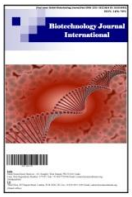

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Microbial Diversity of Nigerian Sludge and Its Potential for Use as Biofertilizer

Opeyemi Fatunla[1,2*], Joseph Essien[1,2], Utibe Ofon[1], Solomon Shaibu[2,3] and Emmanuel Dan[2,3]

[1]Department of Microbiology, University of Uyo, Uyo, Nigeria.
[2]International Centre for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Nigeria.
[3]Department of Chemistry, University of Uyo, Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JE and UO managed the analyses of the study. Authors SS and ED managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Sludge samples were collected from a wastewater treatment plant in Nigeria for characterization and evaluation for agricultural applications. Conventional and Molecular techniques were adopted for the isolation and identification of indigenous microorganisms and resulting isolates were characterized and identified by consulting Bergey's manual of determinative bacteriology and subjected to further screenings to assess their biofertilizer potential using standard microbiological techniques. The viable cells obtained were enumerated and were found to be in the range of 1.03 ± 0.09 $x10^3$ cfu/g to 7.45 ± 0.78 $x10^3$ cfu/g for heterotrophic Bacteria and 1.63 ± 0.74 $x10^3$ cfu/g for fungal community. The Molecular analysis carried out revealed a rich assemblage of diverse species of microorganisms with Bacteria (99.40%) being the most dominant group, followed by Fungi (0.39%) and others (0.21%). Thirty (30) isolates belonging to four (4) Phyla was recovered culturally and identified with Firmicutes 9(30%) being the most dominant group, followed by

**Corresponding author: E-mail: opeyemifatunla@uniuyo.edu.ng;*

Proteobacteria 8(26.7%) and Zygomycota 1(3.33%) was the least dominant. The phosphate solubilization index range from 0.86 to 6.3 for bacterial and 2.5 to 3.8 for fungal isolates respectively. The molecular analysis also revealed microbes adept at improving soil fertility to include those in the order Rhizobiales and Actinomycetales. Although pathogens are of a concern in the land application of sludge, our findings have revealed rich microbial consortia of heterotrophic microorganisms whose beneficial attributes can be harnessed to produce nutrient rich biofertilizer and soil amendment.

Keywords: Sludge; characterization; molecular sequencing; biofertilizer.

1. INTRODUCTION

The fact that microbes play a direct or an indirect role albeit incognito, in many biological activities that is beneficial to mankind has been established and cannot be overemphasized [1-3]. Microbes has served as a powerful tool in modern agriculture by being useful especially in combating crop diseases, production of plant growth promoting substances and in bio-augmentation. Diverse species of soil microorganisms has been found to be closely associated with the rhizosphere of plants where they stimulate plant growth through several mechanisms. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). The search for PGPR and investigation of their modes of action are increasing at a rapid pace as efforts are made to exploit them commercially as biofertilizers [4,5]. Biofertilizer has been identified as a cheap alternative to chemical fertilizer in a bid to increasing soil fertility while also boosting production of crop in sustainable farming. Biofertilizers contain live microbes, which are adept at converting nutritionally important elements from their unavailable form to forms easily available and accessible to plants during biological processes [4]. The utilization of biofertilizers has several advantages over conventional chemicals for agricultural purposes. World agriculture has been found to be heavily dependent on synthetic fertilizers as the source of plant nutrients to meet the increasing demand for food [3]. However, persistent and uncontrolled use of chemical fertilizers often results in unexpected negative impacts on the environment. Poor soil fertility and imbalanced soil microbial activity could be an implication of unabated use of chemical fertilizers. Thus, the use of organics including biofertilizer has gained attention of recent in the sustainability of soil fertility and plant productivity [3]. As many as 99% of the microorganisms present in certain environment cannot be detected by regularly used cultural techniques, therefore, protocols, most especially molecular techniques have been developed to assess the unculturable microbial diversity in order to forestall the existing barriers that prevent the estimation of biodiversity. In the past decades, new biochemical and molecular techniques have been developed to identify and classify microorganisms especially bacteria. Measuring microbial diversity is difficult because of the limited knowledge about bacteria species and classification through families and orders. Molecular techniques broaden our knowledge about microbial diversity and help the taxonomy of species. Measuring and monitoring soil microbial communities can lead us to better understanding of their composition and function in many ecosystem processes [6]. Sewage sludge is the solid, semi-solid, or liquid residue generated from the treatment of domestic sewage. It is very rich in nutrients (nitrogen and phosphorus), organic matter and some trace elements needed for plant growth. The treatment of the large amounts of wastewater produced in the society of today generates large quantities of sludge. The wastewater is treated in such a way that undesirable substances are separated from the water. The first treatment is often mechanical and it removes the bigger particles from the wastewater. Substances can also be removed biologically which is often the case in for example nitrogen and carbon, which is measured in biological oxygen demand (BOD). Chemical treatment is sometimes used and it encourages small particles and dissolved substances to form larger particles which facilitate separation. This is called chemical precipitation. Sludge is formed when these larger particles clump together during suitable separation methods [7]. All the sludge that is separated during these treatment methods (mechanical, biological and chemical) are referred to as raw sludge, which has to undergo varies kinds of further treatment.

