Proceedings of the Nigerian Academy of Science – PNgAS. Vol. 17, No 1s, 2024
Official Journal of the Nigerian Academy of Science

https://doi.org/10.57046/XKHK1386

Original Research Article

Profiling the biocontrol agents, nitrogen-fixing bacteria, and indole acetic acid-producing bacteria in anthill soil

Emmanuel E. Imade[1], *Ben J. Enagbonma[2], Dorin I. Ediagbonya[1], Emmanuel U. Molokwu[1], Cynthia Nwodo[1], Elo-Oghene Ejuwehwe[1], & Chijioke S. Udokwu[1]

Affiliation

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria.

[2]Department of Environmental Management and Toxicology; Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria

***For correspondence**: **email**: benjamin.enagbonma@uniben.edu; **tel**: +234 (0) 913 324 8167

Abstract

Due to the adverse ecological impacts of prolonged and excessive use of agrochemicals, many researchers have called for the adoption of eco-friendly materials in farming. Consequently, the aim of this study is to profile the biocontrol agents, nitrogen-fixing, and indole-acetic-acid-producing bacteria present in anthill soil. Anthill soils and adjacent soils were collected, and their physicochemical properties were analysed using standard analytical methods. Viable bacteria were isolated and screened for plant growth-promoting (PGP) activity using standard biochemical, morphological, and bacteriological methods. The PGP capacity of the isolates was evaluated using standard protocol for nitrogen fixation and indole-acetic-acid production, while antagonistic effect against plant pathogens was evaluated using the disk diffusion assay. Results revealed that *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Bacillus* sp. isolated from the anthill soil demonstrated PGP characteristics including IAA production and nitrogen fixation. *Bacillus* sp. exhibited zones of clearance with values of 20.00 ± 1.50 mm, 20.00 ± 1.75 mm, 17.00 ± 1.33 mm, and 17.00 ± 1.33 mm against *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., and *Fusarium* sp., respectively. This study demonstrated that anthill soils contain beneficial microbes with the potential to stimulate plant growth and suppress soil-borne plant pathogens.

Keywords: *Artificial fertilizer, bioengineer, environmental degradation, soil analysis, soil bacteria.*

INTRODUCTION

Soil nutrients are depleting as the global population rises, and farmers are unable to adequately improve the soil health due to a scarcity of arable lands, which prevents fallowing (Amoo et al. 2021). Thus, the primary approach for boosting crop production is the use of synthetic agrochemicals (Zhang et al. 2017). However, the prospective attainment of this stratagem is low due to the adverse ecological impacts of prolonged and excessive use of agrochemicals. As a result, many researchers have advocated for the use of eco-friendly materials in farming (Enebe and Babalola 2022; Enagbonma et al. 2024). This has pushed subsistence farmers to augment their farmland with inexpensive and readily available materials in an effort to increase agricultural yield (Chisanga et al. 2019; Trujillo-Tapia and Ramírez-Fuentes 2016).

https://doi.org/10.57046/XKHK1386

In contemporary ages, soils engineered by bioturbators like termites (Enagbonma et al. 2020b) and ants (Katun et al. 2020) have been presented to local farmers in sub-Sahara region for cultivating vegetables and fruits on top of anthills (Apori et al. 2020; Mitchell et al. 2019). This recommendation is based on the high nutrient contents of these soils (Fernandez-Bou et al. 2019; Enagbonma et al. 2021). Soil ants build anthills by excavating soils from below which they mix with a blend of their faeces, saliva, and partially digested food (Turay et al. 2022a). During anthill construction, there is an ascending movement and repositioning of organic and mineral resources along the soil profile (Santamaría et al. 2020). This improves the chemical and physical properties of anthill soils, which has led to claims by a number of authors that anthill soils are richer in soil minerals and nutrients than adjacent soils (Urbańczyk and Szulc 2023; Turay et al. 2022b, a).

Nutrient cycling and parasite control are two critical functions ecosystems perform for humans. Nitrogen is one of the most extensively studied macronutrients due to its diverse interactions with ecosystem functioning (Jetten 2008). Along its biogeochemical pathway, nitrogen is reduced to ammonium cation (NH^{+}_{4}), and oxidized into nitric oxide (NO), nitrite ion (NO^{-}_{2}), nitrous oxide (N_2O), and nitrate (NO^{-}_{3}) (Guo et al. 2013). Nitrogen disperses through processes such as erosion, leaching, wind, or transport by animals, affecting various parts of the Earth's system (Gu et al. 2013). The nitrogen cascade is interrupted only when nitrogen is integrated into clay minerals, humus, or biomass. Due to its multiple links to the environment, nitrogen control on farms is challenging, as it gradually returns to the atmosphere as N_2O, NH_3, NO_2, and NO (Mosier et al. 2002).

