Analysis of Thermophilic and Halophilic Bacteria in Traditonally Processed Locust Beans Sold in Ekiti and Kwara State, Nigeria.

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ABSTRACT

Locust beans is one of the most essential condiment and ingredient of any soup in Nigeria. Many household and eateries make use of this condiment on daily basis, considering its taste and addition to every soup, coupled with its health benefit. Being a natural ingredient, some medical practitioner had successfully convince their patient to abstain from many other artificial soup ingredient and stick to locust beans for their food. The Process and preservation of this locust beans involves cooking, drying and salting, which ordinarily should have killed every bacterial in the process, though, this is subject to further verification. Heating and salting is one of the oldest food preservation methods, which is usually regarded as a technique to control microbial activity. It is important to be able to estimate the risk of undesirable microorganism growth, halophilic pathogens such as Vibrio parahaemolyticus at low temperature and thermophilic bacteria Bacillus sp. that survive the pasteurization procedure, thereby called for this studies on isolation of thermophilic bacterial and halophilic bacteria from locally fermented food. The objective of this study is to identify the possible thermophiles and halophiles from the traditionally processed locust beans sample collected from Ekiti and Kwara State. Considering the similarities and peculiarities in the processing and preservation period and method of locust beans in the two different states, it becomes necessary to examine the possible influence of this peculiarities on the presence of the two bacterial in question (thermophilic and halophilic), which makes it a comparative analysis. The study assessed the bacteria isolates in locally fermented locust beans in Ekiti and Kwara State, through descriptive research of the experimental design. Locust beans with salt and without salt were collected and the identification of the bacterial isolates were done by standard microbiological methods. Result revealed that the bacterial isolated includes: Staphylococcus aureus, Enterococcus mundtii, Acinetobacter iwofii and Acetobacter aceti. The identified isolated bacterial were very similar as regard the two different samples from the two states (Kwara and Ekiti). Where Staphylococcus aureus and Enterococcus mundtii have the highest frequency of occurrence 31.8% Acinetobacter iwofii have 22.7% and Acetobacter aceti have the lowest occurrence of 13.6 %. Also, studies revealed that a wide range of thermophilic and halophilic organisms are found in food. The study concluded that locally processed locust beans though preserved by heat and salt still contains some microbes that are deleterious to human health as well as the environment. It was therefore recommended base on the findings, that the locust beans be better preserved by freezing. In this wise, thermophiles that cannot survive in a temperature below 20_0C will die while the presence of halophiles which is added by salt as one of the most common preservation method will be prevented. Regular factory and market inspection should be encouraged by regulators such as environmental health officers, to ensure good hygiene in processing the locust bean.

Keywords: Bacterial, Locust beans, Halophilic, Thermophilic, Aureus.

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BACKGROUND

The use of locust bean in Nigeria can only be traced to the 14th century. Most especially in the Southwestern part of the country, where it has been their only soup condiment apart from pepper. Even when salt had yet been introduced, talk less of the emergence of artificial condiments. The Nigerians depends on the locust beans, which is gotten from the seed of a fruit in a particular tree and been taking through different processes such as picking, pilling, drying, cooking and heating, fermentation and salting, becomes the most natural and safest condiment ever (Ademola, et al. 2018)

The Yorubas most especially, belief, that the more you take from it the better, while very many use this condiment for soup, some do eat it ordinary. Many believe, that taking it ordinarily cures many eye problems naturally. Even now when different artificial seasoning had surfaced, yet, very many, cannot still do without adding locust bean to their soup or food. Most at times, diabetic patient who are advised to abstain from taking seasoning through their food, always runs back to locust beans, which sometimes solely give them the needed taste all the seasonings put together could have given (Adekoya, et al. 2019).

They are characterized by a lack of membrane-bound organelles and a single circular chromosome. Bacteria play crucial roles in various ecological processes, such as nutrient cycling, and can be both beneficial and harmful to humans. According to Prescott et al. (2020), bacteria are unicellular microorganisms that lack a defined nucleus and membrane-bound organelles.

