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Nucleotide Sequencing of Bacteria Associated With Ready To Eat Suya (Tsire) – Roasted Meat Sold At Okitipupa And Ore Communities of Ondo State, Nigeria

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Abstract: Suya meat samples for this study were purchased at different locations of Ore and Okitipupa communities to evaluate its microbial load and advise safety consumption measures for its hundreds of millions consumers. Serial dilutions of the homogenized suya meat were prepared and cultured on MacConkey, Nutrient and Blood agar incubated at 37^{O}C for 24 hours. DNA of pure isolates were done by extraction with solution–based JENA Bioscience Bacteria DNA Preparation kit. rRNA gene of isolates was carried out using universal primers for bacteria. The PCR products were then purified using ethanol precipitation and thereafter sequenced with automated DNA sequencing machine. The optimal tree with the sum of branch length are as follows: 3.23634780, 3.49366796 and 3.54608845. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 13 amino acid sequences, and there were a total of 433 positions in the final dataset. Results revealed the identity of isolates as *Lysinibacillus pakistanensis* SN6-4 (MT071723.1), *Alcaligenes* sp. DST I94 (MH793390.1), *Proteus mirabilis* OG020 (MK641335.1) with 70 – 100% ribosomal RNA homology. It is then concluded that although isolation of bacteria with antimicrobial characteristics in suya is an advantage, suya also has opportunistic pathogenic bacteria that is raising health concerns hence consumers should be guided to ensure that they consume well roasted ready to eat suya to prevent food poisoning.
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1. Introduction

Suya is a spicy meat skewer which is a popular food item in West Africa. It is also eaten in Sudan, referred to as "Agashe" (El-sheikh , 2018). Suya is generally made with skewered beef, ram, or chicken. Innards such as kidney, liver and tripe are also used. Suya can be found across West Africa, along Nigeria, Cameroon and Niger Republic (Culture trip – Nigeria, 2017). Meat is excellent in supplying high quality protein, vitamins and mineral salts (Afolabi and Odubanjo, 2015) thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities.

Consumption of meat could be traced back in history to the period when primitive man ate raw flesh of animals. Meat is a perishable food and it is very rich in protein thereby making it ideal for the growth of wide range of spoilage bacteria. The growth of microbes in meat is governed by a number of intrinsic and extrinsic factors. Intrinsic properties of meat, such as pH and moisture can promote microbial growth, whereas temperature is an extrinsic factor. Fresh meat has a high water content that is favorable for the growth of microorganisms. It also generally contains bacteria, including those that can cause diseases. The animals naturally carry bacterial species like *Salmonella* and *E. coli* in their intestines, and raw meat can become contaminated during the slaughter process. Equipment and tools used in the processing meat can also become contaminated with microbes and spread those to the raw meat. (Catherine, 2020). Bacteria multiply rapidly at temperatures from 40 °F to 140 °F. Pathogenic bacteria do not necessarily multiply in meat leading to illness. Some species such as *Staphylococcus aureus* tend to be outcompeted by other harmless flora or spoilage bacteria that lead to a bad odor that cause most consumers to discard the meat.

Public concern has risen due to numerous food scandals such as those surrounding food borne diseases which remain a substantial burden. The spoilage challenge could be met with an improved global food and safety control system. One rapid improvement would be a rapid and accurate detection system for microbial spoilage. This technique should

ideally also be non-destructive and give accurate results in real time for application in highly automated food processing environment (Catherine, 2020) The suya meat is either sold at a joint or hawked. When sold at joints, the suya meat is kept warm over a fire source. When hawked, the suya meat is carried about in an open basin from one place to another thereby exposing it to dust and other toxic effects of the environment hence allowing harmful microorganisms find their way into the meat and causing food poisoning. Food poisoning is an illness with acute gastro enteritis as a major symptom caused by the ingestion of containing harmful pathogenic microorganisms. Some of the microorganisms present in suya meat that causes food poisoning when consumed are *staphylococcus aureus, Salmonella typhi, Escherichia coli, bacillus spp, klebsiella spp, clostridium botulinum, and streptococcus.* These microorganisms induce toxic substances into the meat which makes it unfit for eating and also reducing the taste and nutrient value, (Eke *et al.,*2013; Genevieve,1974)

2. Material and Methods

Samples of the suya meat were collected with a sterile well zipped pack bag from four different selling points each at different points of both Ore and Okitipupa, Ondo state, Nigeria. The samples were wrapped and placed inside a Ziplock bagwell labelled and transported to the microbiology laboratory where microbial analysis was carried out on each of the samples.

Preparation of Diluent

From each of the samples,1 gram of already homogenized roasted meat was dispensed into 10ml of distilled water which was kept and used as the sample stock. With the aid of a sterile pipette 9ml of distilled water was put into different test tubes which was used to carry one in ten serial dilutions.