Many microorganisms produce indole-acetic-acid (IAA), a common product of L-tryptophan metabolism which they synthesize in order to influence the physiological processes of the host for their own advantage (Widawati 2020). IAA promotes root elongation, increases the number of lateral roots and root hairs, which play a crucial role in water and nutrient uptake (Laird et al. 2020). IAA kindles cell elongation by decreasing wall pressure, increasing water absorption into the cell, boosting cell osmotic content, and enhancing protein synthesis (Elsoud et al. 2023). Additionally, IAA delays leaf abscission, promotes flowering, and accelerates fruiting (Keswani et al. 2020).

While knowledge of the chemical and physical properties of anthill soils has increased, the same cannot be said of the microbial diversity and their roles as biocontrol and biofertilizer agents (Wasoontharawat 2017). Research has shown that soil microbial diversity is a crucial indicator for evaluating environmental functions and processes like decomposition, mineralization, and plant growth, by suppressing disease-causing organisms (Enagbonma et al. 2020a; Enagbonma et al. 2019; Enagbonma et al. 2021). Thus, in this research, we aim to profile the biocontrol agents, nitrogen fixing and indole acetic acid producing bacteria present in anthill soil. Biocontrol and biofertilizer materials consist of live microorganisms which stimulate the amount of essential minerals to plants, regulate soil biological processes which promote plant health, and produce biochemicals (like antibiotics) that combat viruses, nematodes, bacteria, and fungi which are capable of affecting plant (Pirttilä et al. 2021; Bajracharya 2019; Imade and Babalola 2021). In this study, we also aim to test the hypothesis that anthill soil bacteria will exhibit a favourable response to indole acetic acid production, nitrogen fixation, and suppression of plant soil pathogens.

https://doi.org/10.57046/XKHK1386

MATERIALS AND METHODS

Study locations and soil collection

Soil coil was used to obtain 50g of soils from eight anthills at a depth of 0 - 15 cm from two locations (that is; four anthills from each location were sampled) in Benin City, Nigeria. The locations were Ugbowo (Lat. 6^0 23^1 45^{11} North, long. 5^0 36^1 54^{11} East) and Ekosodin (Lat. 6^0 23^1 42^{11} North, long.5^0 36^1 49^{11} East). For comparison, the corresponding adjacent soil samples which were 10 m away from the anthill (Enagbonma et al. 2021) were also collected from Ugbowo and Ekosodin at 0 – 15 cm depth. This depth (0 – 15 cm) was selected since the bulk of microbial diversity and activity occurs between 0 – 15 cm depth (Enagbonma and Babalola 2022). During the sampling period, the obtained samples were temporarily stored in cooler cases with ice packs and transported to the laboratory the same day for physicochemical analysis and isolation of bioagent.

Examination of soil properties

After eradicating woody materials and debris from the collected samples, 20 g of the soil samples were used for physicochemical evaluation. The pH of the soil was tested by creating a slurry of soil at a water to soil ratio of 2.5:1 and measuring using a pH metre, while the nitrogen content was assessed using the Kjeldhal method. Extracts of the exchangeable cations (calcium and magnesium), obtained from 1 mole of ammonium acetate were analyzed with an atomic absorption spectrophotometer (AAS). The available phosphorus was estimated using the spectrophotometric method, and organic carbon content was assessed following the technique described by Okoduwa et al. (2022) and Wakung'oli et al. (2020).

Microbial isolation and characterization

One gram of soil was used for serial dilution up to the 5^{th} dilution. The aliquot was then inoculated onto various sterile agar plates, including eosin methylene blue agar, MacConkey agar, nutritional agar, and plate count agar, followed by incubation at 37 °C for 24 hours. Distinct colonies were then sub-cultured to get pure culture for further investigation. Bacterial isolates were described based on their morphological and biochemical characteristics. This included Gram staining, methyl red test, coagulase test, catalase test, Voges-Proskauer test, triple sugar iron test, urease test, indole test, oxidase test, and citrate utilisation test (Okoduwa et al. 2022; Luo et al. 2022).

Screening of anthill soil bacteria for PGP features

Screening for IAA production

IAA production screening was conducted by reacting a liquid culture of bacterial isolates grown in 500 mg/L L-tryptophan with Salkowski's reagent (50 mL of 35% perchloric acid + 1 mL of 0.5 M $FeCl_3$ solution) and tryptic soy broth (1 g/L MES hydrate, pH 6). The inoculated broth was incubated for 72 hours at 30 °C in a rotary shaker. After incubation, the broth was centrifuged for 15 minutes at 1200 × g. Then, 2.0 mL of Salkowski reagent was mixed with 1.0 mL of the supernatant, and the mixture was incubated for 25 minutes at room temperature. IAA production was confirmed by the appearance of a pink color after incubation (Widawati 2020).

