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PROFILING THE PHOSPHATE SOLUBILIZING AND NITROGEN-FIXING BACTERIA FROM THE RHIZOSPHERE OF *Talinum fruticosum* (L.) Juss

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ABSTRACT

Rhizospheric microorganisms play crucial roles in enhancing crop production and soil fertility maintenance and with little or no information on *Talinum fruticosum* rhizosphere microbiome. This research aimed to profile the phosphate-solubilizing and nitrogen-fixing bacteria (PSB and NFB) inhabiting the *Talinum fruticosum* rhizosphere, a common vegetable crop in many tropical and subtropical regions. Soils were extracted from the *Talinum fruticosum* plants grown in Ekosodin Secondary School Farm at Ekosodin, Benin City, Nigeria. Isolation and characterization of PSB and NFB were carried out using cultural, morphological and biochemical, selective media tests. *Bacillus* sp., *Serratia* sp., *Enterobacter* sp., and *Alcaligenes* sp. isolated from the *Talinum fruticosum* rhizosphere were screened for their phosphate solubilizing and nitrogen fixing abilities. Out of many isolates implicated in *Talinum fruticosum* rhizosphere, only *Bacillus* sp.was found to be positive for both nitrogen fixing and phosphate solubilization test while *Enterobacter* sp.was positive for only nitrogen fixing test. This study supports other studies that the rhizosphere of *Talinum fruticosum* hosts some plant growth-promoting bacteria which when harnessed may lead to sustainable agricultural strategies for improving crop yield.

Keywords: *Biofertilizers, Chemical fertilizers, Environmental health, Environmental sustainability, Food security*

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INTRODUCTION

A healthy soil is essential for creating a dynamic nutrient cycling to optimally support plant growth (Babalola *et al*., 2020). While soil is commonly thought of by plant physiologists as being a place where plants get nutrients, it is also home to a multifaceted ecosystem that includes microorganisms like bacteria, fungi, and archaea (Enagbonma *et al*., 2023). It is commonly understood that microbes (either pathogenic or beneficial) have a significant involvement in numerous biotic processes necessary to maintain the soil's biological and physiochemical stability (Morris and Blackwood, 2024). For instance, some strains of *Pseudomonas* sp., *Bacillus* sp., *Rhizobium* sp., and Mycorrhizal fungi facilitate nutrient cycling by decomposing organic matter into essential nutrients like nitrogen and phosphorus, which plants require for growth (Kuila and Ghosh, 2024; Vincze *et al*., 2024). Additionally, microbes enhance soil structure by producing sticky substances like polysaccharides that bind soil particles together, preventing erosion (Ali *et al*., 2024). Their metabolic activities also contribute to soil pH balance and detoxification of pollutants, thereby supporting a healthy soil environment (Agarwal *et al*., 2024). The study aims to identify and characterize PSB and NFB in the rhizosphere of *Talinum fruticosum*. This profiling helps understand microbial interactions crucial for plant growth and soil fertility. Insights gained can aid in sustainable agriculture practices, enhancing nutrient availability and plant health.

For crops to incorporate these imperative nutrients (nitrogen and phosphorus), they will have to depend on the proliferation of soil bacteria, notably the NFB and PSB that possess the biochemical mechanisms necessary to break down and mineralize organic compounds of phosphorus and nitrogen (Jiao *et al*., 2024). They release inorganic forms of nitrogen and phosphorus (such as phosphate and nitrate) into the soil which are mostly needed by plants (Zhao *et al*., 2024).

These PSB and NFB (examples of PGPB) live in the tissues of plants and the rhizosphere (Ghoreshizadeh *et al*., 2024; Maulida *et al*., 2024). Plant growth-promoting bacteria improve the plant's development by direct mechanisms like nitrogen fixation and phosphate solubilization. They may be employed as biological fertilizers and biological-pesticides therefore, reducing the total reliance on chemical fertilizers which prolonged and excess application can cause eutrophication of water bodies, soil degradation, and air pollution (Amoo *et al*., 2021).

To address these adverse environmental impacts of prolonged and excess application of chemical fertilizers and guarantee food security in light of the expanding population (Babalola and Enagbonma, 2024), it is vital to use eco-friendly approaches like rhizosphere microbiome that supply plants with nutrients while also being beneficial to the environment (Enagbonma *et al.*, 2024). With little or no information on *Talinum fruticosum* rhizosphere bacteria as a viable source of Biofertilizers, this pioneering study aimed at profiling the PSB and NFB in the rhizosphere of *Talinum fruticosum*, a resilient and nutritious leafy green native to the West Africa, Caribbean, Mexico, and Central and South America (Adediji, 2019). *Talinum fruticosum* thrives in warm climates, producing edible, succulent leaves rich in vitamins C and A and common in local food (Edema-Eyen *et al*., 2023).