Preparation of Media for Culturing

Nutrient, MacConkey, Blood and Eosin Methylene Blue Agar were prepared by standard methods and used in culturing when needed.

Culturing of Organism

Spread plate technique was used, in which 0.1ml of the appropriate dilution factors 10^{-3} and 10^{-5} as well as the stock used for each sample was spread plated on the surface of the media in the petri-dish with sterile spreader rod. The plates containing Nutrient agar were incubated at 37℃ for 24hours. After incubation, different culture characteristics were observed.

Determination of Bacterial Load

Total plate count of bacteria in each sample was determined using a colony counter and calculated as colony forming units per ml (CFU/ml) with the formula: CFU/ml = No. of colonies x Dilution factor/ volume of inoculum.

Streak Plate Method

Portion of each dilution was placed the surface of an agar medium and then streaked with a sterile wire loop. Actuate inoculation was accomplished by streaking the agar surface with a back and forth sweeping motion of the wire loop and by turning the plate at angles 90℃. The isolated colonies were individually sub cultured to another media to obtain pure cultured to isolate which were studied on different media.

Isolation of Pure Cultures of Bacteria

Pure cultures were obtained by taking one loopful of a colony and aseptically inoculating onto sterile Nutrient agar and incubated at 37℃ for 24hours. Pure culture was stored in cryogenic vials with sterile nutrient agar in slanting position to obtain agar slants. These were used for differential tests and biochemical test.

Colony Morphology

Isolated organisms from the different plates were characterized by their colony morphology which includes their colony shape (i.e. circular, filamentous, rhizoid etc.), edge, elevation, color, transparency.

Staining Techniques Used

Gram Stain

The specimen is collected from the growth of the organisms in the cryogenic vile and smeared on a clean glass free slide and allowed to air dry. Gram staining was done. Gram positive stained dark blue, while gram negative bacteria appear pink red.

Biochemical Test Used In the Identification of the Isolates.

Catalase Test

A loopful of the test culture was placed on a drop of normal saline solution and smeared evenly on a clean slide. A drop of hydrogen peroxide was poured on to the smear and inspected for gas bubbles. Rapid effective sciences indicated oxygen gas production and a positive test.

Coagulase Test

Two forms of coagulates occur a free coagulate which is detected by the coagulate tube test and a slide test slide coagulate test- This is used to

differentiate *staphylococcus aureus* from other *staphylococcus* clumping when a colony dropped on a plasma positive.

Sugar fermentation with Durham tubes (Glucose, Lactose, Maltose and Sucrose)

Phenol red glucose broth was prepared and 5ml of the broth was added to test tubes then Durham tubes were inserted into the test tubes so as to detect gas production. The test tubes were autoclaved at 121^0c for 15mins. The tubes after removing from the autoclave were inoculated with colonies of the isolates using inoculating loop. The test tubes were then incubated at 37^0c for 24hrs. The observations were recorded.

Citrate test

Simmon's citrate agar media was prepared and sterilized using autoclave, then5ml of the media was poured into the culture tubes and slants were prepared. Simmon's citrate agar slants were inoculated with isolates from each plate identified as gram negative. The un-inoculated tubes were kept as control; the tubes were incubated at 37^0C for 48hours.Slant culture was observed for the growth and coloration of the medium and was recorded。

Urease test

Urease agar medium was prepared and sterilized using autoclave. The medium was allowed to solidify in the slanting position to form a slope. The slants were inoculated with test organism.The tubes were incubated at 37°C for 48hrs. The observation was done and recorded appropriately.

16S rDNA EXTRACTION AND AMPLIFICATION FROM BACTERIA ISOLATES

The nucleotides sequence obtained were compared with other nucleotides sequences using BLASTn tools of the National Centre for Biotechnology Information. The phylogenetic tree was constructed using the results obtained from BLASTn. The evolutionary history was inferred using the Neighbor-Joining method described by Saitou N. and Nei M. (**1987**). The evolutionary distances were computed using the Poisson correction method described by Zuckerkand and Pauling (**1965**) and are in the units of the number of amino acid substitutions per site. Therefore, the evolutionary analyses were conducted in MEGA X Kumar S. *et al*., (**2018**). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) were determined following the method described by Felsenstein J. (**1985**).