They are unicellular prokaryotic microorganisms that lack a membrane-bound nucleus and other membrane-bound organelles. They are among the earliest forms of life on Earth and exhibit diverse metabolic capabilities (Tortora et al., 2017). Bacteria are essential contributors to various environmental processes, including nitrogen fixation, decomposition of organic matter, and nutrient cycling. Their ecological roles make them crucial for maintaining the balance of ecosystems (Madigan et al., 2018).

Food bacteria, particularly lactic acid bacteria (LAB) and certain strains of Bacillus and Clostridium, play a vital role in food fermentation processes. These bacteria contribute to the production of various fermented foods such as yogurt, cheese, sauerkraut, pickles and locust beans. They enhance flavor, texture, and nutritional properties while also contributing to food preservation (Tamang et al., 2016).

Some food bacteria, such as pathogenic strains of Escherichia coli, Salmonella, and Listeria, can pose significant risks to food safety. These bacteria can cause foodborne illnesses when present in contaminated food, leading to symptoms ranging from mild gastrointestinal discomfort to severe health issues (Doyle et al., 2015).

Thermophiles, however, are groups of heat loving microbes thriving at high temperature. Also Thermophiles or heat-loving organisms are generally defined as those organisms that grow optimally (T) above 50°C, while those growing optimally above 80 °C are referred to as Hyperthermophiles (Dekic, et al. 2018).

Madigan et al., (2018) in their wisdom defined thermophiles as microorganisms that exhibit optimal growth and metabolic activity at elevated temperatures, typically ranging from 45°C to 80°C (113°F to 176°F) or even higher. They are often found in geothermally heated environments, such as hot springs, hydrothermal vents, and geysers, where they have evolved to withstand and utilize the high temperatures.

Thermophiles prefer temperatures higher than those suitable for mesophiles, the majority of organisms. They may fall into different categories based on their preferred temperature range, such as moderate thermophiles, extreme thermophiles, and hyperthermophiles (Agbobatinkpo, et al. 2019).

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Thermophiles can be further described as extreme thermophiles (T >65°C) or hyperthermophiles (T >80°C) and most of the latter are archae-bacteria. (Li, et al. 2023). Thermophiles can be categorized into moderate thermophiles (growth optimum, 50–60^{0}C), extreme thermophiles (growth optimum, 60–80^{0}C), and hyper thermophiles (growth optimum, 80–110^{0}C). Thermophilic microorganisms can be classified as Gram-positive or Gram-negative, they can exist under aerobic or anaerobic conditions, and some of them can form spores. Due to their increased importance, potential applications, and roles in different fields (Dekic, et al. 2018).

Thermophiles have been isolated from different ecological zones (e.g., hot springs and deep sea) of the earth. The organisms with the highest growth temperatures (103–110^{0}C) are members of the genera Pyrobaculum, Pyrodictium, Pyrococcus, and Melanopyrus belonging to Archaea; within Fungi, the Ascomycetes and Zygomycetes classes have high growth temperatures, while, in case of bacteria, Thermotoga maritime and Aquifex pyrophilus exhibit the highest growth temperatures of 90 and 95^{0}C, respectively. It is however interesting to know that none of the thermophiles can survive in a temperature lower than 20^{0}C (Dekic, et al, 2018).

Halophiles are microorganisms, including bacteria and archaea that are well-adapted to saline conditions. They are capable of growing in environments with salt concentrations that are often inhibitory or lethal to most other organisms. Halophiles can be found in various saline habitats, such as salt flats, salt mines, and hypersaline environments like salt pans and saltwater bodies (Madigan et al., 2018). Halophiles exhibit varying degrees of salt tolerance, and they are classified into different categories based on their optimal salt concentration requirements. These categories include slight halophiles, moderate halophiles, and extreme halophiles (Zhang, et al. 2018). Top of Form

Halophiles on the other hand, are microorganisms that can survive, grow and reproduce in extreme saline conditions. Salting is one of the oldest food preservation methods, which is usually regarded as a technique to control microbial activity. However, some studies revealed that salt itself contains a wide range of halophilic organisms, including bacteria and archaea that are unique to these environments, as well as salted and fermented foods (Chun, et al, 2020)

In recent years, novel halophilic archaea namely Halopiger thermos to lolerans (Minegishi et al., 2016), Haloparvum alkali to lerans Kondo et al., 2016, and Halarchaeum grantii Shimane et al., 2015. were isolated from commercial salts using culture-based methods. Indeed, a variety of halophilic archaea were found in high salt-fermented foods and human intestines, suggesting that halophiles play an important role in food processing and preparation.