Thus, in this current study, critical cultural, biochemical characterization and molecular identification of the diverse species of microbes in sludge were carried out and based on these

findings; work has been done to access the biofertilizer potential of these microorganisms.

2. MATERIALS AND METHODS

2.1 Study Site

Sludge samples were obtained from Lower Usuma Dam Wastewater Treatment Plant (LUDWTP) located in Abuja Nigeria between latitude 9°01' 12" N and longitude 7°25' 16" E (Fig. 1). It has a capacity to process 120 million litres of waste water and provide Abuja and its neighboring areas with the same amount of clean drinking water per day.

2.2 Collection of Samples

Grab sludge samples (25 Litres) were collected from the study site using standard Microbiological technique described by APHA [8]. The samples were collected with the aid of a sterile hand trowel and stored in sterile amber bottles. All samples were transported in ice-cooled chest to the laboratory for analysis. Extensive physicochemical characterization of the sludge was carried out and previously reported by Fatunla K et al. [9].

2.3 Isolation of Culturable Micro-organisms

A sub-sample (50 g) of raw sludge was added to 450 ml of sterile phosphate-buffered saline (PBS) solution aseptically and homogenized. As described by Cappuccino JG et al. [10], 10^{-1}–10^{-3} dilutions were made using sterile PBS solutions. Total culturable heterotrophic bacterial and fungal counts were determined by pour and streak plate techniques respectively following the methods of APHA [8]. Viable cells obtained after isolation and enumeration were purified using nutrient agar medium (NA). Following repeated sub-culturing by streak method Cheesbrough M [11], pure colonies of the isolates obtained were kept in McCarthy bottles containing freshly prepared agar in slants and incubated at 30 ± 2°C for 18 to 24 hours before storage at 4°C for future use. Identification of isolates will be carried out on the basis of its growth characteristics on differential media and biochemical properties using standard protocol as described by Cheesbrough M [11] and Holt JG [12].

