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SALT TOLERANCE CAPACITY OF BACTERIA ISOLATED FROM IRRIGATED SOIL IN KANO, NIGERIA

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ABSTRACT

This study characterized and screened NaCl tolerance in some bacterial isolates from irrigated soil of Bagwai, Kano State, Nigeria. The soil used for the study was sampled from Irrigation sites using random sampling method. The physicochemical properties and bacteriological characteristics of the soil sample were determined using culture, morphological, biochemical and molecular methods. The soil pH and electrical conductivity were 7.33 and 8.02 dSm^{-1} respectively. The total nitrogen and available phosphorus from the study area were 0.118% and 3.96 $mgkg^{-1}$ respectively. The organic carbon recorded a very low content of 0.183%. Moisture content was 080% while temperature was found to be 29^{o}C respectively. Bacteria isolated were identified as *B subtilis*, *S rhizophilia* and *K pneumoniae*. Further comfirmation using 16s rRNA sequencing showed the occurrence of *Stenotrophomonas rhizophilia* SBANHCu 14 (99.55%) and *Klebisella pneumoniae* GX 14 (98.68%). All the two bacterial isolates were able to tolerate salt concentration up to 20% but optimum tolerance was observed at 5%.

Keywords: Bacteria, Irrigated Soil, Salt, Tolerance

INTRODUCTION

Adaptation is the adjustment of organisms to their environment in order to improve their chances at survival in that environment. Different living organisms are affected with salinity but provide a mechanism to amend the abiotic stress environment such as changes in salinity, humidity, pH and temperature (Jaymin *et al*., 2013).

Soil contains a lot of microorganism including which are found in various natural environment and shows variability of adaptations, among which is bacteria. Bacteria occupied large part of the ecosystem, with unique characteristics such as very small, unicellular, primitive and non-chlorophyll. Dilution method was found to be suitable method to isolate the bacterium living cells in the soil (Gowsalya *et al*., 2014) Under arid or semi-arid conditions and in regions of poor natural drainage, there is a hazard of salts accumulation in soil (Juan *et. al.,* 2010). Soil salinity is considered as one of the major and widely spread environmental problems that limit crop production and lower soil productivity, particularly in arid and semi-arid environments. Thus, salinity has been linked with the rise in groundwater table resulting from excessive irrigation and poor drainage in large-scale perennial irrigation system (Madyaka, 2008). The aim is to study salt tolerance capacity of bacteria isolated from irrigated soil in Kano, Nigeria.

Halophilic and halo tolerant microorganisms are able to thrive and grow in saline and hypersaline environments. Mangroves are typically tropical fragile coastal ecosystems of inter-tidal zones of river deltas and back water areas (Sanjay *et al*., 2014). In the recent time, many approaches have been applied to alleviate soil salinity problems (Costa *et al*., 2018; Gangwar *et al.,* 2020).According to Kuamr and Verma (2019) and Mishra *et al.,* (2019), other available methods includes bioremediation and phytoremediation.

The plant growth-promoting rhizobacteria (PGPR) which mostly heterogenous bacteria contribute a lot in remediating salinity which plays a vital role in plant physiological activities. Some of the rhizobacteria which are beneficial includes genera of *Alcaligenes, Pseudomonas, Azospirillum, Bacillus, Klebsiella, Azotobacter, Enterobacter, Burkholderia, Arthrobacter and Serratia* that aid in plant growth through various mechanisms (Kenneth *et al*., 2018; Sharma *et al.,* 2021). These bacteria serve as biofertilizers and involved in the recycling of plant nutrients which help in phytostimulation and phytoremediation (Zhang *et al*., 2018). The PGP bacteria not only increase production of exopolysaccharides, siderophores, alter pH, modify toxic metals and solubilize phosphorus but also involve in stress alleviation and secretion of indole-3-acetic acid (IAA), cytokinin and gibberellins and the development of antibiotic resistance (Kedmir *et al*., 2018; Barnawal *et al.,* 2019).

Other important role of bacteria is enhancement of soil fertility production of antimicrobial products and removal toxic substances in soil and water (Haque, 2019). Furthermore, native adaptation of floras to their environment is regulated by genetic variation of microorganisms in relation to plants. (Rodriquez and Redman, 2008). Also, bacteria availability in Rhizosphere of a plant play a vital role to improve the growth of various crops, which are cultivated in wide-ranging root-zone salinities. However, there is possibility of bacteria potential for bioremediation of salt from plant rhizosphere. Hence this study was undertaken for the isolation and molecular characterization of the halophilic bacteria from salt affected soils (Nushair *et al.,* 2018). Furthermore, the soil bacteria play a very vital role in biogeochemical cycles of require nutrients resulting in better crop yield. In this regard, the quest for eco-friendly and sustainable agriculture with emphasis on the application of beneficial microorganisms is increasing at frequent levels (Rodriquez and Redman, 2008).