3. Results

Table 1 : Microbial Loads on Suya meat sold at different Locations of Okitipupa

Samples	Stock (S)	10^{-3}	10^{-5}
SI_1	*260 x 10^7	*17.0	*4.0
SI_2	250 x 10^7	16.0	4.0
$S2_1$	280 x 10^7	17.0	3.0
$S2_2$	250 x 10^7	16.0	4.0
$S2_3$	260 x 10^7	15.0	3.0
$S3_1$	250 x 10^7	18.0	4.0
$S3_2$	230 x 10^7	15.0	5.0

*Data are means of three replicate

Table 2 : Microbial Loads on Suya meat sold at different Locations in Ore

Samples	Stock (S)	10^{-3}	10^{-5}
$S4_1$	*250 x 10^7	*18.0	*6.0
$S4_2$	230 x 10^7	16.0	3.0
$S4_3$	240 x 10^7	15.0	3.0
$S5_1$	230 x 10^7	20.0	3.0
$S5_2$	230 x 10^7	21.0	3.0
$S6_1$	250 x 10^7	21.0	5.0
$S6_2$	270 x 10^7	19.0	6.0
$S6_3$	260 x 10^7	20.0	4.0
S7	250 x 10^7	21.0	4.0
S8	240 x 10^7	23.0	4.0
$S9_1$	250 x 10^7	23.0	4.0
$S9_2$	270 x 10^7	19.0	4.0
$S10_1$	240 x 10^7	24.0	5.0
$S10_2$	280 x 10^7	22.0	4.0
$S10_3$	280 x 10^7	21.0	4.0

*Data are means of three replicate

Table 3: Results of Biochemical tests on the bacterial isolates

Isolate	Gram test	Form	Cat	Cit	Ure	Oxi	Lac	Glu	Suc	Mal	Suspected Organism
$S1_1$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S1_2$	-	Rod	+	+	+	-	-	-	-	-	*Proteus mirabilis*
$S2_1$	-	Rod	+	+	+	-	-	-	-	-	*Proteus mirabilis*
$S2_2$	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
$S2_3$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S3_1$	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
$S3_2$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S4_1$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S4_2$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S5_1$	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
$S5_2$	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
$S6_1$	-	Rod	+	+	+	-	-	-	-	-	*Proteus spp(mirabilis)*
$S6_2$	-	Rod	+	+	+	-	-	-	-	-	*Proteus spp(mirabilis)*
$S6_3$	-	Rod	+	+	+	-	-	-	-	-	*Proteus spp(mirabilis)*
S7	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
S8	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S9_1$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S9_2$	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
$S10_1$	-	Rod	+	+	+	-	-	-	-	-	*Proteus spp(mirabilis)*
$S10_2$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*
$S10_3$	+	Rod	+	+	-	+	-/+	+	+	+	*Lysinibacillus fusiformis*

Keys: Gram test – Gram staining test, Form- shape under microscopic view, Cat – Catalase test, Cit – Citrate test, Ure- Urease test, MR- Methyl red test, Coa- Coagulase Ind- Indole test, Oxi- Oxidase, Lac- Lactose, Glu- Glucose, Suc- Sucrose, Mal- Maltose, + = positive, - = negative, -/+ = variable.