Screening for nitrogen fixation activity

A one-day-old culture of bacterial isolates grown on nutrient agar was streaked on a Jensen's nitrogen free medium otherwise known as NFM (formulated via the addition of: 20g/L sucrose, 1g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L NaCl, 0.1 g/L $FeCl_3$, 0.005g/L $Na_2MoO_4.2H_2O$, 2g/L $CaCO_3$, 15g/L agar). Plates were incubated at 28 °C for 1-7 days. Growth on the nitrogen-deficient

https://doi.org/10.57046/XKHK1386

medium confirms the ability to fix nitrogen (Estrada-De Los Santos et al. 2001)

Screening of bacteria for antimicrobial activities against pathogens

The ability of anthill-borne *Bacillus* sp. to inhibit the *in vitro* growth of selected plant pathogens was investigated using an agar well diffusion assay. The test bacteria were *Enterobacter* sp., *Serratia* sp., and *Pseudomonas* sp., while the fungal isolates were *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., and *Fusarium* sp. For the well diffusion test, Mueller-Hinton Agar was used for bacteria and Sabouraud dextrose agar was used for fungi. The inoculum was prepared by suspending the test bacteria/fungi in a sterile saline solution (0.85%). The turbidity of the suspension was adjusted with a spectrophotometer at 530 nm to achieve a final concentration equal to that of a 0.5 McFarland standard. The agar was swabbed uniformly with sterile cotton buds after being inoculated with 1 ml of the organism suspension. A sterile cork borer was used to create three 6 mm diameter wells, and 20 µL of the antagonistic agent were placed into each well. After 24–48 hours of incubation at 35 °C, the plates were inspected for the presence of inhibition zones. Positive inhibition was determined when the diameter of the clear zone surrounding the wells was at least 0.5 mm (Imade et al. 2022).

Statistical analysis

All assays were prepared in triplicates. Descriptive statistics and Analysis of variance (ANOVA) were used to evaluate the data obtained from the study using statistical package for the Social Sciences ® v.21, PAST v. 2.17c, and Microsoft Excel v. 2010.

RESULTS

Examination of the chemical and physical properties of soils obtained from anthill and adjacent soils

The assessment of the physicochemical properties of the soil revealed that the concentrations of P, OC, Mg, OM, Ca, TKM, and K in soil samples from anthills were greater than the values in adjacent soils. On the contrary, the amounts of sand, pH, and sediment in nearby soil (S1 and S2) were greater than the amounts in soil samples from anthill (A1 and A2) (Table 1).

Table 1: Chemical and physical properties of soils obtained from anthill and adjacent soils

Soil parameters	A1	S1	A2	S2
pH	5.85 ± 0.38	6.50 ± 0.10	6.23 ± 0.19	6.68 ± 0.13
OC (%)	1.77 ± 0.05	1.38 ± 0.21	0.82 ± 0.10	1.46 ± 0.01
OM (%)	3.01 ± 0.08	2.39 ± 0.36	2.52 ± 0.02	1.43 ± 0.14
TKN (%)	1.97 ± 0.32	1.34 ± 0.26	2.25 ± 0.12	1.40 ± 0.31
P (mg/L)	35.84 ± 12.94	25.63 ± 4.42	39.83 ± 3.29	15.18 ± 3.53
Ca (mg/L)	0.32 ± 0.03	0.23 ± 0.03	0.33 ± 0.05	0.14 ± 0.02
K (mg/L)	6.05 ± 0.25	5.19 ± 0.49	5.21 ± 0.03	3.05 ± 0.16
Mg (mg/L)	0.94 ± 0.04	0.83 ± 0.07	0.90 ± 0.05	0.80 ± 0.35
Sand (%)	93.5 ± 1.12	95.25 ± 1.30	92.50 ± 1.12	94.00 ± 1.22
Silt (%)	1.48 ± 0.26	2.45 ± 0.31	1.46 ± 0.25	1.78 ± 0.34
Clay (%)	4.28 ± 1.27	3.06 ± 1.81	4.83 ± 1.43	5.45 ± 0.70

Total bacterial count and occurrence in anthill and adjacent soils

Generally, the entire bacterial count in A1 (6.67 ± 0.07) was significantly higher than the entire bacterial count in S1 (6.32 ± 0.09). While the entire bacterial count in A2 (6.42 ± 0.05) was higher

https://doi.org/10.57046/XKHK1386

than the entire bacterial count in S2 (6.39 ± 0.04), but the difference was not significant (Fig. 1). Isolates observed in all soil samples by using the standard bacteriological, morphological, and biochemical methods included *Corynebacterium* sp., *Klebsiella pneumoniae*, *Citrobacter* sp., *Micrococcus luteus*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Shigella boydii*, and *Bacillus* sp. (Fig 2). However, *Salmonella* sp., *Serratia* sp., and *Enterobacter* sp. predominated the adjacent soils.