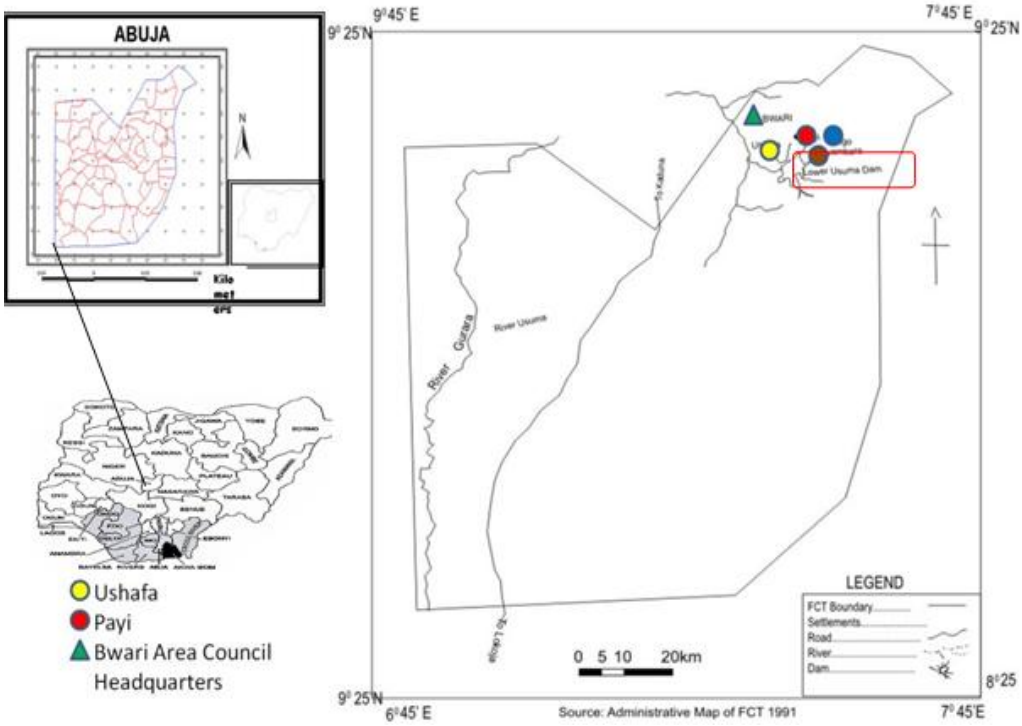

Fig. 1. Delineated map of Abuja, showing the location of lower usuma dam water treatment plant, Abuja, Nigeria

2.4 Molecular 16S rRNA Whole Community Genomic Sequencing of Sludge Sample

DNA Extraction from Sludge Sample was performed using ZYMO soil DNA extraction Kit (Model D 6001, Zymo Resaerch, USA) following to the manufactures instructions. The filtered DNA obtained was then used for PCR and DNA sequencing. DNA sequencing was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of all microorganism present in the soil sample using sequencing primer -16S: 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'-ATTACCGCGGCTGCTGG-3'. The sequencing was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of all microorganisms present in the sludge sample using automated PCR cycle- Genome Sequencer™ MiSeq (Illuminar). Analysis and alignment was performed using Vecton NTI suite 9 (InforMax, Inc.). Overall bioinformatic analysis was done using NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1 [13].

2.5 Screening for the Bio-fertilizer Potential of Isolates

All isolates were screened initially based on their capability to solubilize phosphate when cultured on modified pikovskaya's medium (0.50gL^{-1} yeast extract, 10.0 gL^{-1} dextrose, 5.0 gL^{-1} Ca_3PO_4, 5.0 gL^{-1} $(NH_4)_2SO_4$, 0.1 gL^{-1} $MgSO_4$.$7H_2O$, 0.2 gL^{-1}, KCl, 15.0 gL^{-1}agar agar and 1000mL of distilled water at pH 7.0) as described by Karpagam T et al. [14]. Isolates showing phosphate solubilizing ability were spot inoculated at the centre of Pikovskaya's agar plate and incubated at 37°C. Diameter of clearance zone were measured successively after 24 hours, up to 7 days. The Phosphate Solubilization Index (PSI) which is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter were measured [14].

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{Colony diameter} + \text{halo zone diameter}}{\text{Colony diameter}}$$

3. RESULTS

The quantitative estimates of the microbial population (Table 1) have shown that the culturable bacterial community present in 1 g of sludge samples were in the range of 1.03 ± 0.09 x10^3 cfu/g to 2.19 ± 0.88 x10^3 cfu/g for Total Heterotrophic Bacterial Count, 1.50 ± 0.42 x10^3 cfu/g to 2.27 ± 0.03 x10^3 cfu/g for Salmonella Count, 3.45 ± 0.49 x10^3 cfu/g to 5.45 ± 0.35 x10^3 cfu/g for Total Coliform Count, 2.60 ± 0.14 x10^3 cfu/g to 7.45 ± 0.78 x10^3 cfu/g for Pseudomonad Count and 1.63 ± 0.74 x10^3 cfu/g for Total Yeast and Mould Count. Further examinations were carried out on the cultures and comparison of results with Bergey's Manual showed that of the eight gram-negative bacilli isolated, two were identified as genus of *Pseudomonas* while the remaining six belong to the following genera *Salmonella, Shigella, Aeromonas, Klebsiella, Citrobacter,* and *Providentia*. All gram-positive bacilli isolated were identified as genus of Bacillus, while the Gram Positive cocci isolated were identified as genus of *Micrococcus, Staphylococcus, Streptococcus, Enterococcus* respectively (Table 2).