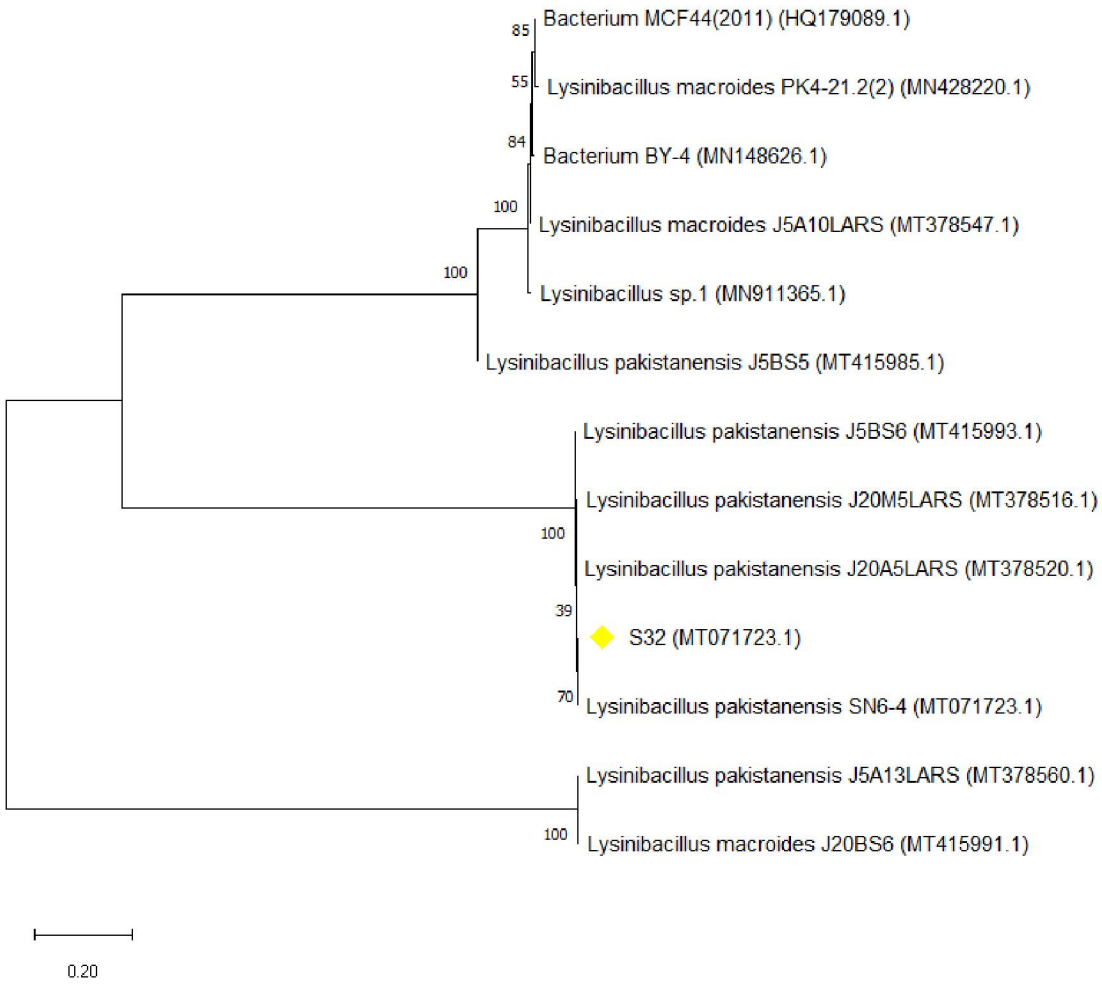

Fig 1: Phylogenetic tree of evolutionary relationships of taxa by Neighbor-Joining