Screening for salt tolerance was previously based on morphological features alone without considering biochemical and molecular characteristics, which are not very reliable since they may be affected by environmental factors. Islam *et al.,* (2015) used 3 SSR markers as a molecular tool to identify 5 tolerant rice varieties out of the 25. Molecular research revealed that, DNA markers closely linked to traits related to salt tolerance has become a key objective in most breeding programs (Krishnamurthy *et al*., 2016; Sakina *et al*., 2016., and Reddy *et al*., 2017).

MATERIALS AND METHODS

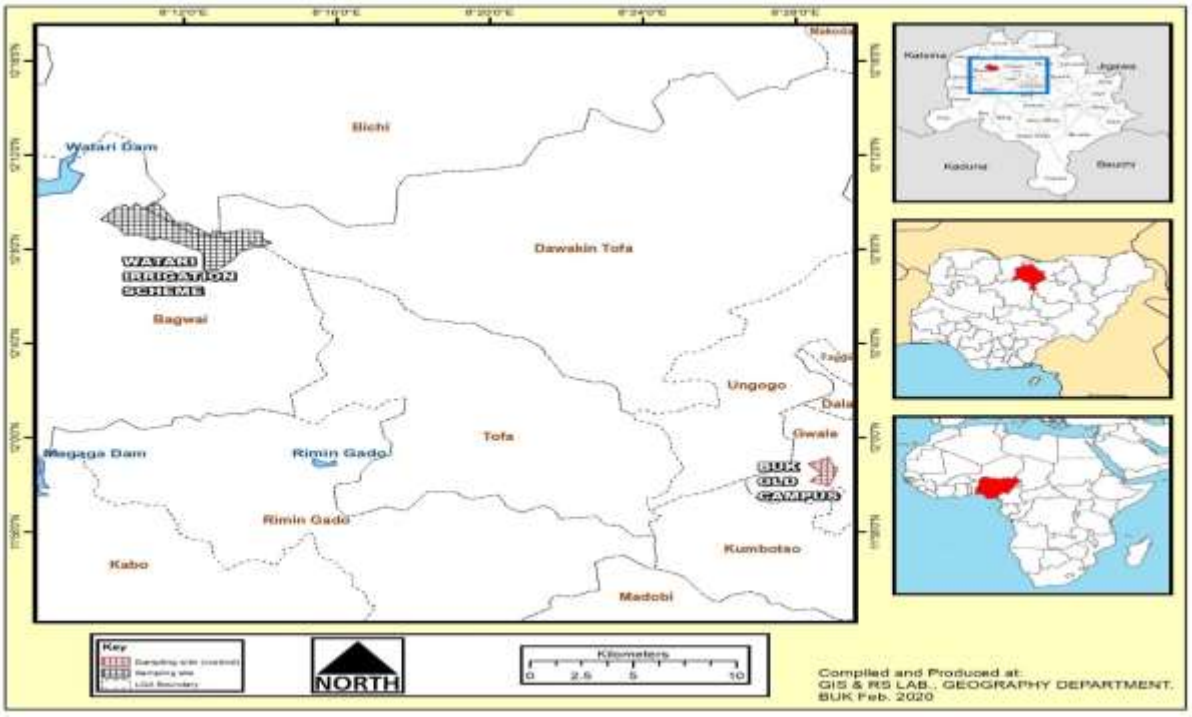

Figure 1: Map of the Study Area

Collection of Soil Samples

Soil samples were randomly collected and bulked during the month of April, 2019. Two hundred grams (200g) at a depth of 0-20cm using soil auger was collected and put into freshly purchase new polyethene bags. The sample was transported to the Microbiology Research Laboratory for some physicochemical and bacteriological analyses (Bilyaminu, 2019).

Determination of Soil Physicochemical Parameters

Physicochemical properties of the soil samples such as pH, temperature, electrical conductivity, moisture content, available phosphorous, organic carbon and nitrogen were determined using the procedure described by Eno *et al.,* (2009).