Halophilic microorganisms are mostly prokaryotes but include some eukaryotes as well. They have the ability to withstand the denaturing effects of salts as well as to manage and maintain the equilibrium

between the high environmental osmotic pressure and the low water activity (w_a) level outside the cell, compared to that within the intracellular (in the case of prokaryotes) or intercellular (in the case of

eukaryotes) regions. The halophilic microorganisms that have been most extensively studied are Aphanothece halophytica (cyanobacteria), Halobacterium spp. (archaea), Dunaliella salina (green alga) and Hortaea werneckii (fungus) (Li, et al. 2023).

Microorganisms belonging to this group can be considered as one of the toughest (most specialized) kinds of organisms living on this planet, being able to adapt themselves to growing in high salt concentrations (Kimura and Yokoyama, 2019). It has recently been reported that halophilic archaea exist in salted food products or fermented foods as well. In addition, people have become aware that halophilic archaea exist

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much closer to the surrounding human environments (Pswarayi and Gänzle, 2022).

However, based on the fact that the **Halophilic archaeal** and **Thermophilic archaeal** strains isolated until now are very diverse, it needs to be considered that the possibility of having a wide variety of halophilic archaea and thermophilic archaea in food products is higher than what would have been expected in the past (Akanni, et al. 2018).

METHODS

This study design made use of experimental method of analysis to carry out microbiological analysis on locust bean sold in different areas and took a form of conclusive study which was based on laboratory methods.

Sampling

The sampling frame consisted of Oja markets in a specific geographical section where locust beans was randomly selected using purposive sampling method Oja tuntun markets in Ilorin metropolis, and Oja Oba in Ado Ekiti, Ekit State. Little quantity of a traditionally fermented locust beans were collected from eight

(8) different spots in Oja tuntun markets, as well as Oja Oba, in Ado Ekiti.

Little quantity of the locust bean collected from the Osogbo the capital of the nearest state (Osun State), which shared boundary with both Ekiti and Kwara state was used as the control sample for the study. The samples were kept in clean and sterile polythene bags to prevent contamination and the samples were transferred to the laboratory immediately for analysis. The cheese samples were collected in the afternoon between the hours of 12.00pm – 04.00 pm in the space of two days, respectively.

Process of Isolation and Identification Bacteria.

The isolation of bacteria was completed within 24 hours of samples collection. This was carried out by mixing 1g of the locust beans samples with 9mL of sterile distilled water and diluted serially up to 10^{-10}. This was repeated for all the water samples. 0.2mL (aliquot) of the suspension was plated out of Mueller – Hinton agar supplemented with different concentrations of salts. The plates were incubated 35^0C for 24 hours. Distinct colonies growing on each plate were counted selected, subcultured and stored on slants. Pure cultures of all the isolates were subjected to biochemical test. In order to know the identity of the isolated organisms the following tests were carried out:

Colonial Morphology

The shape, size, pigmentation, elevation and marginal characteristics of the bacteria species were examined on agar plates after appropriate incubation periods.

Gram stain

Smears of 18-24 hours old cultures of bacteria isolates on clean glass slides were heat fixed and stained with crystal violet for about 30-60seconds. The dye was drained and specimen fixed with Lugol's iodine for 30 seconds respectively. The slides were rinsed with tap water, decolourised with 95% ethanol for about 10-15 seconds and again washed with tap water. The slides were counterstained with safranin for 30 seconds, then rinsed, air dried and examined under the microscope using the oil immersion lens for Gram reaction and cellular morphology. Gram positive organisms stained blue to purple while gram negative stained pink to red.