The sequencing results of 16S rRNA gene based analysis showed a total of fifteen (15) Operational Taxonomic Unit (OTUs) in the sludge sample. As shown in Fig. 2, phylum Firmicutes was the most abundant with a relative abundance of 65.39% represented mainly by the families Listeriaceae, Lactobacillaceae, Bacillaceae and Clostridiaceae (Fig. 3) followed by phylum Proteobacteria with a relative abundance of 10.31% represented mainly by the families Enterobacteriaceae, Rhizobiaceae and Bradyrhizobiaceae (Fig. 3). The least abundant phylum in the sludge sample was Euryarchaeota with a relative abundance of <0.01% represented mainly by the families Methylobacteriaceae and Thermococcaceae (Fig. 3).

A typical result of a confirmation assay for phosphate solubilizing potentials of the 10 isolates (selected due to their ability to solubilize phosphate in Pikovskaya's broth medium was carried out on Pikovskaya's agar plates. Phosphate solubilization index ranged from 0.86 to 6.3 for bacterial isolates (Fig. 5) and from 2.5 to 3.8 for fungal isolates (Fig. 6) after incubation for a period of 24hrs to 120hrs. Streptococcus pyogenes had the highest solubilization index of 6.3 at incubation period of 24hrs followed by Shigella sp with solubilization index of 3.9 at the same incubation period. The least (0.86) solubilization index was observed in Enterococcus faecium after 24 hours of incubation (Fig. 5). Among the fungal isolates, Candida sp had the highest solubilization index of 3.8 at incubation period of 96 hours followed by Candida famata with solubilization index of

3.0 at incubation period of 72hours. On the other hand, *Rhizopus* sp had the least solubilization index of 1.3 at incubation period of 48 hours (Fig. 6).

4. DISCUSSION

As revealed by the metagenomics analysis (Figs. 2-4) carried out in this study, *Rhizobia* species of the genera Rhizobium, Mesorhizobium, Bradyrhizobium were found in abundance in LUDWTPS. Rhizobia species were probably gotten from the soil via the influent wastewater from which sludge was produced. They are normal flora of soil where they work in close association with leguminous plants to fix nitrogen [15]. The role of the Rhizobia group in biological nitrogen fixation cannot be overstated. Nitrogen is one of the prime elements required essentially for the synthesis of enzymes, proteins, chlorophyll, DNA and RNA. And hence, Nitrogen plays a critical role in determining the health of living organisms including microbes and plants. For nodulating legumes, the Nitrogen demand is fulfilled through symbiotic Nitrogen fixation (SNF) wherein atmospheric N2 is converted to usable forms by nitrogenase of Rhizobia [16]. The biological nitrogen fixation accounts for about 65% of the total nitrogen currently utilized in agricultural practices which of course is believed to be continuously required in future sustainable crop production systems [17]. Other plant growth-enhancing traits for which Rhizobia have been exploited includes synthesis of siderophore and solubilization of inorganic phosphorous [18] and as biocontrol agents. Candida sp, Aspergillus sp and Penicillum sp represent the fungal communities encountered in this study. Although these strains were not found to be pathogenic, the presence of other pathogenic species has been reported in sewage sludge [19].

Table 1. Microbial counts of the sludge samples obtained from lower Usuma dam water treatment plant Abuja Nigeria

Sample	THBC X 10^3 mean count ± S.D (CFU/g)	TYMC X 10^3 mean count ± S.D (CFU/g)	TCC X 10^3 mean count ± S.D (CFU/g)	TSC X 10^3 mean count ± S.D (CFU/g)	TPC X 10^3 mean count ± S.D (CFU/g)	TSSC X 10^3 mean count ± S.D (CFU/g)
1	2.19 ± 0.88	1.63 ± 0.74	3.45 ± 0.49	4.40± 1.60	3.96 ± 3.5	1.50 ± 0.42
2	1.34 ± 0.04	-	5.45 ± 0.35	4.90 ± 1.13	2.6 ± 0.14	2.27 ± 0.03
3	1.03 ± 0.09	-	-	2.55 ± 0.35	7.45± 0.78	-