Enumeration and Isolation of Bacteria l

Dilution technique was used to reduce the density of organisms present in the soil to a countable number by diluting the highly concentrated sample. Dilutions of each soil sample were prepared using seven folds dilution techniques. Ten grams (10g) of soil sample was suspended in 90 ml sterile distilled water and made up to 10^{-7} level dilution. Before soil particle settled down, 1 mL of suspension from each test tube was transferred to next test tube of each series. An aliquot of 0.5ml each of the soil suspension from dilution 10^{-1} to 10^{-7} pour plates was made in NaCl (5%) supplemented nutrient agar and incubated at 37°C for 72 h and the colony forming units (CFU) were enumerated. Pure culture of the isolated bacteria was made and identified on basis of Bergey's Manual of Systematic Bacteriology (Debarati *et al.,* 2016).

Morphological Characterization

Gram staining was used to determine the nature of the bacterium (Todar *et al.,* 2005). Biochemical test were carried out to find the enzymatic activity of isolated organism (Musliu and Salawudeen, 2012).

Screening of Isolates for Salt Tolerance

A Series of nutrient broth tubes supplemented with various salt concentrations ranging from 5 to 20 % were used to determine requirement of salt for growth. In each series, tubes were labeled as 5, 10, 15 and 10% NaCl concentrations with the designation of isolates. Each tube was inoculated with 0.1 mL culture of selected isolates and incubated at 30ºC for 72 h. After incubation, the optical density of broth was recorded at 600nm using a spectrophotometer. (Nanis *et al.,* 2018).

Molecular Analysis of Bacterial Isolates

DNA Extraction

The genomic DNA was isolated and its 16s rRNA gene was amplify by the use of universal primer. The initial denaturation of DNA strands at 94ºC for 2 min, annealing with primers at 55ºC for 1 min and extension at 72ºC for 10 mins (Gowsalya *et al.,* 2014).

The 16s rRNA Genes Sequencing

Amplified strands were sequenced by RNA sequencer - 3037xl DNA analyzer using BigDye® terminator v3.1 cycle sequencing kit. Aligned sequences were converted to genograms using sequence analysis software version 5.2. These sequences were compared with the sequences in NCBI database using basic local alignment search tool (BLASTIN) Gowsalya *et al.,* (2014).

RESULTS

Physicochemical Properties of Soil.

. The soil sample obtained was found to have a pH and electrical conductivity of 7.33 and 8.58dSm^{-1} respectively. The moisture content and temperature were found to be 0.80 % and 29ºC respectively. The phosphorus, organic carbon and nitrogen were recorded as 3.96 mgkg^{-1}, 0.183% and 0.118% respectively (Table 1).

Table 1: Physicochemical Properties of Watari Soil Sample

Parameters	Values
pH (1:2.5)	7.33
Temperatue (°C)	29.0
Electrical Conductivity (dSm^{-1})	8.58
Moisture Content (%)	0.80
Available Phosphorus ($mgkg^{-1}$)	3.96
Total Nitrogen (%)	0.118
Organic Carbon (%)	0.183
Exchangeable Potassium ($cmolkg^{-1}$)	0.56

Mean Bacterial Colony Forming Units per Gram ($cfug^{-1}$) of Soil Samples

Table 2 shows the results of mean bacterial colony forming units per gram **($cfug^{-1}$)** of soil samples analyzed. The results indicated that Watari had an average counts of (4.9×10^7).

Table 2: Average Bacterial Colony Forming Units per Gram ($cfug^{-1}$) of Soil Samples

Replication	Watari
1	4.78×10^7
2	4.74×10^7
3	5.10×10^7
Average	4.90×10^7

Culture, Morphological and Biochemical Properties of the Bacterial Isolates

Table (3) shows the morphological and biochemical properties of the different bacterial isolates. The isolates identified were *Bacillus subtilis, Stenotrophomonas rhizophilia Klebsiella pneumoniae.* One *was* grams positive while the two were gram negative. All the biochemical test were presented in Table 3.