To achieve this study aim, isolation, identification and enumeration of the total bacteria residing in *Talinum fruticosum* rhizosphere as well as screening the isolates for phosphate-solubilizing activity and nitrogen-fixing activity were

done. With *Talinum fruticosum* rhizosphere reported to grow well in almost all seasons, this study then hypotheses that *Talinum fruticosum* rhizosphere will be laden with PSB and NFB that are beneficial to plant health.

MATERIALS AND METHODS

STUDY LOCATIONS AND SOIL SAMPLING

Samples of the *Talinum fruticosum* rhizosphere soil were randomly obtained at six different sites (Tfr1, Tfr2, Tfr3, Tfr4, Tfr5, and Tfr6) at Ekosodin Secondary School Farm at Ekosodin, Benin City. Rhizosphere soil samples were extracted from *Talinum fruticosum* rhizosphere at 0 - 15cm depth (Gong *et al*., 2023). The rhizosphere samples collected were placed in ice-cooled boxes, properly labelled, and transported to the laboratory, where they were stored at a temperature of 4^{o}C within 24 hours for isolation, identification, and enumeration of the total bacteria residing in *Talinum fruticosum* rhizosphere as well as screening the isolates for phosphate solubilizing activity and nitrogen-fixing activity.

BACTERIAL ISOLATION AND ENUMERATION

The rhizosphere soil samples were weighed and diluted serially using 10-folds, where 10g of soil was added to 90 ml of sterile saline water from which 1ml of the aliquot was serially diluted by transferring to the test tubes containing 9 ml of sterile dilute water. Thereafter, an inoculum volume of 0.1 ml from the fourth tube was put into sterilized petri dishes then the nutrient agar was added (augmented by 1% fluconazole to inhibit fungal growth). Replicates of samples were prepared for bacterial plates cultured using the pour plate method. The formula for the dilution factor and the enumeration of the bacterial isolates is given below in equations (1), and (2) previously used by Okoduwa *et al*. (2022).

$$Dilution\ factor\ = \frac{final\ volume}{aliquot\ volume} \qquad (1)$$

$where: final\ volume = aliquot\ volume\ (sample\ volume)\ + diluent\ volume$

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (2)$$

PHENOTYPIC IDENTIFICATION OF BACTERIA FROM RHIZOSPHERE SAMPLES

Pure cultures of the bacterial isolates were acquired from the subculture of a single colony from the successful pour plate technique and were characterized using cultural, morphological, and biochemical tests. The Gram reaction, urease, indole, catalase, oxidase, citrate utilization, oxidase, and respective reactions of bacteria on triple sugar iron agar, were carried out to identify bacterial isolates (Abou-Shanab *et al.*, 2003). For growth on differential media, *Bacillus cereus* agar, *Pseudomonas* agar, Mannitol salt agar, and Eosin methylene blue agar were used.

NITROGEN FIXATION ACTIVITY TEST.

A 24-hour bacterial culture was subcultured on Jensen's Nitrogen medium plates, composed of 15g/L agar, 2g/L $CaCO_3$, 0.005g/L $Na_2MoO_4.2H_2O$, 0.1g/L $FeCl_3$, 0.5g/L $MgSO_4.7H_2O$, 1g/L K_2HPO_4, 20g/L sucrose, and 0.5g/L NaCl. Incubation of plates for 1-7 days was done at 28°C. Nitrogen fixation was confirmed by growth in the nitrogen-deficient medium, as described by Weselowski et al. (2016)

CONFIRMATION TEST FOR PHOSPHATE-SOLUBILIZATION ACTIVITY

Pikovaskayas agar was prepared according to a modified protocol as used by Sanchez-Gonzalez *et al*., (2022). The medium was autoclaved at 121°C for 15 minutes, then cooled. Sterile agar was poured into Petri dishes, and bacterial cultures were grown in replicates on Micromaster plates at 30°C for 3 days. Phosphate solubilization was visually assessed by the presence of clear zones around colonies after 1-3 days. The halozones diameter and corresponding bacterial colonies were calculated, and the solubilization index (SI) was measured via the previously described formula (Doilom *et al.*, 2020).

$$\mathbf{SI} = \frac{\text{diameter of colony} + \text{diameter of halozone}}{\text{diameter of colony}} \qquad (3)$$

STATISTICAL ANALYSIS

Triplicate experiments were analyzed using descriptive statistics and ANOVA, with data processed through SPSS, PAST, and Excel software.