Spore staining

A biochemical characteristic of many bacteria is the ability to reduce nitrates. The product, nitrite, is then

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tested by a special reagent. The test organisms were inoculated separately into tubes containing nitrate peptone water and Durham tubes and incubated at 35^0C for 2 days. Test for nitrate reduction was determined by the Certain bacteria produce endospores and this technique is used to detect the presence of such spores. Smears of 48hours old culture of isolates were heat fixed on different glass slides. These were flooded with malachite green and heated over a beaker of boiling water for 10 minutes respectively. More stain was added continuously to the slides to avoid drying. The slides were subsequently washed and counter stained with safranin for 20 seconds, washed, blot-dried and examined under the oil immersion lens. While the vegetative portion of the organism stained pink to red, the spored stained green.

Motility test

This test was carried out using Edwards and Wing motility test medium. The semi-solid medium was inoculated with the different bacterial isolated by stabbing with a sterile inoculating needle at the centre of the medium column to over half the depth. The motile organisms grew and spread out from the line of puncture while the non-motile organism grew only along the line of puncture.

Catalase production

Most aerobic microorganisms are capable of producing the enzyme catalase although to different extents. The principle of this test is that when organisms containing catalase enzyme are mixed with hydrogen peroxide (H_2O_2), gaseous oxygen is released. Suspensions of 18 hours old culture of the test organisms were made with distilled water on a clean glass slide. A few drops of hydrogen peroxide were added using a dropping pipette. The evolution of gas bubbles caused by the liberation of free oxygen indicated the presence of catalase enzyme.

Oxidase test

This was carried to detect the presence of cytochrome oxidase in the microbes. The overnight broth cultures of isolates, were inserted in Bactident oxidase test strips. The strips were withdrawn at once and left for 10minutes for colour change. Colour change from yellow to dark purple confirmed the presence of oxidase. The oxidase test strips were impacted with 1% tetramethy-p-phenyldiamine solution.

Indole production

Some microbes are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. The latter reacts with 4-dimethylaminobenzaldehyde to form a dark red dyestuff. This procedure involved growing the isolates in tryptone broth for 48 hours at 35^0C, after which 1-2mL of chloroform were added to the broth culture and mixed gently. About 2mL of Kovac;s reagent were then added, shaken gently and allowed to stand for 20 minutes. A cherry-red colour at the reagent layer indicated indole production.

Citrate utilization

Simmons's citrate medium is a nutrient substrate that offer ammonium salts as the only source of nitrogen and citrate as the only carbon source. The degradation of citrate leads to alkalinisation of the medium, which is indicated by the pH indicator, bromothymol blue, changing colour from green to deep blue. Slants of Simmons's citrate agar were inoculated with light inoculums of the isolated and incubated at 35^0C for 5 days. Colour change from green to blue indicated a positive result.

Nitrate reduction

A biochemical characteristic of many bacteria is the ability to reduce nitrates. The product, nitrite, is then tested by a special reagent. The test organisms were inoculated separately into tubes containing nitrate peptone water and Durham tubes and incubated at 35^0C for 2 days. Test for nitrate reduction was determined

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by the addition of 1ml each of reagents 1 and 2 of the modified Greiss-Ilosvay's reagent. The presence of gaseous nitrogen and indicated by the development of a pink, purple or maroon colour within a few minutes. Presence of gas in the Durham tubes also suggested production of gaseous nitrogen and consequently, a positive result.

Urease activity

Urea, a common organic nitrogen source for many microbes, can be hydrolysed to ammonia and carbon dioxide. The latter produces an alkaline condition in the medium, which is indicated by a colour change of the pH indicator. Slants of Christensen's urea agar medium were inoculated with the isolates and incubated at 35^0C for 5-7 days, watching daily for any colour changes. The development of colour change from pink to red showed a positive urease activity.

Methyl Red Voges Proskauer (MR-VP) test

These are actually two tests in one. In the methyl res test, a medium that contains a little carbohydrate fermentable by microorganisms is used. Some micro-organisms normally ferment carbohydrates accompanied with acid production and hence, the colour of methyl red retains its red (acid) colour while others ferment carbohydrates without acid production and hence the methyl red changes to yellow. Some of these products include acetonic (acetyl methyl carbinol), 2,2-butanediol or diacetyl. The presence of these metabolic product is established by means of Barritus of APHA reagents. In the strongly alkaline environment of these solutions, acetone and 2,3-butanediol are oxidized to diacetyl, which in turn reacts with the reagent to form guanidine. This is the basis of VP test.