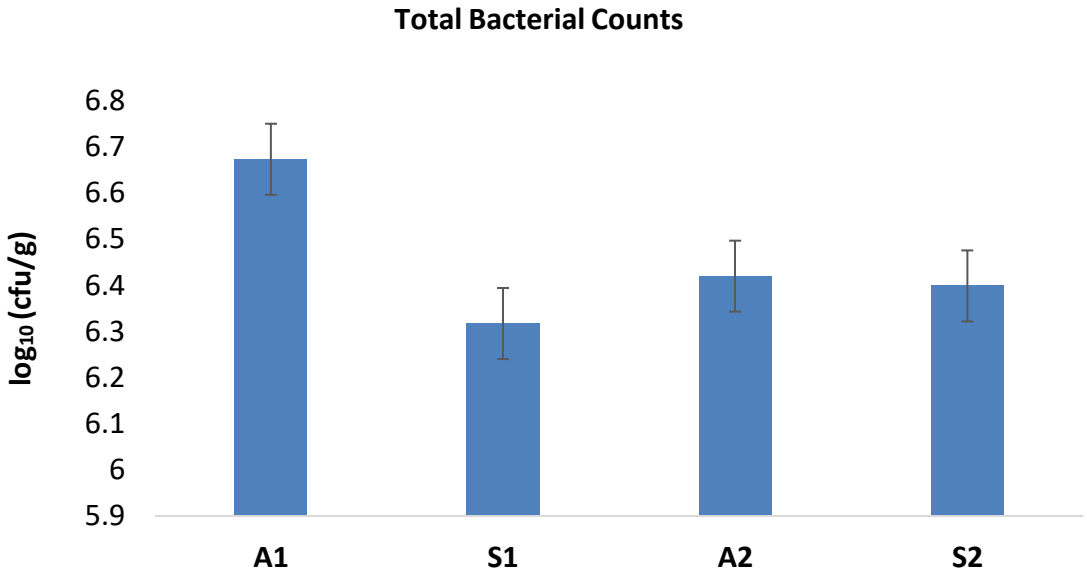

Figure 1: Aggregate bacterial count in A1, A2, S1, and S2. A1 and A2 represent soil samples from anthills at Ekosodin and Ugbowo, correspondingly, whereas S1 and S2 represent adjacent soils from Ekosodin and Ugbowo, respectively.

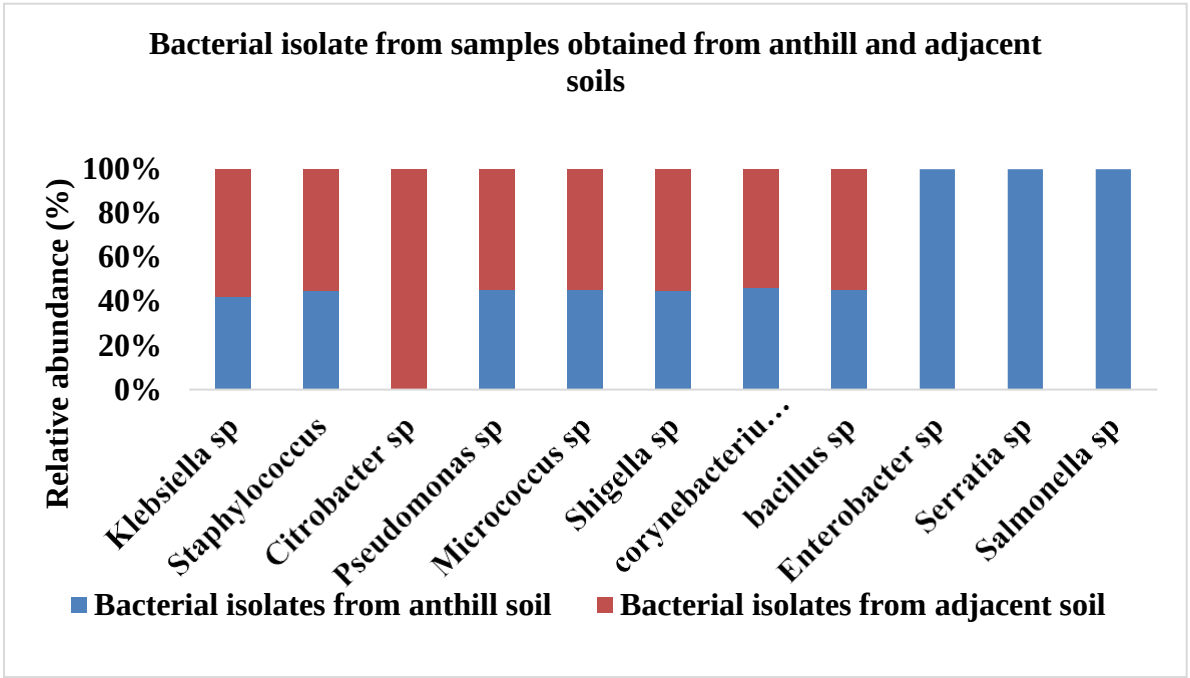

Figure 2: Proportion rate of bacterial isolate from samples obtained from anthill and adjacent soils

https://doi.org/10.57046/XKHK1386

Plant growth-promoting features

All bacterial isolates extracted from anthill and adjacent soils were shown to possess 100% capacity for nitrogen fixation, while 42.8% of the isolates demonstrated the capability to produce indole-acetic-acid. **(Table 3).**

