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Inoculations of *R. erythropolis* and *B. subtilis* Stimulate Indigenous Bacteria and Improve the Properties of Low-fertilized Agricultural Soils

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ABSTRACT

Biodiversity and the number of bacteria present in the soil are two of the main parameters of soil quality, especially for agricultural purposes. Analysis of the low-fertilized soils suggested that the number and diversity of the bacterial communities in this soil are low. Hence, various methods have been used to stimulate bacterial activity and improve agricultural soil conditions. One of the popular methods is the inoculation of bacteria such as *B. subtilis* and *R. erythropolis*. These bacteria are potential species as bio-inoculants in soil management. However, the effectiveness of these bacteria in stimulating the activity of bacterial communities and improving soil properties of the low-fertilized soil is still sparsely explored. Therefore, this study aimed to analyze the impact of the inoculation of *B. subtilis* and *R. erythropolis* on the bacterial community structure and soil properties of low-fertilized soil. The soil used is agricultural soil for tobacco farming activities using agrochemicals. Bacterial community structures were analyzed using the environmental DNA (eDNA) method. The soil properties analyzed were total nitrogen, carbon, phosphorous, potassium, and pH. This study suggests that *B. subtilis* and *R. erythropolis* may affect the bacterial community structure and increase the number of bacteria to reach the ideal limit

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for fertile soil. Adding bacterial inoculants could stimulate the growth of bacteria and the nutrient cycle in the soil environment, resulting in improved soil fertility.

Keywords: B. subtilis, microbial ecology, *R. erythropolis*, soil fertility, soil inoculation

INTRODUCTION

World food demand shows an increasing tendency toward food supply. According to Alexandratos and Bruinsma (2012), the food demand for developing countries will increase by 60% in 2030 and double in 2050. Various efforts have been made to improve food production, including agriculture (Bargaz et al., 2018). One of the main factors in increasing agricultural yields is tillage, which makes it more productive (Amini & Asoodar, 2015). Hence, chemical fertilizers and pesticides are often used on various agricultural lands. Conventional agriculture increases along with the intensification of agricultural production. High harvest targets primarily triggered this increase. Currently, the use of agrochemicals in agricultural land has become inevitable and continues to increase yearly (Aktar et al., 2009). However, conventional agricultural practices have been implicated in diminishing soil fertility by reducing the overall bacterial abundance. Such farming systems can alter the composition of beneficial microorganisms within the soil community, thereby influencing critical nutrient-cycling processes, such as nitrogen fixation and phosphorus solubilization (Yadav et al., 2023). Prolonged and unregulated utilization of conventional agricultural systems has been demonstrated to detrimentally impact soil fertility and ecosystem integrity, leading to alterations in soil microflora composition (Dhanker et al., 2021).

These materials can be a way to provide nutrients for plant growth quickly. With the availability of nutrients, plant growth can be accelerated more easily. However, the use of agrochemicals raises various problems. The increasing price of agrochemicals and their decreasing availability make it difficult for farmers to carry out their agricultural business (Jacquet et al*.,* 2022). The use of agrochemicals at high prices also causes the profits of agricultural businesses to decrease even though the number of harvests can be maintained (Popp et al., 2013). The use of agrochemicals that interrupt a series of processes in the natural cycle of nutrients in the soil causes changes in soil conditions over time. The long-term application of agrochemicals can change soil properties and microbial activity (Maximillian et al., 2019; Meena et al., 2020). Agrochemicals can reduce microbial activity (Prashar & Shah, 2016), which plays an essential role in the decomposition of organic matter and provides nutrients in the soil (Bollag, 2008).

The high biodiversity of microbes is one of the leading indicators that can be used to analyze soil conditions (Kim et al*.,* 2022). Various studies to assess soil conditions and increase its fertility are based on microbial biodiversity in the soil. The decrease in activity and the number of microbes causes a decrease in nutrient cycles in the soil, so soil

fertility decreases (Campbell, 2008). Hence, the low number of bacteria and the diversity of bacteria became one of the main characteristics of low-fertilized soils (Adhikari et al., 2014; Babalola & Glick, 2012; Xin-ling et al., 2021). Low-fertilized soils can be improved by stimulating the microbes in this soil (Jacoby et al., 2017). One alternative technique is providing bacterial inoculants to reduce agrochemicals and stimulate bacteria in nutrient cycles (Kumar et al., 2022). Soil inoculation with microbes operates through several mechanisms. One such mechanism involves reinstating natural microbial communities, which conventional agricultural practices may disrupt (Hermans et al., 2023). Another mechanism entails the recruitment and accumulation of beneficial microorganisms triggered by plant-pathogen interactions, leading to disease suppression (Wen et al., 2023). Moreover, soil inoculation can instigate directional shifts in soil and plant communities, fostering ecosystem restoration in degraded environments (Han et al., 2022). Various techniques, including soil biofertilization, trap crop utilization, and seed coating, are employed for soil inoculation (Han et al., 2022). These techniques offer valuable insights into root-microbe interactions, aiding in the comprehension of soil inoculation mechanisms. Understanding the intricacies of soil inoculation with microbes is imperative for devising strategies to enhance agricultural practices and ecosystem rehabilitation efforts. Inoculation with beneficial microbes, such as *B. subtilis* and *R. erythropolis*, has been proposed as an effective practice for enhancing plant growth and soil health (Angelina et al., 2020). *B. subtilis* is widely used as a Growth Promoting Rhizobacteria (Kamou et al., 2015), while *R. erythropolis* is a hydrocarbonoclastic bacteria (Liu et al., 2015).