The isolates were inoculated into 10ml of MR-VP medium and incubated at 350C for 3days. After incubation, the tests were performed in the following way;

a. MR TEST – five drops of methyl red indicator were added to the culture. A red colour indicated a positive reaction.

RESULTS

The sample result were analyzed using the basic statistical methods of frequency distribution table and simple percentage for sample collected from Oja Tuntun in Kwara Sate.

Table 1: Cultural and Biochemical Characterization of the Bacterial Isolates Encountered in Sampled Kwara State Locust Bean.

Sample	Cell Shape	Gram Reaction	Catalase Reaction	Sim Reaction	Mr	Vp	Sugar Fermentation GLU MAL MAN LAC SUC C T N T R	Urease	Citrate	Possible Organisms
SS1	Cocci	+	_	–+	–	+	A	A	A	A
	Rod	_	_	—	–	+	NC	NC	G	NC
	Cocci	+	+	–+	–	+	A	A	A	A

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SS2	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_			+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS3	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS4	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Ovoid	_	_	–+	–	+	A	AG	AG	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
SS5	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Ovoid	_	_	–+	–	+	A	NC	G	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS6	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
SS7	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS8	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Ovoid	_	_	–+	–	+	A	NC	NC	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus

Result 2

The sample result were analyzed using the basic statistical methods of frequency distribution table and simple percentage for sample collected from Oja Oba in Ekiti Sate.

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Table 2: Cultural and Biochemical Characterization of the Bacterial Isolates Encountered in Sampled Ekiti State Locust Bean

Sample	Cell Shape	Gram Reaction	Catalase Reaction	Sim Reaction	Mr	Vp	Sugar Fermentation GLU MAL MAN LAC SUC C T N T R					Urease	Citrate	Possible Organisms
Ss1	Cocci	+	_	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	–+	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
SS2	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_	–		+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS3	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS4	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Ovoid	_	_	—	–	+	A	AG	AG	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
SS5	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Ovoid	_	_	—	–	+	A	NC	G	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS6	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus
SS7	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii

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	Rod	_	_	—	–	+	NC	NC	G	NC	NC	–	+	Acinetobacter iwoffii
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
SS8	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Enterococcus mundtii
	Ovoid	_	_	—	–	+	A	NC	NC	AG	AG	–	+	Acetobacter aceti
	Cocci	+	+	—	–	+	A	A	A	A	A	–	ND	Staphylococcus aureus

The above tables shows the similar results of locust sample collected in Oja Tuntun and Oja Oba. It comprises large number of bacteria isolated from the sample as shown in the above tables.

In the first center sampled Enterococcus mundtii, Acinetobacter iwofii and Staphylococcus aureus was isolated. Staphylococcus aureus Acinetobacter iwofii and Enterococcus mundtii bacteria was isolated in sample 2. The sample 3 of locust were found to contained Staphylococcus aureus, Acinetobacter iwofii and Enterococcus mundtii. While in sample 4 Enterococcus mundtii, Acetobacter aceti and Staphylococcus aureus. Likewise, in sample 5 it was found to contained Staphylococcus aureus, Acetobacter aceti and Enterococcus mundtii .

Sample 6 showed the presence of Staphylococcus aureus, Acinetobacter iwofii and Staphylococcus aureus. Also Enterococcus mundtii, Acinetobacter iwofii and Enterococcus mundtii was isolated in sample 7. Lastly, sample 8 contained Enterococcus mundtii, Acetobacter aceti and Staphylococcus aureus. The results were very similar in terms of the isolated bacterial except from the SIM reactions which are mostly negative in the sample collected from Ekiti State, unlike that of Kwara State sample which are of the combination of both negative and positive SIM reaction.

The SIM (Sulfide, Indole, and Motility) test is a biochemical test used in microbiology to assess the metabolic characteristics of microorganisms. The three components of the SIM test include the detection of hydrogen sulfide (H2S), indole production, and motility (—, –, and –+) respectively. **(—)** indicates no hydrogen sulfide production. The absence of blackening in the medium indicates that the microorganism does not produce hydrogen sulfide. **(–)** also denotes no indole production. The absence of a color change (usually red) after the addition of Kovac's reagent indicates that the microorganism does not produce indole. While **(--+)** means Motile, i.e. growth is observed spreading away from the line of inoculation, indicating that the microorganism is capable of movement.