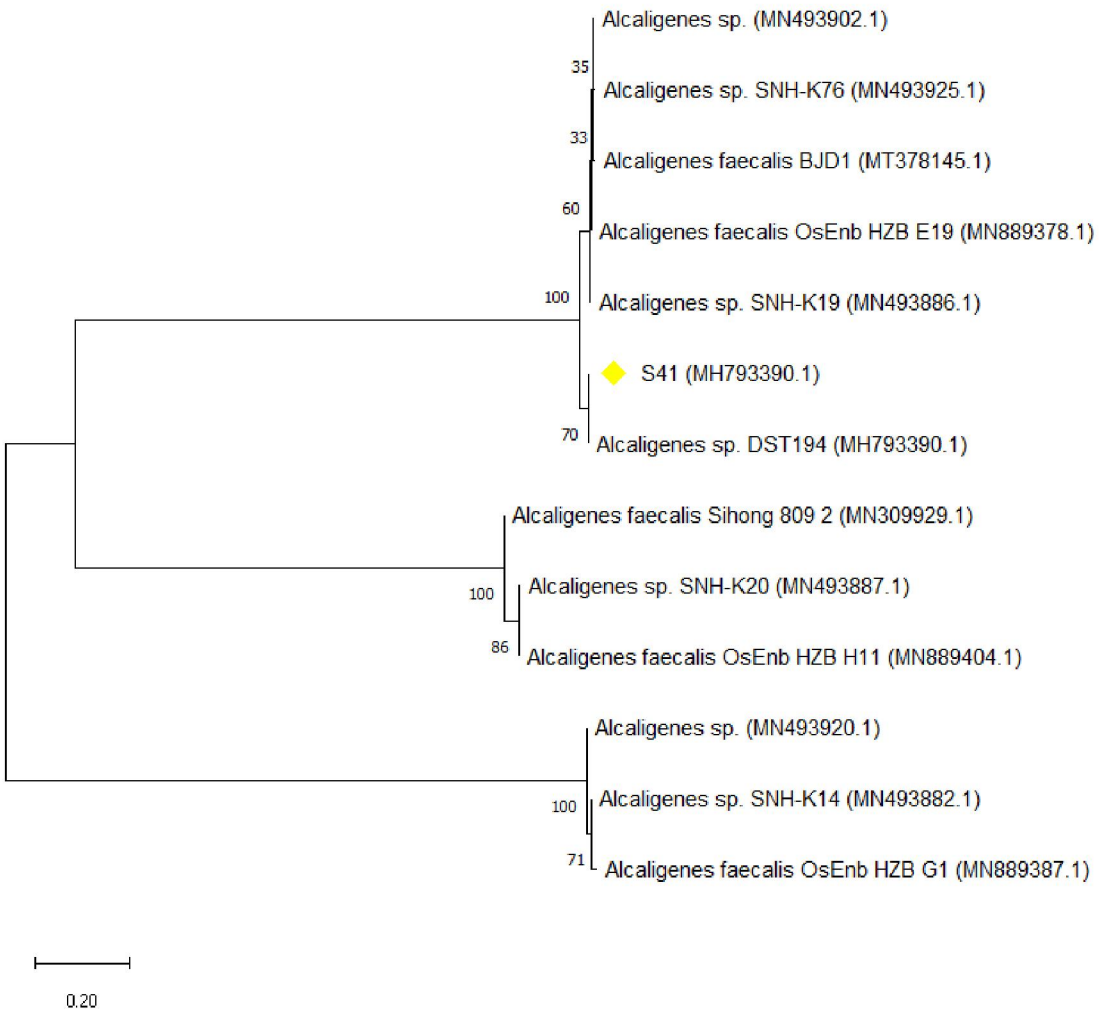

Fig 2: Phylogenetic tree of evolutionary relationships of taxa by Neighbor-Joining.

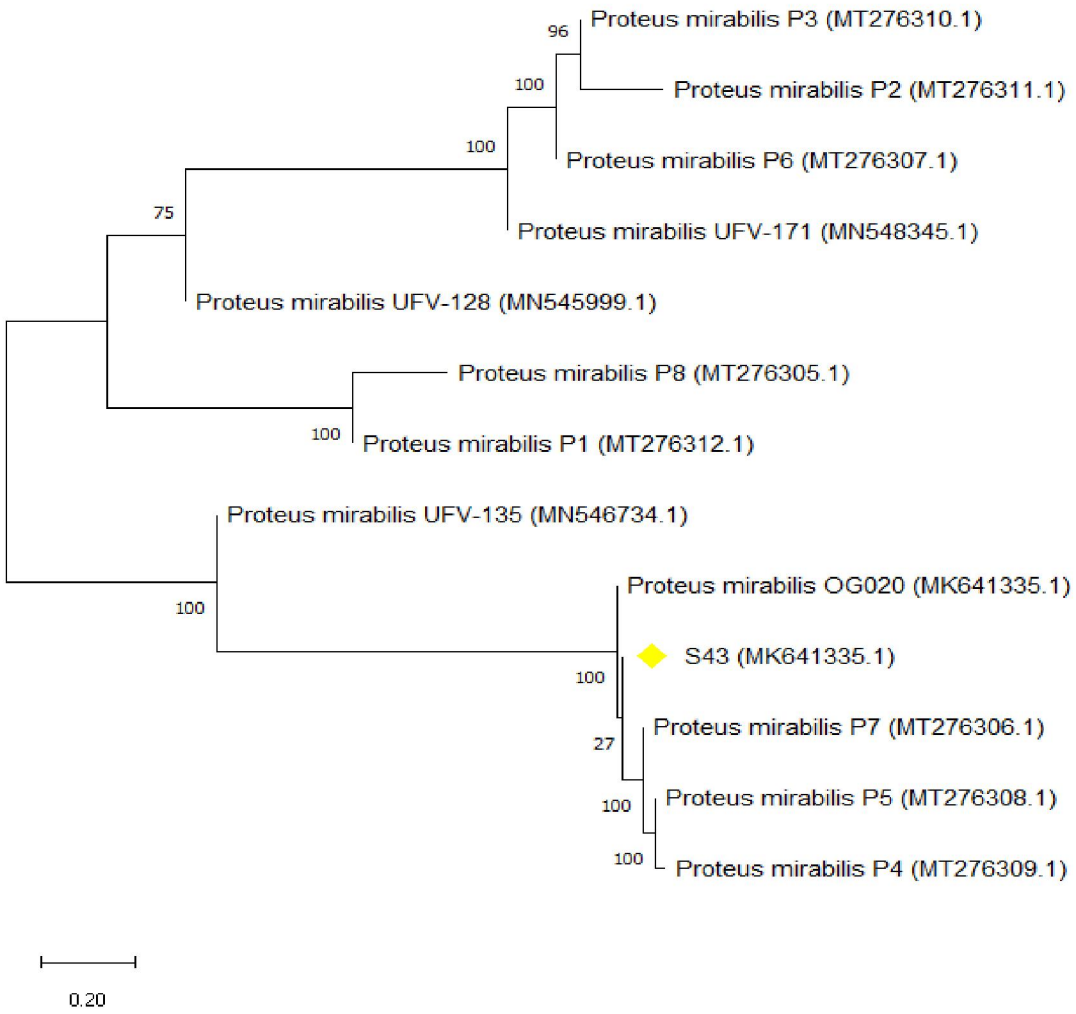

Fig 3: Phylogenetic tree of evolutionary relationships of taxa by Neighbor-Joining

The optimal tree with the sum of branch length = 3.23634780, 3.49366796 and 3.54608845 in Fig1, Fig2 and Fig3 respectively was determined. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 13 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 433 positions in the final dataset.