Table 3: Culture, Morphological and Biochemical Characteristics of Bacterial Isolates from Watari Soil Samples

	CODES		
TEST	**SL3**	**SL4**	**SL5**
Morphology	Rod Shaped	Rod Shaped	Rod Shaped
GT	+	-	-
MR	-	-	-
VP	+	-	+
CIT	+	+	+
CAT	+	+	+
OX	-	-	-
UR	-	-	+
TSI	-	+	+
IN	+	+	+
EO	*Bacillus Subtilis*	*Stenotrophomonas rhizophilia*	*Klebsiella pneumoniae*

Key: Positive = +, Negative = -, GT. = Grams Test, CAT = Catalase, OX. = Oxidase, CIT = Citrate, VP. = Voges Proskaur, MR. = Methyl Red, UR. = Urease, IN. = Indole, EO. =Expected Organisms

Screening of Bacterial Isolates for NaCl Tolerance

In the present study, all the isolates showed salt tolerance at different salt concentration, while three were able to showed high potential more especially at 5% NaCl (*B subtilis, S rhizophilia* and *K pneumoniae*). *K pneumoniae* tolerated 15% NaCl better than other isolates. At 20% only *S rhizophilia* was able to tolerate the concentration better (Figure 2).

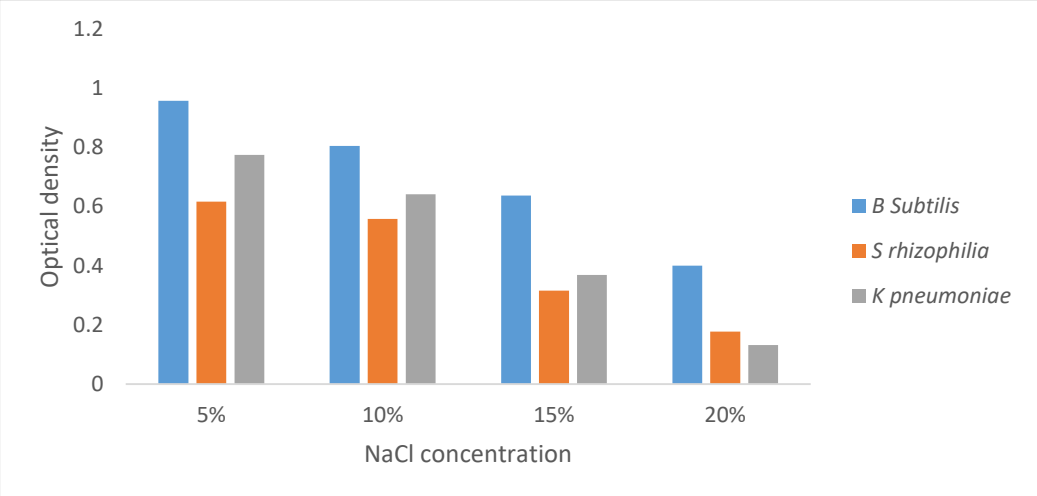

Figure 2: Sodium Chloride Tolerance Capacity of the Bacterial Isolates

Molecular characteristics of the Selected Bacterial Isolate

After bacteria DNA gene extraction and PCR, PCR products obtained for each of the bacteria were analysed on 1.5% agarose gel and after visualization by Gel Doc (BioRAD, USA), it was observed that 16SrRNA gene had created 1500bp bands (Figure 3). The results of sequencing were registered in a gene bank as new strain (Table 4). Besides the biochemical characterization, two bacterial isolates were identified by 16s rRNA sequencing and identification was confirmed after a BLAST search result. Overall, 98-100%

sequence similarities were obtained with already known sequences of NCBI database and isolates were identified accordingly. The SL4 and SL5 showed identity as *Stenotrophomonas rhizophilia* (98%) and *Klebisiella pneumonia*e (99%), with strain SBANHCU14 and strain GX14 respectively. Sequences from isolates SL4 and SL5 were deposited in Gene Bank 165-rRNA under the accession numbers KR259223. I and KU937377.1 respectively.