Table 3: Indole acetic acid and nitrogen fixating bacterial isolates obtained from anthill and adjacent soils

Bacterial isolates	Indole acetic acid production	Nitrogen fixation
Pseudomonas aeruginosa	+	+
Klebsiella pneumoniae	+	+
Shigella boydii	-	+
Bacillus sp.	+	+
Micrococcus luteus	-	+
Staphylococcus sp.	-	+
Corynebacterium sp.	-	+

KEY: + (Present/Positive) - (Absent/ Negative) Antagonistic activities of anthill soil

Biocontrol activity against test bacteria and fungi

Biocontrol activity of the anthill soil-borne bacteria was identified via decreased radial growth of the test potential pathogens. The *Bacillus* sp. was able to inhibit the growth of some bacterial and fungal isolates. The inhibition zone was recorded in mm. The test bacteria in this study included *Enterobacter* sp., *Serratia* sp. and *Pseudomonas* sp. Zones of clearance were observed for *Pseudomonas* sp. and *Enterobacter* sp. with values of 20.00 ± 1.75 mm and 17.00 ± 1.33mm, respectively. Furthermore, the tested fungal isolates in this study include *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp. and *Fusarium* sp. with inhibition zones of 17.00 ± 1.33 mm 20.00 ± 1.75 mm, 20.00 ± 1.50 mm, and 17.00 ± 1.33 mm against *Aspergillus niger*, *Trichoderma* sp., *Penicillium* sp., and *Fusarium* sp. respectively. The zone of clearance for the bacterial isolates were 12 ± 16 mm, 0 ± 00 mm, and 14 ± 09 mm for *Enterobacter* sp., *Serratia* sp., and *Pseudomonas* sp., respectively. This is presented in Figures 4 and 5.