Values are means of duplicate plate counts with standard deviation

Key: THBC: Total Heterotrophic Bacterial Count; TYMC: Total Yeast and Mould Count; TCC: Total Coliform Count; TSC: Total Staphylococcal Count; TPC: Total Pseudomonad Count; TSSC: Total Salmonella Shigella Count; S. D = Standard deviation

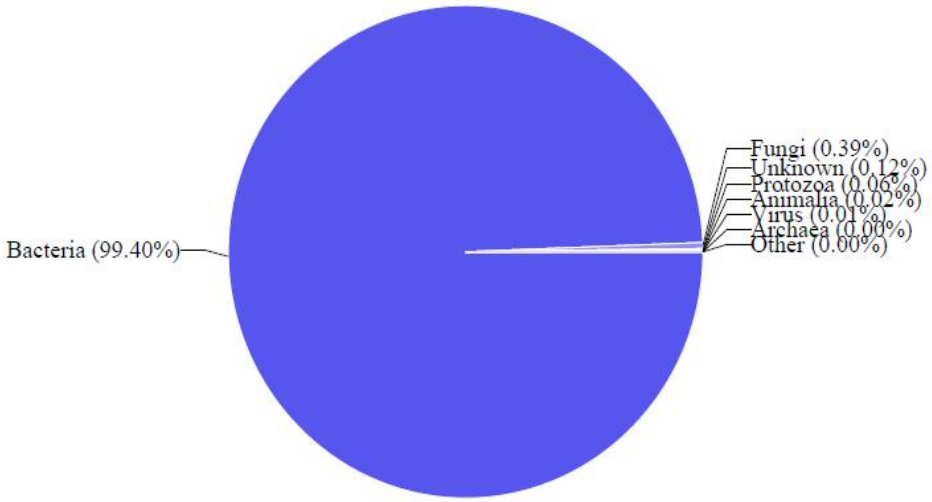

Fig. 2. Distribution and percentage occurrence by phylum of the whole microbial community in sludge sample obtained from lower Usuma dam Water treatment plant located in Abuja Nigeria

Table 2. Culturable microbial communities recovered from sludge obtained from Lower Usuma dam Water treatment plant located in Abuja Nigeria

Phylum	Family	Genera	Species
Firmicutes	Micrococcaceae	Micrococci	*Micrococcus*sp
	Bacillaceae	Bacilli	*Bacillus cereus, Bacillus megaterium*
	Streptococcaceae	Streptococci	*Streptococcus pyogenes, Streptococcus* sp
	Enterococcaceae	Enterococci	*Enterococcus faecium, Enterococcus* sp,
	Staphylococcaceae	Staphylococci	*Staphylococcus aureus, Staphylococcus epidermidis,*
Proteobacteria	Enterobacteriaceae	Salmonellae, Shigellae, Klebsiellae, Citrobacter, Providentiae	*Salmonella* sp, *Shigella sp* *Klebsiella oxytoca, Citrobacter diversus, Providentia rettgeri*
	Pseudomonadaceae	Pseudomonae	*Pseudomonas aeruginosa, Pseudomonas flourescens*
	Aeromonadaceae	Aeromonae	*Aeromonas* sp
Ascomycota	Saccharomycetaceae	Candida	*Candida famata, Candida sp, Candida succiphila*
	Trichocomaceae	Penicillium	*Penicillium* sp
Zygomycota	Mucoraceae	*Rhizopus*	*Rhizopus* sp

