

Several findings have reiterated that bacteria could be isolated from vegetables due to poor handling and storage. Thus, the slicing and exposing of onions for hours should not be encouraged, because pathogenic bacteria capable of eliciting bacterial food poisoning can be isolated from such onions.

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Competing interest

The authors declared no competing interest.

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