4. Discussions

Bacteria loads of suya samples from Ore and Okitipupa communities are as shown on Tables 1 and 2. The microbial load of suya varies with locations as shown by the different values obtained at Okitipupa and Ore. A suya sample from S2 spot at Okitipupa ($S2_1$) has the highest value of 2.80 x10^9 CFU/ml while the least bacteria count was recorded at point $S3_2$ (Okitipupa) with 2.3 x10^9CFU/ml. The variation in number of bacteria across suya samples from different locations as well as those from same spots corroborate the earlier finding of Eke *et al.,* (2013) who also

reported that total viable count obtained from suya at various points varies across locations in Ekpoma township (South – South Nigeria). Chukwura and Majekwu (2002) also posited that microbiological analysis of meat samples in Awka urban of Anambra State, indicated contamination of meat samples with various bacterial species of intestinal origin while some are contamination from intestine of slaughtered animals.

The presence of *Lysinibacillus pakistanensis* SN6-4 (MT071723.1), *Alcaligenes* sp. DST I94 (MH793390.1), *Proteus mirabilis* OG020 (MK641335.1) in the tested samples show that there are pathogenic and opportunistic bacteria in the meat which are of health concerns (Hema, 2010, Al-Zahrani *et.al.,* 2013). It is opined that the consumption of contaminated brawn as in the case of suya meat in many sub-Saharan communities has been identified as the cause of many health issues (Joe, 2019, Zahra *et.al*., 2020). Alcaligenes spp. are opportunistic human pathogens causing sporadic cases of pneumonia, septicemia, peritonitis, and urinary tract and other infections while they are lethal against nematode *Rhabditis blumi* (Hae *et.al.,* 2011). The presence of *Alcaligenes* sp. DST I94 (MH793390.1) in the meat samples aligns with the findings of Mahamuda (2017) who submitted that contamination of meat samples by human and animal waste can serve as source of pathogenic bacteria causing different diseases and confirmed *alcaligenes* spp alongside *Klebsiella* spp., *Pseudomonas* spp and *Proteus* app as one of the microorganisms causing intestinal disorders or diarrhea in developing countries. The Isolation of *Proteus mirabilis* OG020 (MK641335.1) in the suya samples correlate with the findings on the epidemiological investigations that indicated that meat products, bean products, fish, and cold dishes are commonly associated with food poisoning related to . *mirabilis* Wang *et al.*, 2010; Cao *et al.*, 2011; Jiang *et al.*, 2017). Hence, *P. mirabilis* may pose a relatively great threat to food safety and public health. *P. mirabilis* is considered as the causative pathogen in hospital cross-infection associated with urinary catheter (O'Hara *et al.,* 2000; Hola *et al.,* 2012). However, some novel strains have been reported to pose risks to human health and may cause serious disease in patients (Rozalski *et al.*, 1997; Wang *et al.*, 2010, Qiyun *et.al.,* 2013). In recent years, more and more food poisoning cases associated with *P. mirabilis* had been reported in China. In these food poisoning incidents, clinical symptoms of the patients infected with *P. mirabilis* included abdominal pain, diarrhea, nausea, and dizziness (Wang *et al.*, 2010; Shi *et al.*, 2014, 2016, Ghadah *et.al.,* 2013).

Our finding of *Lysinibacillus pakistanensis* SN6-4 (MT071723.1) in various suya samples from various sites is in line with that of Rifat *et.al.* 2013 who isolated a strain of *Lysinibacillus* is from spoiled fruits and vegetable wastes and that bacteriocin producing *Lysinibacillus* sp., nov., were isolated from food samples. However, Ahmad *et al.,* 2011 submitted that *Lysinibacillus* has emerged as an interesting microbe in the field of bacteriocin because of its inhibitory potentials while Varish and Mohammed 2015 also submitted that *Lysinibacillus sp.,* nov., isolated from food samples shows a significant antibacterial as well as antifungal activity with selective and greater inhibitory effect against gram positive as well as gram-negative pathogenic indicators. This means the presence of *Lysinibacillus pakistanensis* SN6-4 (MT071723.1) is helpful for making suya safe for consumption as its inhibitory to bacterial and fungal growth. Al-Othman *et.al*., 2013 also reported bacteria with inhibitory advantageous properties in food . CSF of *Lysinibacillus* JX416855 has also been reported to inhibit gram-positive pathogens *B. pumilus* (14mm), *B. subtilis* (13mm), *S aureus* (16mm), *B. cereus* (15mm) and *L.monocytogenes* (13mm) and gram negative *R. planticola* and *P.aeruginosa*. These results confirmed that *Lysinibacillus* JX416855 found very effective against gram negative bacteria. *Lysinibacillus* isolate is also reported to have been found potential fungal antagonistic against *Aspergillus niger, Aspergillus flavus, F.oxysporium* and *Trichoderma spp*. Biocontrolling (ahmad *et al.,* 2014) and insecticidial nature of Lysinibacillus has been described (Hu *et al*., 2008; Melnick *et al.,* 2011). Bacteriocins, like acidocin have been extracted with butanol and tested against fungi showing significant antimicrobial activity (Abo-Amer, 2007). These findings explains possible reasons why a good number of bacteria usually associated with ready to eat meat such as *Staphylococcus aureus, Listeria* spp, *Salmonella Enteritidis, Escherichia coli O157:H7,* and *Campylobacter* were absent in this research work as the antibacterial bacteriocin of *Lysinibacillus pakistanensis* SN6-4 (MT071723.1) has possibly eliminated them from the suya samples.