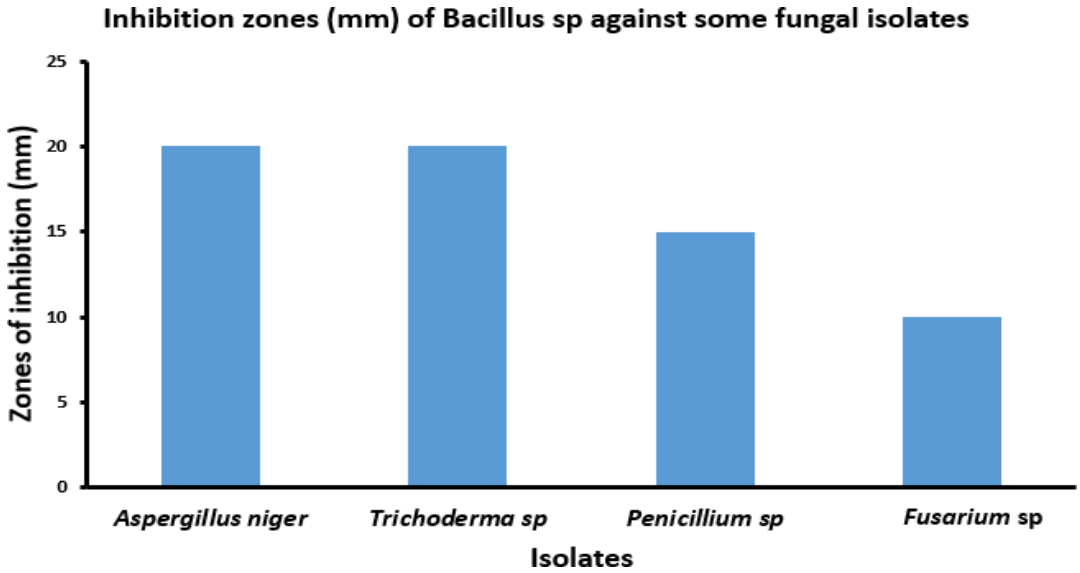

Figure 4: Inhibitory effect of *Bacillus* sp. against some fungal pathogens

https://doi.org/10.57046/XKHK1386

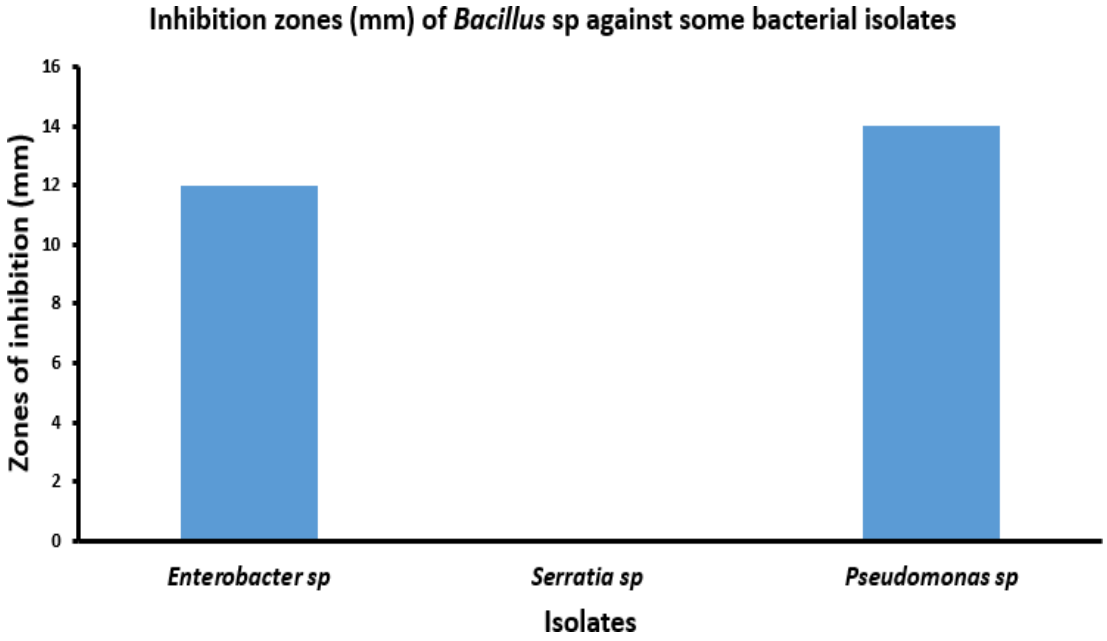

Figure 5: Inhibitory effect of *Bacillus* sp. against some bacterial pathogens

DISCUSSION

This investigation assessed the potential of anthill soil as a likely reservoir for biocontrol agents and biofertilizers. Our research outcomes supported various assertions that claim that anthill soils are gold mine for soil nutrient compared to adjacent soils (Chisanga et al. 2017; Kristiansen and Amelung 2001). These concentrations of nutrients in anthill soils are instigated by the bioturbation of ants and their distribution to the nearby soil is impacted by soil erosion, the kind of anthill and resident species (Ouattara et al. 2023; Nkem et al. 2000). The high nutrient concentrations in anthill soils could also account for high bacterial count in anthill soils when likened to the nearby soils. In addition, anthills are composed of partially digested food, ant saliva, and ant faeces, which contain numerous bacteria from the ant's mouthparts and stomach. Consequently, anthill soils are a fertile breeding ground for microbes (Katun et al., 2020b).

In this study, we found that anthill soils had higher clay content compared to the adjacent soils, which were sandier. This may also have contributed to the elevated bacterial levels in anthill soils, as the particle size distribution of soil has a fundamental effect on the activity of microorganism populations (Fang and Achal 2020). The ability of anthill soil bacteria in supporting plant growth were observed in this study. This became evident with the occurrence of some PGPB strains (*Salmonella* sp., *Serratia* sp., *Enterobacter* sp., *Corynebacterium* sp., *Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Shigella* sp., *Micrococcus* sp., and *Bacillus* sp.) in anthill soils. These organisms are capable of nitrogen fixation and indole-acetic acid (IAA) production. *Pseudomonas aeruginosa* solubilizes IAA which provides added benefits for their use as biocontrol agents in agricultural management (Wasoontharawat 2017).

Among the isolates, Pseudomonas sp. and Enterobacter sp. exhibited the highest biological control activity against Bacillus sp. These bacterial strains release volatile and diffusible metabolites, contributing significantly to biological control by employing various mechanisms against plant

https://doi.org/10.57046/XKHK1386

pathogens (Yang 2019). The bacterial isolates exhibit antagonistic properties by discharging extracellular cell wall degrading enzymes like chitinase and -1,3-glucanase, as well as antifungal compounds (Kumari et al. 2022; Xiao et al. 2009). It has been demonstrated that the presence of these soil-valuable microorganisms and rich nutrient materials in anthill soil increases crop yield and can therefore be used as biological fertilisers and biocontrol agents.

CONCLUSION

This study reveals the biocontrol agents, nitrogen-fixing bacteria, and indole acetic acid-producing bacteria that exist in anthill soil. The research findings validated the hypothesis that anthill soil is a "gold mine" of soil nutrients and beneficial microbes due to ant soil-turning activities. *Bacillus* sp. and *Pseudomonas* sp. were identified as plant growth-promoting bacteria, with 100% capacity for nitrogen fixation and 42.8% ability to produce indole acetic acid. These results suggest that anthill soils hold significant potential as a source of biocontrol agents and biofertilizers and warrant further investigation to fully exploit their benefits.

Acknowledgements: Authors appreciate the University of Benin for the serene laboratory given for this project.

Authors' contributions: All authors contributed equally and approved the final manuscript.

Funding: The Alexander von Humboldt Foundation (AvH), The Federal Ministry of Education (BMBF) and The African-German Network of Excellence (AGNES) supported this project.