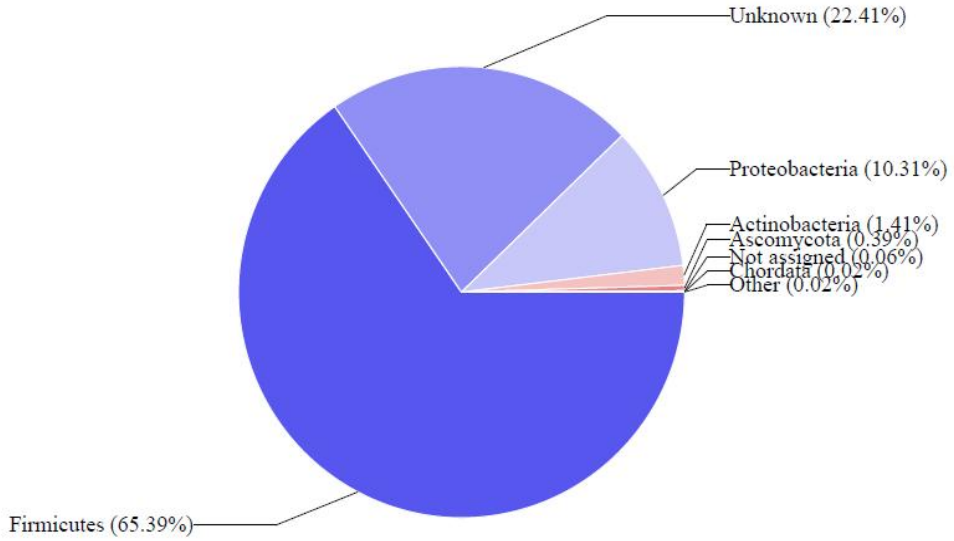

Fig. 3. Distribution and percentage occurrence by family of the whole microbial communities in sludge sample obtained from lower Usuma dam Water treatment plant located in Abuja Nigeria

Phosphorus is a plant macronutrient that plays a significant role in plant metabolism, ultimately reflected on crop yields. It is important for the functioning of key enzymes that regulate the metabolic pathways [20]. The uptake of phosphorus by the plant is only a small fraction of what is actually added as phosphate fertilizer. The remaining phosphorus is later converted to insoluble forms of phosphates and lost in the soil due to adsorption, precipitation, or conversion to organic phosphates. Soil microorganisms play an important role in making the phosphorus available to plants by mineralizing the organic phosphorus in the soil. Several researchers have reported the crucial role of microbes in making phosphorous available to plants in utilizable form

Vatsyayan N et al. [2], Yoon MH et al. [3], Karpagam T et al. [14], Poonam AS et al. [21], Mardad I et al. [22]. The phosphate solubilizing activity is determined by the microbial biochemical ability to produce and release organic acids, which through their carboxylic groups chelate the cations (mainly Ca), bound to phosphate converting them into the soluble forms Kpomblekou K et al. [23]. In this study, the solubilization index recorded ranged from 0.92 to 6.3. PSB1 (Bacillus sp) 3.0, PSB3 (Bacillus megaterium) 3.0 and PSB 8 (Enterococcus sp) 6.3 exhibited the best phosphate solubilisation on pikovskaya medium. Although phosphate solubilisation index was generally higher than those reported by Yoon MH et al. [3] and Karpagam T et al. [14]. PSF1 (Candida sp) 3.7 and PSF3 (*Candida succiphila*) 2.7 exhibited the best phosphate solubilisation on Pikovskaya medium. The research findings have shown that members of genus Bacillus displayed higher solubilisation capabilities over the course of the experiment compared to Enterococcus and the fungal solubilizers. This finding agrees with the work of Vatsyayan N et al. [2], Yoon MH et al. [3], Karpagam T et al. [14], Mardad I et al. [22] and Chen YP et al. [24]. Hence, presence of these organisms in rhizosphere might be beneficial to plant phosphorous nutrition and growth. The findings have also shown that few sludge microbes have poor phosphate solubulization efficiency (PSE), and it is suggestive of their weak or little potential to enhance or stimulate heterotrophic mineralization of organic sources of nutrients.