Table 3: Frequency Distribution of Bacteria Isolated from Locust Sample

SAMPLE										
Organisms	Ss 1	Ss 2	Ss 3	Ss 4	Ss 5	Ss 6	Ss 7	Ss 8	Frequency Of Distribution	Percentage
Enterococcus Mundtii	+	+	+	+	+	_	++	+	7	31.8
Staphylococcus Aureus	+	+	+	+	+	++	_	+	7	31.8
Acinetobacter Iwoffii	+	+	+	–	–	+	+	–	5e	22.7
Acetobacter aceti	–	–	–	+	+	–	–	+	3	13.6

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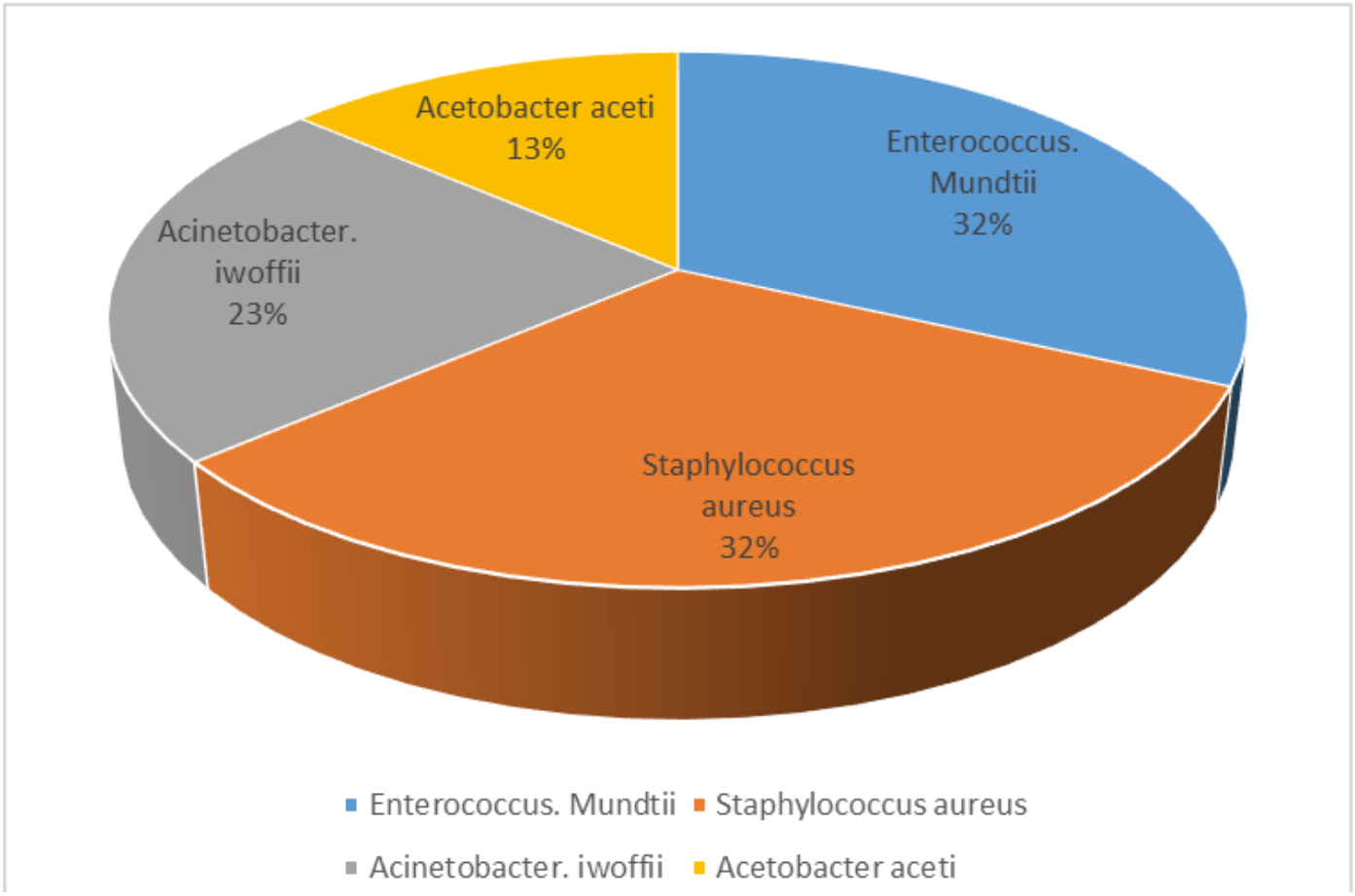
Based on the frequency of occurrence or distribution, Table 3 shows that four major organisms were found in all the samples. These are Staphylococcus aureus, Enterococcus mundtii, Acinetobacter iwofii and Acetobacter aceti. The Table shows that Enterococcus mundtii, is found to be present in all seven samples out of the eight samples which are in SS 1, SS 2, SS 3, SS 4, SS 5, SS 7and SS8 but existed in sample 6.