Data availability: The authors declare that all relevant data supporting the findings of this study are included in this article.

Ethical declarations and consent to participate: This research does not involve animal and human trials as was as social media data and it follows the ethical requirements for publication.

Consent for publication: Not related.

Competing interests: Authors declared no conflict of interest.

References

Amoo AE, Enagbonma BJ, Ayangbenro AS, & Babalola OO (2021). Biofertilizer: An eco-friendly approach for sustainable crop production. Food Security and Safety: African Perspectives:647-669

Apori SO, Murongo M, Hanyabui E, Atiah K, & Byalebeka J (2020). Potential of termite mounds and its surrounding soils as soil amendments in smallholder farms in central Uganda. BMC Research Notes 13:1-6

Bajracharya AM (2019). Plant growth promoting rhizobacteria (PGPR): Biofertiliser and Biocontrol agent-Review article. Journal of Balkumari College 8:42-45

Chisanga K, Mbega E, & Ndakidemi PA (2019). Socio-economic factors for anthill soil utilization by smallholder farmers in Zambia. Sustainability 11 (18):4849

Chisanga K, Ndakidemi PA, Mbega E, & Komakech HC (2017). The potential of anthill soils in

https://doi.org/10.57046/XKHK1386

agriculture production in Africa: A review.

Elsoud MMA, Hasan SF, & Elhateir MM (2023). Optimization of Indole-3-acetic acid production by Bacillus velezensis isolated from Pyrus rhizosphere and its effect on plant growth. Biocatalysis and Agricultural Biotechnology:102714

Enagbonma BJ, Ajilogba CF, & Babalola OO (2020a). Metagenomic profiling of bacterial diversity and community structure in termite mounds and surrounding soils. Archives of Microbiology 202:2697-2709

Enagbonma BJ, Amoo AE, & Babalola OO (2020b). Deciphering the microbiota data from termite mound soil in South Africa using shotgun metagenomics. Data in brief 28

Enagbonma BJ, Amoo AE, & Babalola OO (2021). Biopedturbation by termites affects respiration profiles of microbial communities from termite mound soils. Journal of Soil Science and Plant Nutrition 21 (3):2115-2123

Enagbonma BJ, Aremu BR, & Babalola OO (2019). Profiling the functional diversity of termite mound soil bacteria as revealed by shotgun sequencing. Genes 10 (9):637

Enagbonma BJ & Babalola OO (2022). Metagenomics shows that termite activities influence the diversity and composition of soil invertebrates in termite mound soils. Applied and Environmental Soil Science 2022

Enagbonma BJ, Fadiji AE, & Babalola OO (2024). Anthropogenic fertilization influences a shift in barley rhizosphere microbial communities. PeerJ 12:e17303. doi:10.7717/peerj.17303

Enebe MC & Babalola OO (2022). Functional diversity of bacterial communities in the rhizosphere of maize grown on a soil under organic and inorganic fertilization. Scientific African 16:e01212

Estrada-De Los Santos P, Bustillos-Cristales Ro, Caballero-Mellado J (2001). Burkholderia, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Applied and environmental microbiology 67 (6):2790-2798

Fang C & Achal V (2020). Physico-chemical aspects and complete bacterial community composition analysis of wasp nests. Sustainability 12 (7):2652

Fernandez-Bou AS, Dierick D, Swanson AC, Allen MF, Alvarado AGF, Artavia-León A, Carrasquillo-Quintana O, Lachman D, Oberbauer S, & Pinto-Tomás AA (2019). The role of the ecosystem engineer, the leaf-cutter ant Atta cephalotes, on soil CO2 dynamics in a wet tropical rainforest. Journal of Geophysical Research: Biogeosciences 124 (2):260-273

Gu B, Chang J, Min Y, Ge Y, Zhu Q, Galloway JN, & C (2013). The role of industrial nitrogen in the global nitrogen biogeochemical cycle. Scientific Reports 3 (1):2579

Guo J, Peng Y, Wang S, Ma B, Ge S, Wang Z, Huang H, Zhang J, & Zhang L (2013). Pathways and organisms involved in ammonia oxidation and nitrous oxide emission. Critical reviews in

https://doi.org/10.57046/XKHK1386

environmental science and technology 43 (21):2213-2296

Imade EE, Ajiboye TO, Fadiji AE, Onwudiwe DC, & Babalola OO (2022). Green synthesis of zinc oxide nanoparticles using plantain peel extracts and the evaluation of their antibacterial activity. Scientific African 16:e01152

Imade EE & Babalola OO (2021). Biotechnological utilization: the role of Zea mays rhizospheric bacteria in ecosystem sustainability. Applied Microbiology and Biotechnology 105 (11):4487-4500

Jetten MS (2008). The microbial nitrogen cycle. vol 10. Wiley Online Library

Katun A, Abdulmumin A, Yahaya M, Habeeb N, & Bala A (2020). Anthill inhibiting bacteria, a promising source of bio efficacy. Journal of Advance Medical and Pharmaceptical Science 22 (12):14-21