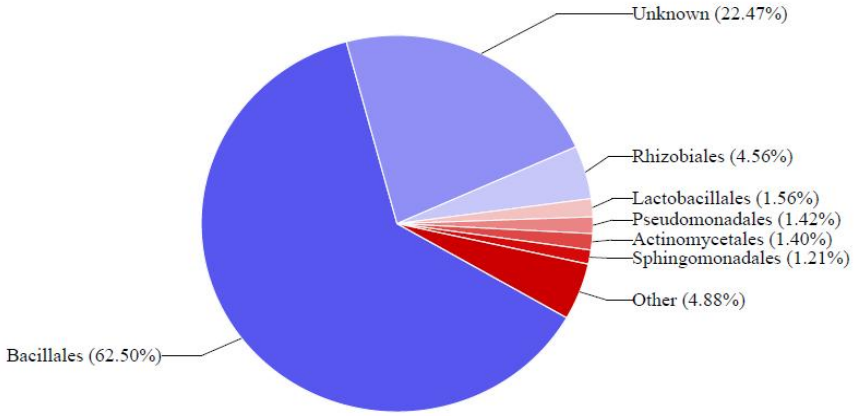

Fig. 4. Distribution and percentage occurrence by order of the whole microbial communities in sludge sample obtained from Lower Usuma dam Water treatment plant located in Abuja Nigeria

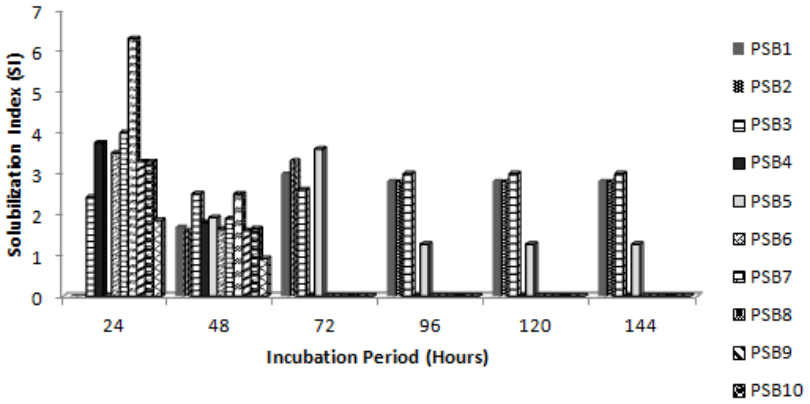

Fig. 5. Solubilization index of phosphate solubilizing bacterial isolates on pikovskaya plates. Values given are the means (n = 3) ± standard deviation

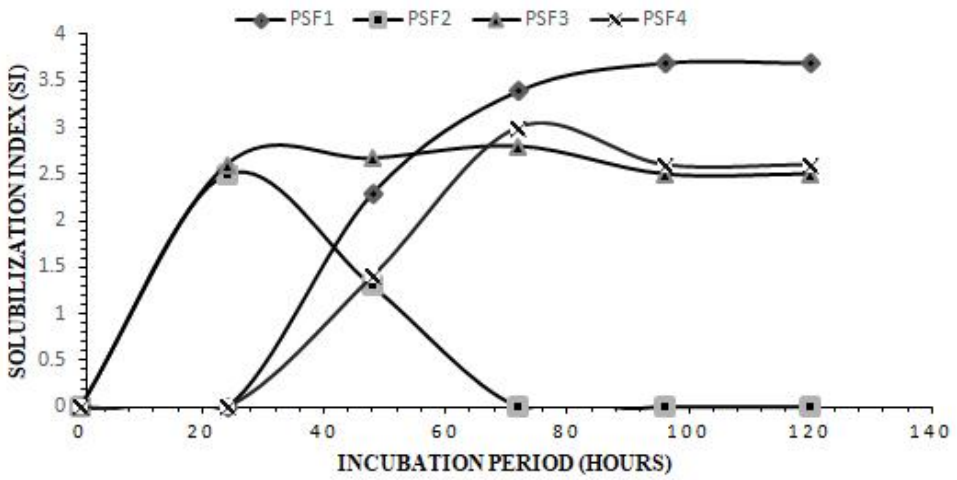

Fig. 6. Solubilization index of phosphate solubilizing fungal isolates on pikovskaya plates. Values given are the means (n = 3) ± standard deviation