RESULTS

TOTAL BACTERIA COUNT

The total mean bacterial count (log10 cfu/g) in the *Talinum fruticosum* rhizosphere soil obtained at Ekosodin Secondary School Farm include Tfr1 = 5.36 ± 0.00, Tfr2 = 5.13 ± 0.04, Tfr3 = 5.47 ± 0.00, Tfr4 = 5.49 ± 0.02, Tfr5 = 5.48 ± 0.01 and Tfr6 = 5.46 ± 0.00 respectively (Figure 1).

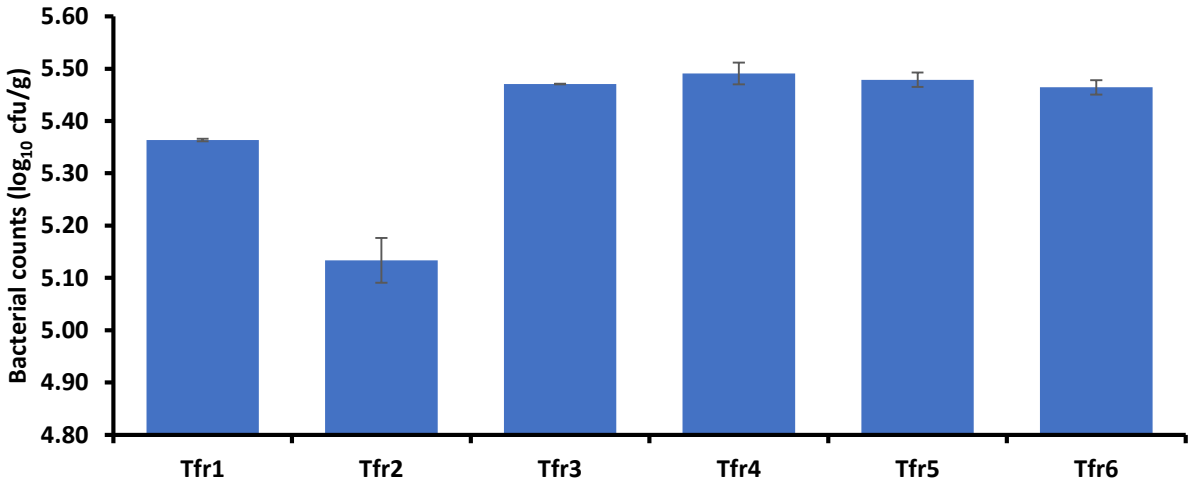


Figure 1: Total bacterial counts from rhizosphere soil (log_{10} cfu/g)

PERCENTAGE ABUNDANCE OF BACTERIA ISOLATED FROM *Talinum fruticosum* RHIZOSPHERE SOIL

The bacterial isolates obtained from *Talinum fruticosum* (and their relative abundance) include 6.25% (*Alcaligenes* sp.), 12.50% (*Enterobacter* sp.) 18.75% (*Serratia* sp.), and 62.50% (*Bacillus* sp.) as shown in Figure 2.

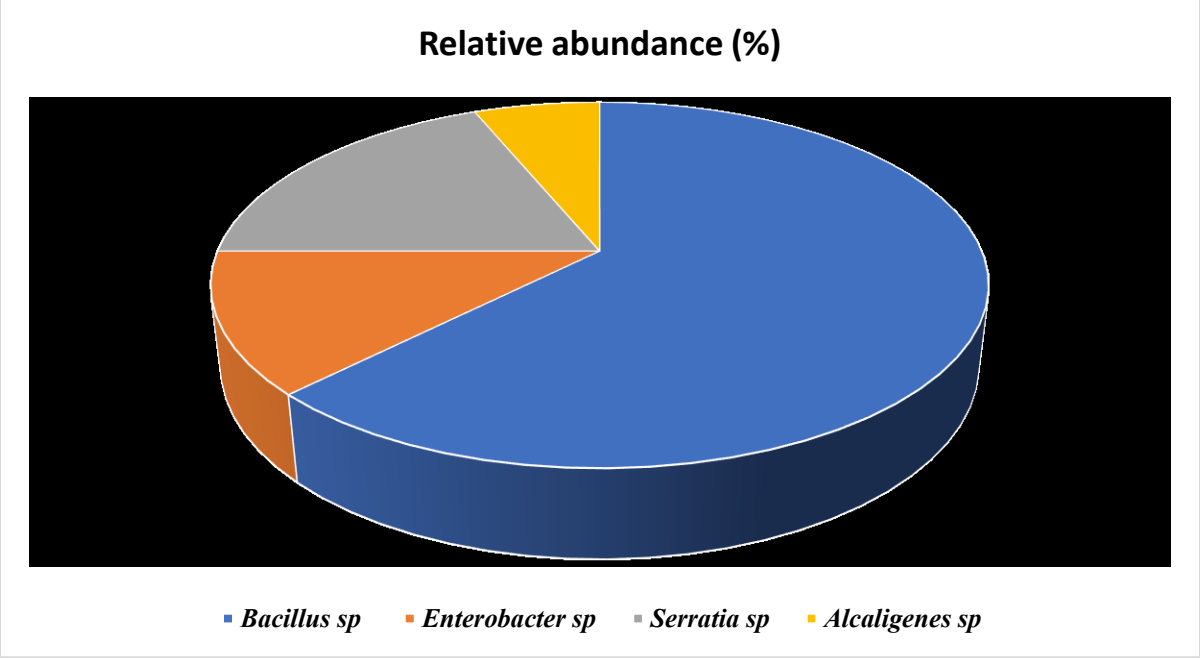


Figure 2: Percentage abundance of bacteria isolated from *Talinum fruticosum* rhizosphere soils