Keswani C, Singh SP, Cueto L, García-Estrada C, Mezaache-Aichour S, Glare TR, Borriss R, Singh SP, Blázquez MA, & Sansinenea E (2020). Auxins of microbial origin and their use in agriculture. Applied Microbiology and Biotechnology 104:8549-8565

Kristiansen SM & Amelung W (2001). Abandoned anthills of Formica polyctena and soil heterogeneity in a temperate deciduous forest: morphology and organic matter composition. European Journal of Soil Science 52 (3):355-363

Kumari P, Rastogi N, Singh HB, & Rajput RS (2022). Plant growth-promoting bacteria associated with the nests of the seed-harvester ant, *Trichomyrmex scabriceps*.

Laird TS, Flores N, & Leveau JH (2020). Bacterial catabolism of indole-3-acetic acid. Applied Microbiology and Biotechnology 104:9535-9550

Luo L, Zhao C, Wang E, Raza A, & Yin C (2022). *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. Microbiological Research:127016

Mitchell J, Bissett J, & Macdonald I (2019). Seasonal abundance of the harvester termite, Hodotermes mossambicus (Hagen)(Isoptera: Hodotermitidae), and its impact on vegetation in a semi-arid grassland in Zimbabwe. African Entomology 27 (1):201-217

Mosier A, Doran J, & Freney J (2002). Managing soil denitrification. Journal of soil and water conservation 57 (6):505-512

Nkem J, De Bruyn LL, Grant C, & Hulugalle N (2000). The impact of ant bioturbation and foraging activities on surrounding soil properties. Pedobiologia 44 (5):609-621

Okoduwa I, Enagbonma B, & Imade E (2022). Effects of pharmaceutical effluents on soil microbiome and physicochemical parameters. Journal of Applied Sciences and Environmental Management 26 (8):1363-1367

https://doi.org/10.57046/XKHK1386

Ouattara K, Yeo K, Kouakou LM, & Dekoninck W (2023). Ant nests effect on organic matter, carbon, and nitrogen flux in the soil under grasses tufts in Lamto savannah (Côte d'Ivoire). Agrosystems, Geosciences & Environment 6 (2):e20374

Pirttilä AM, Mohammad Parast Tabas H, Baruah N, & Koskimäki JJ (2021). Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms 9 (4):817

Santamaría C, Lachaud J-P, & Armbrecht I (2020). Effects of nest building by the dominant hunting ant, *Ectatomma* sp. 2 (*E. ruidum complex*), on Andean coffee plantations. Pedobiologia 79:150626

Trujillo-Tapia MN & Ramírez-Fuentes E (2016). Bio-fertilizer: an alternative to reduce chemical fertilizer in agriculture. Journal of Global Agriculture and Ecology 4 (2):99-103

Turay BS, Bah AM, Amara DMK, Kallon VI, & Kanu SA (2022a). Germination, growth and yield responses of eggplant and okra grown on anthill and termite mound soils. Biology and Life Sciences Forum 16 (1):13

Turay BS, Bah AM, Amara DMK, Kallon VI, & Kanu SA Germination, Growth and Yield Responses of Eggplant and Okra Grown on Anthill and Termite Mound Soils. In: Biology and Life Sciences Forum, 2022b. vol 1. MDPI, p 13

Urbańczyk DA & Szulc W (2023). Effect of anthills on changes in the physico-chemical properties in sandy soil. Soil Science Annual 74 (1):162336

Wakung'oli M, Amoo AE, Enagbonma BJ, & Babalola OO (2020) Termite societies promote the taxonomic and functional diversity of archaeal communities in mound soils. Biology 9 (6):136

Wasoontharawat M (2017). Isolation and screening phosphoric solubilizing bacteria from organic anthill fertilizer and phosphorus release capacity. SNRU Journal of Science and Technology 9 (2):454-464

Widawati S. Isolation of Indole Acetic Acid (IAA) producing Bacillus siamensis from peat and optimization of the culture conditions for maximum IAA production. In: IOP Conference Series: Earth and Environmental Science, 2020. vol 1. IOP Publishing, p 012025

Xiao X, Liu K, & Liao Z (2009). Disease-control effect and mechanism research of biological organic fertilizer on tomato bacterial wilt. Journal of Agro-Environment Science 28 (11):2368-2373

Yang W (2019). Components of rhizospheric bacterial communities of barley and their potential for plant growth promotion and biocontrol of Fusarium wilt of watermelon. Brazilian Journal of Microbiology 50 (3):749-757

Zhang Y, Shen H, He X, Thomas BW, Lupwayi NZ, Hao X, Thomas MC, & Shi X (2017). Fertilization shapes bacterial community structure by alteration of soil pH. Frontiers in Microbiology 8. doi:10.3389/fmicb.2017.01325