5. CONCLUSION

The research findings have shown the nitrogen fixing bacteria from the paraphyletic group Rhizobia represented by Bradyrhizobiaceae, Rhizobiales and Mesorhizobium and bacteria from the Bacillus genera to represents the bulk of heterotrophic bacteria in sludge. The role of these versatile microorganisms in biogeochemical cycling has been researched extensively and established. Although sewage sludge is rich in plant nutrients, the heterotrophic potential of these beneficial microorganisms could be harnessed to enhance the use of sludge as a biofertilizer and soil conditioner for land reclamation and bioremediation, especially of the crude oil contaminated wetlands in the Niger Delta region of Nigeria.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bashan Y. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv. 1988;16:729-770.
2. Vatsyayan N, Ghosh AK. Isolation and characterization of microbes with biofertilizer potential. IOSR J. Env. Sci., Toxicol. Food Technol. 2013;7(4):05-09.
3. Yoon MH, Walpola BC. In vitro Solubilization of inorganic phosphates by phosphate solubilizing microorganisms. Afri J. Microbiol. Res. 2013;7(27):3534-3541.
4. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 2003;255:571–586.
5. Whipps JM. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 2001;52:487–511.
6. Stefanis C, Alexopoulous A, Voidaiou C, Bezirlzoglou E. Principal methods for isolation and identification of soil microbial communities. Folia Microbiologica. 2012; 58(1):1-13.
7. Casey TJ. Unit treatment processes in water and wastewater engineering. John

Wiley & Sons Ltd, Chichester, England; 1997.
8. APHA. Standard methods for examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York; 2005.
9. Fatunla K, Inam E, Essien J, Dan E, Odon A, Kang S, Semple K. Influence of composting and thermal processing on the survival of microbial pathogens and nutritional status of Nigeria sewage sludge. International Journal of Recycling of Organic Waste in Agriculture. 2017;6:301-310.
10. Cappuccino JG, Sherman N. Microbiology: A laboratory manual. 6th Edition. Pearson Education, Inc., Singapore. 2002;544.
11. Cheesbrough M. District laboratory practice in tropical countries part 2. Cambridge University press, UK. 2006; 45-70.
12. Holt JG. Facultative anaerobic gram-negative rod. In: Bergey's manual of Determinative Bacteriology. 9th Ed. Williams and Wilkins, Baltimore. 1994; 99-179.
13. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 1991;173(2):697–703.
14. Karpagam T, Nagalakshmi PK. Isolation and characterization of phosphate solubilizing microbes from agricultural soil. Int. J. Curr. Microbiol. App. Sci. 2014;3(3): 601–614.
15. Hassen AI, Bopape FL, Trytsman M. Nodulation study and characterization of rhizobial microsymbionts of forage and pasture legumes. W. J. Agricul. Res. 2014; 2(3):93-100.
16. Shiferaw B, Bantilan MC, Serraj R. Harnessing the potential of biological nitrogen fixation for poor farmers: technological policy and institutional constraints and research need. In: Serraj, R. (ed) symbiotic nitrogen fixation: Prospects for enhanced application in tropical agriculture. Oxford and IBH, New Delhi. 2004;3.
17. Matiru VN, Dakora FD. Potential use of Rhizobial Bacteria as promoters of plant growth for increased yield in landraces of african cereal crops. Afri. J. Biotechnol. 2004;3:1–7.
18. Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA. Plant growth promotion by phosphate solubilizing fungi. Arc. Agr. Soil Sci. 2010;56:73–98.
19. United State Environmental Protection Agency. Environmental regulations and technology. Control of Pathogens and Vector Attraction in Sewage Sludge (Including Doestic Septage), Under 40 CFR part 503; 1989.
20. Theodorou ME, Plaxton WC. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiol. 1993;101:339-344.
21. Poonam AS, Ghosh AK. Screening and assessment of phosphate solubilising microbes as potential biofertilizer, isolated from selected wetland and rain-fed ecosystem of Bihar. Asian J. Exp. Biol. Sci. 2012;3(2):397-406.
22. Mardad I, Aurelio Serrano A, Soukri A. Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a moroccan mineral phosphate deposit. Afri. J. Microbiol Res. 2013;7(8):626-635.
23. Kpomblekou K, Tabatabai MA. Effect of organic acids on release of phosphorus from phosphate rocks. Soil Sci. 1994; 158:442–453.
24. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Els. J. App. Soil Eco. 2006;34: 33–41.

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