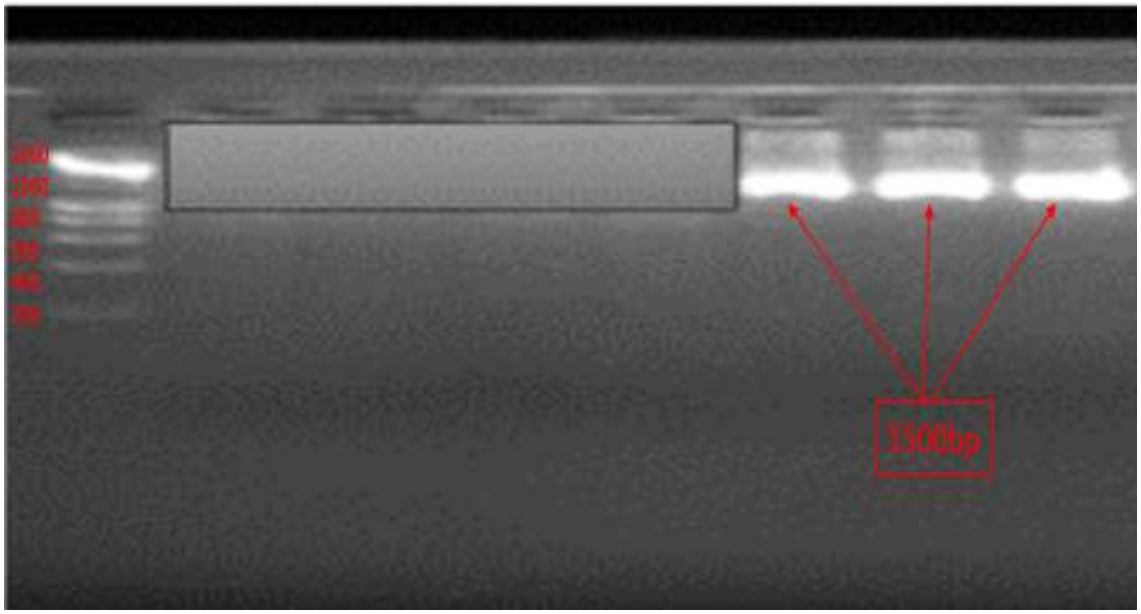

Figure 3: Gel Electrophoresis of the Bacterial Isolates

Table 4: Identification of Isolates on the basis of 16S rRNA sequence

Isolates	Identified as	Strains	Identity (%)
SL4	*Stenotrophomonas rhizophilia*	SBANHCu14	99.55
SL5	*Klebsiella pneumoniae*	GX14	98.68

DISCUSSION

The soil of the study site showed low nutrient content. Adamu (2013) reported similar findings from the same irrigation site and the low nutrients could be attributed to poor agricultural practices and management. The average $cfug^{-1}$ of the soil was reported to be 4.9×10^7. The findings are in consistent with the works by Nanis *et al.* (2018) that salinity caused declined in bacterial population. However, the result of this study shows that the $cfug^{-1}$ of the bacteria was considered as a healthy number (Poul and Nair, 2008). Different bacterial isolates were obtained from the study site, three were identified and characterized based on cultural, morphological and biochemical properties. The bacterial isolates were characterized as both gram positive and gramnegative bacteria with the predominant of gram negative. The gram positive bacteria are, *Bacillus subtilis*, while gram negative bacteria were *Stenotrophomonas rhizophilia* and *Klebsiella pneumoniae*. The presents study is in agreement with Hingole (2016) also recorded *Bacillus subtilis* and *Klebsiella pneumonia*e among the bacterial isolates that stimulated growth of maize in saline soil. Samina *et al.* (2010) isolated *Stenotrophonomas* spp in corn in his greenhouse research and field experiment. These shows that the higher the NaCl concentration, the microbial growth decreases due to high salt stress and decreasing optimal growths as a result of high salt concentration reported by Abdulkarim *et al.* (2009) and Hajmeer (2006). Furthermore, suggested that it may be due to hyper osmotic effect on the bacterial and osmotic shock on the organisms, may have led to the growth suppression. *B subtilis* also showed a higher optimal growth, than *Stenotrophomonas rhizophilia* and *Klebsiella pneumoniae* at all the NaCl concentrations; this also confirmed the adaptive ability of *B subtilis* to perform better in a NaCl stressed environment. Omotoyinbo, (2016) explain that the exponential phase of growth which shows a pattern of balanced growth where in all the cells are dividing regularly by binary fission, and growing by geometric progression indicates why at high salt concentration there was low microbial growth.

The gel electrophoresis showed all the three bacterial isolates were observed at 1500bp band while the molecular characterization showed two among the three isolates used for the studies were identified by 165rRNA sequences as *Stenotrophonoma rhizophilia* (SL4) and *Klebsiella pneumoniae* (SL5) .Based on sequences, strain BLAST search result showed that the strains SBANHCU14 and strain GX14 are more closely related to the species of *Stenotrophonomas rhizophilia* with (98%) and *Klebsiella pneumoniae* with (99%) respectively (Table 4) This finding is similar to that of Hingole (2016).

CONCLUSION

The physicochemical properties of the saline soil showed low nutrient availability with high sodium content. The bacterial count has 4.90×10^7 $cfug^{-1}$ and the optimum growth of salt tolerance was observed in *B subtilis*. The isolated and identified bacterial isolates were *B subtilis, S rhizophilia* and

K pneumonia where the gel electrophoresis of the isolates was at 1500bp band.

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