Conclusion

Food ingestion is a major way for human beings to obtain enough nutrient for basic living; therefore, the quality and safety of food have recently become a major concern. Although, food safety is a global concern, it is more of a concern in developing countries like Nigeria where "farm to table" food are commonly consumed alongside improperly processed food. Due to complexities in microbial interactions, a number of novel microbial issues associated with food safety and contaminations are now being acknowledged.

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References

[1]Abo-Amer AE (2007). Molecular characterization of antimicrobial compound produced by *Lactobacillus acidophilus* AA11. *Acta. Microbiol. Imm H*., 54: 107- 119.

[2]Afolabi F.T and Odubanjo O.R (2015). Microbial Assessment of Chicken and Beef Suya Samples in Oyo, Nigeria. *Nat Sci* 2015;13 (11):74-77. (ISSN: 1545-0740). http://www.sciencepub.net/nature;

[3]Al-Othman M.R., Mahmoud MA., Abd El-Aziz A.R.M. Effectiveness of nontoxigenic *Aspergillus flavus* and *Trichoderma harzianum* as biocontrol agents on aflatoxin B1 producing by *Aspergillus flavus* isolated from Cashew. *Life Sci J* 2013;10 (4):1918-1922]. (ISSN:1097-8135). http://www.lifesciencesite.com. 254 doi:10.7537/marslsj100413.254

[4]Austin, Brian (2014). "The Family Alcaligenaceae". In Rosenberg, Eugene; DeLong, Edward F.; Lory, Stephen; Stackebrandt, Erko; Thompson, Fabiano (eds.). The Prokaryotes. Springer Berlin Heidelberg. pp. 729–757. doi:10.1007/978-3-642-30197-1_397. ISBN 9783642301964.

[5]Batt,C.A(2014) Encyclopedia of Food Microbiology (Second Edition) 38 – 41.

[6]Cao, B., Li, R., Xiong, S., Yao, F., Liu, X., Wang, M., et al. (2011). Use of a DNA microarray for detection and identification of bacterial pathogens associated with fishery products. *Appl. Environ. Microbiol*. 77, 8219–8225. doi: 10.1128/AEM.05914-11

[7]Catherine S (2020): Microbes in raw meet. https://www.azolifesciences.com/article/Microbes-in-Raw-Meat.aspx;

[8]Chukwura EI, Majewu CN (2002) . Prevalence of Microbial Contaminants of Suya meat sold in Akwa Urban, *Journal of Tropical Microbiology* (11): 89 – 91.

[9]Culture trip- Nigeria, (2017): https://theculturetrip.com/africa/nigeria/articles/a-brief-history-of-suya-west-africas-answer-to-shish-kebab/;

[10]El-sheikh (2018). "Agashe: Sudan's Homage to its African Roots". 500 Words Magazine

[11]Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

[12]Genevieve G Y (1974) Gastroenteritis microbiology .Mc Graw hill book 5th edition Pp 150 – 163.

[13]Ghadah Abusalim, Sulaiman Ali Alharbi, Hesham Khalil, Milton Wainwright and Mohammad A. Khiyami.Adhesion of Staphylococcus epidermidis and Staphylococcus aureus to intravascular cannulae. *Life Sci J*2013;10(4):981-988]. (ISSN:1097-8135). http://www.lifesciencesite.com. 126 doi:10.7537/marslsj100413.126

[14]Gilbert K.J Kendall, M and Hobbs Bc (1969) isolation method for microbiologist 2nd edition, publisher London 87-151.