NITROGEN-FIXING AND PHOSPHATE-SOLUBILIZING ACTIVITIES

Of all the isolated bacteria, just *Bacillus* sp. and *Enterobacter* sp. gave a positive result on the nitrogen-fixing test while *Alcaligenes* sp. and *Serratia* sp. gave a negative result. Furthermore, only *Bacillus* sp. tested positive for the phosphate capacity test while *Alcaligenes* sp., *Enterobacter* sp, and *Serratia* sp. were negative (Table 1).

Table 1: Nitrogen-fixing and phosphate-solubilizing bacteria test

Isolates	Nitrogen-fixing activity test	Phosphate solubilizing test
Bacillus sp.	+	+
Enterobacter sp.	+	-
Serratia sp.	-	-
Alcaligenes sp.	-	-

KEY: + (Positive / Present) - (Negative /Absent)

DISCUSSION

The application of NFB and PSB in agriculture can go a long way in reducing the reliance on agrochemicals which have a lot of environmental consequences (Wani *et al*., 2007). This study shows that *Bacillus* sp*., Enterobacter* sp*., Serratia* sp*.,* and *Alcaligenes* sp. were present in *Talinum fruticosum* rhizosphere soil and some strains of these bacteria can promote seedling growth as reported by Podile (1994) when He bacterized peanut rhizosphere with *Bacillus subtilis* AF 1. Akinsemolu *et al*. (2024) also reviewed that *Bacillus* sp. which was predominant in our study (Figure 2) is crucial in agriculture and biotechnology for its role in producing antibiotics, enzymes, and other bioactive compounds. Also, it promotes plant growth and suppresses pathogens, enhancing crop health and yield. *Serratia marcescens* RZ-21 plays a key part in agriculture by boosting plant development and well-being (Ma *et al*., 2016). It produces plant hormones, enhances mineral uptake, and protects crops from pathogens through antimicrobial compounds (Kulkova *et al*., 2024). Its ability to degrade organic matter also contributes to soil fertility, making it a valuable microorganism for sustainable farming practices (Fulazzaky *et al.*, 2017). *Enterobacter* sp and *Alcaligenes* sp. are important in agriculture due to their role in nitrogen fixation and phosphate solubilization, enhancing soil fertility and plant growth. Its ability to decompose organic matter improves soil structure and nutrient availability, contributing to sustainable farming practices and increased crop yields (Behera *et al*., 2017; Benjelloun *et al*., 2021). The high number of bacteria in the rhizosphere of *Talinum fruticosum* (Figure 1) indicate a healthy and active soil microbiome. This suggests enhanced nutrient cycling, improved soil structure, and better plant health, as beneficial bacteria can boost plant development, protect against pathogens, and facilitate the availability of essential nutrients (Gkarmiri *et al.*, 2017).

Our results also support other studies which reported that *Bacillus* sp and *Enterobacter* sp (tested positive only for nitrogen) are both phosphate-solubilizing bacteria and nitrogen-fixing bacteria (Table 2) (Mehta *et al*., 2013; Peng *et al*., 2009; Yang *et al.*, 2011). *Bacillus* sp. and *Enterobacter* sp. are both known to possess certain traits that enable

them to interact with their environment in unique ways. For instance, Modi *et al.,* (2022) reported *Bacillus* sp., can convert insoluble phosphates into soluble forms, making them available for uptake by plants, while Shreya *et al* (2023) reported that *Bacillus* sp. and *Enterobacter* sp., convert atmospheric nitrogen (N_2) into a form that can be used by living organisms, such as ammonia (NH_3) or nitrate (NO_3^-). These isolates, *Bacillus* sp. and *Enterobacter* sp., could therefore be potentially used for promoting crop yield.

CONCLUSION

The study successfully identified some phosphate-solubilizing and nitrogen-fixing bacteria in the rhizosphere of *Talinum fruticosum*, highlighting their potential role in enhancing soil fertility and plant nutrition. These findings underscore the importance of microbial communities in sustainable agricultural practices aimed at improving crop yield and environmental sustainability.

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