Similarly, Staphylococcus aureus, is also found in seven samples ie, SS 1,SS 2,SS 3,SS 4,SS 5,SS 6and SS 8 but absent in sample 7. While Acinetobacter iwofii was isolated in five sample of sample (SS 1, SS2, SS3, SS6 and SS7 also excepted in sample (SS4, SS5, and SS 8). Lastly Acetobacter aceti was found in three samples which are SS4, SS5, and SS 8 but existed in other samples.



Pie chart diagram showing the frequency occurrence of predominantly isolated bacteria

DISCUSSION

The presence of various forms of bacteria is obvious in locust beans despite that the traditionally processed food undergone series of heat and salting methods (Adewumi, et al. 2019).

Enterococcus mundtii as a predominant bacteria shown in Table 4 it effects may not be predominant especially on human because the only review was done by Kaufhold and Ferrieri (2014) that Enterococci may cause postoperative endophthalmitis with a poor visual prognosis, isolated two strains of *E. mundtii* from a chronic thigh abscess and sinus mucosa, but they could not determine whether the bacteria were commensals or pathogens. In this regard, to our knowledge, report of *E. mundtii* identified as a pathogen of

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human infectious disease, including endophthalmitis. Staphylococcus aureus which is second predominant bacteria in locust are deleterious to health and should be prevented. This finding is in agreement with that of Ademola et al, (2018).

They in their study established that OTUs spanned three phyla (*Firmicutes, Actinobacteria* and *Proteobacteria*), and nine genera: *Acinetobacter, Aerococcus, Bacillus, Enterococcus, Enterobacter, Lysinibacillus, Micrococcus,* dominated the processing stages of locust bean. Meanwhile the outcome is in contrary to that of Adedeji et al, (2017), with the submission that locust bean is free of bacteria, and that there is no need to educate processor/vendor of locust bean on good hygiene and processing practice.

CONCLUSION

This research showed that there are various types of microorganisms involved in fermentation of locust bean seeds. Diverse groups of bacteria isolates obtained include: Acinetobacter iwofii, Enterococcus mundtii, Staphylococcus aureus and Acetobacter aceti. The isolated bacteria as discovered are similar across the two different samples collected from Oja Tuntun and Oja Oba in Kwara and Ekiti State respectively.

In addition, it may be further concluded that every stage of production is sensitive regarding the load of microorganisms, therefore, there must be proper control to prevent contaminations.

In conclusion, this research work establish, that there is a significant frequency of occurrence in the microbial load content of locally fermented locust beans (iru) as a result of the presence of food pathogens and indicator organisms from cheese samples, which could lead to acute or chronic food poisoning or food-borne illnesses such as gastroenteritis etc.

RECOMMENDATIONS

Base on the findings and in attempt to prevent food poisoning, it is highly recommended that the locust beans be better preserved by freezing. In this wise, thermophiles that cannot survive in a temperature below 20_0C will die while the presence of halophiles which is added by salt as one of the most common preservation method will be prevented. Regular factory and market inspection should be encouraged by regulators such as environmental health officers, to ensure good hygiene in processing the locust bean.

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