[15]H.A. A. Al-Zahrani and El-Hamshary, O. I. M. Microbial quality of bottled water and their molecular characterization in Jeddah, Saudi Arabia. *Life Sci J* 2013;10(4):731-737]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 92 doi:10.7537/marslsj100413.92

[16]Hae Woong Park, Yong Ook Kim, Jae-Seok Ha, Sung Hun Youn, Hyeong Hwan Kim, Anwar L. Bilgrami, and Chul Soo Shin (2011). Effects of associated bacteria on the pathogenicity and reproduction of the insect-parasitic nematode Rhabditis blumi. *Can. J. Microbiol.* 57: 750–758

[17]Hema Ramanathan (2010). Food Poisoning: a threat to humans. Marsland Press. PO Box 180432, Richmond Hill, New York 11418, USA Page 19 -20

[18]Hola, V., Peroutkova, T., and Ruzicka, F. (2012). Virulence factors in Proteus bacteria from biofilm communities of catheter-associated urinary tract infections. *FEMS Immunol. Med. Microbiol*. 65, 343–349. doi: 10.1111/j.1574-695X.2012.00976.x

[19]Hu X, Fan W, Han B, Liu H, Zheng D, Li Q, Dong W, Yan J, Gao M, Berry C and Yuan Z (2008). Complete genome sequence of the mosquitocidal bacterium Bacillus sphaericus C3-41 and comparison with those of closely related Bacillus species. *J. Bacteriol*., 190: 2892-2902.

[20]Jiang, X., Yu, T., Liu, L., Li, Y., Zhang, K., Wang, H., *et al.* (2017). Examination of quaternary ammonium compound resistance in Proteus mirabilis isolated from cooked meat products in China. *Front. Microbiol*. 8:2417. doi: 10.3389/fmicb.2017.02417

[21]Joe Essien (2019). Pathogenic Microbes Growth Pattern Analysis in Staple Suya Meat Consumption Using the Bayesian Networks. doi:10.31695/IJASRE.2019.33146.

[22]Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.

[23]Mahamuda A.E, Shawda S.S and Tasnia A(2017). Microbiological quality analysis of fresh vended fruit juices and water sold in roadside stalls in Dhaka Metropolis by MPN method. Stamford *Journal of Microbiology*.7(1):1-6. DOI:https://doi.org/10.3329/sjm.v7i1.40062

[24]Melnick RL, Suárez C, Bailey BA and Backman PA (2011). Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases. *Biol. Control*., 57: 236-245. Moreno-Arribas

[25]O'Hara, C. M., Brenner, F. W., and Miller, J. M. (2000). Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. *Clin. Microbiol. Rev*. 13, 534–546. doi: 10.1128/cmr.13.4.534-546.2000

[26]Qiyun Fu, Shaotong Zheng. The Analyses of Distribution and Antimicrobial Resistance of Pathogens Isolated from Neurosurgery Ward for Three Years. *Life Sci J* 2013; 10(4): 1620-1623]. (ISSN:1097-8135). http://www.lifesciencesite.com; 214 doi:10.7537/marslsj100413.214

[27]Rifat H., Iftikhar A., Jayoung P,, Muhammed E.,Muhammed I., Young H. C (2015). A moderately boron-tolerant candidatus novel soil bacterium lysinibacillus pakistanensis sp. Nov. Cand., isolated from soybean (glycine max l.) Rhizosphere. *Pak. J. Bot*., 45(SI): 41-50.

[28]Rozalski, A., Sidorczyk, Z., and Kotelko, K. (1997). Potential virulence factors of Proteus bacilli. *Microbiol. Mol. Biol*. Rev. 61, 65–89.

[29]Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

[30]Shi, X., Hu, Q., Lin, Y., Qiu, Y., Li, Y., Jiang, M., et al. (2014). Etiological and molecular characteristics of diarrhea caused Proteus mirabilis. Zhonghua Liu Xing Bing Xue Za Zhi 35, 724–728.

[31]Uraih N and Izuagbe (1990). Food intoxication and food publican health food and industrial 1st edition Pp 58- 61 Rhodes A and Felcher, D.l (1996) Principles of industrial microbiology pergamon oxford Pp 42 – 148.

[32]Varish Ahmad and Mohd Sajid Khan (2015): Therapeutic intervention and molecular characterizations of bacteriocin producing Lysinibacillus sp., nov., isolated from food sample *Pak. J. Pharm. Sci*.. 28 (4) 1337-1344

[33]Wang, Y., Zhang, S., Yu, J., Zhang, H., Yuan, Z., Sun, Y., (2010). An outbreak of Proteus mirabilis food poisoning associated with eating stewed pork balls in brown sauce, Beijing. *Food Control* 21, 302–305. doi: 10.1016/j.foodcont.2009.06.009

[34]Zahra N, Sarwar A, Rashid MS, Ahmad A, Qammar A, Sultan M, Ali Q, Malik A. Microbial analysis and health risk of Listeria spps and S. aureus isolated from cheese and raw milk marketed in Lahore, *Pakistan. Life Sci J* 2020;17(7):55-59]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 9. doi:10.7537/marslsj170720.09.

[35]Zuckerkandl E. and Pauling L. (1965). Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson and H.J. Vogel, pp. 97-166. Academic Press, New York.

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