The provision of bacterial inoculants affects not only soil properties but also the indigenous bacteria in the soil. However, the effect of *R. erythropolis* and *B. subtilis* inoculants on the bacterial community structure and properties of the low-fertilized soils has yet to be widely explored. Changes in the bacterial community structure and its relationship with soil properties after adding bacterial inoculants are critically important aspects of microbial ecology for developing microbial resources, such as efforts to improve food safety and security. Therefore, this study aims to analyze the bacterial community structure and soil properties after inoculating *B. subtilis* and *R. erythropolis* on low-fertilized soil.

MATERIALS AND METHODS

Soil and Material Preparation

This study used soil samples from a tobacco field in Miyazaki Prefecture, Japan. Rice straw from the same prefecture is used as organic matter to be added to the soil. Chemical properties and total bacterial numbers of soil samples and rice straw were analyzed (Table 1). The soil samples were categorized according to the Soil Fertile Index Database criteria established by Adhikari et al. (2014). Standardized soil fertility parameters include a total bacterial count of ≥ 6.0 cells/g soil, total nitrogen content of ≥ 2500 mg^{-1} kg, total carbon

content of ≥ 25000 mg^{-1} kg, total phosphorus content of ≥ 3000 mg^{-1} kg, and total potassium content of ≥ 3000 mg^{-1} kg (Adhikari et al., 2014). Based on these categorizations in the Soil Fertile Index Database, the soil samples did not meet the criteria for being classified as fertile.

Table 1
Chemical properties of soil and rice straw as organic material used in this study

Sample	TB (cells/g-soil)	TC (mg/kg)	TN (mg/kg)	TP (mg/kg)	TK (mg/kg)	C/N ratio	pH
Rice Straw	n.d	470,000	25,000	60,000	18,000	19	6,8
Soil Sample	n.d	9000	530	1000	1200	16	6,9

Note. TB: Total Bacteria; TC: Total Carbon; TN: Total Nitrogen; TP: Total Phosphorous; TK: Total Potassium; n.d: not detected (<1 × 10^6 cells/g-soil)

Before analyzing fresh soil samples, the soil was sieved through a 2 mm mesh sieve. Subsequently, 500 g of soil was placed into a 1 L pot with a moisture content of 30% and mixed with 1% (w/w) rice straw powder that had been pulverized using a chopper machine to eliminate any water content. *R. erythropolis* and *B. subtilis* were cultured in Luria Bertani broth medium (containing 1% peptone, 0.5% yeast extract, and 0.5% NaCl) at 35°C for 48 hours to prepare the bacterial inoculum.

The experimental design consisted of three pots with two replications of each treatment. Then, each bacterial inoculant *R. erythropolis* and *B. subtilis* (ca., 1 × 10^6 cells/g-soil) was added to each soil treatment. In comparison, Soil without bacterial inoculants was used as a control in this study. All soils (i.e., inoculated with bacteria and control) were incubated for 28 days (22°C/14 hr and 18°C/10 hr) in a plant factory incubator at Ritsumeikan University, Japan. The sampling parameter consisted of the total number of bacteria every week, soil chemical properties in week 4, and bacterial community structure in week 4.

Analysis of Chemical Properties

The chemical properties of soils (total carbon, total nitrogen, total phosphorous, and total potassium) were analyzed in the last final incubation on the 28th day or week 4. The soil carbon was determined using a total organic carbon analyzer machine (TOC-VCPH; Shimadzu, Kyoto, Japan) and the solid sample combustion unit (SSM-5000A; Shimadzu, Kyoto, Japan). To analyze the total nitrogen, total phosphorous, and total potassium, the soil was digested in a Kjeldahl digestion unit (Gerhardt, Königswinter, Germany) and then filtered (ADVANTEC No. 6; Toyo Roshi Co. Ltd., Tokyo, Japan). Total nitrogen, total phosphorous, and total potassium in the filtrate were determined using the indophenol blue method for nitrogen, the molybdenum blue method for phosphorous, and the atomic absorption spectrophotometry for potassium (Hitachi, Tokyo, Japan). The pH of soils was measured using the LAQUA pH/ion meter F-72 (Horiba, Ltd., Kyoto, Japan).

Analysis of Total Bacterial Number

The total number of bacteria in the soil every week was estimated by analyzing the environmental DNA (eDNA) with the low stirring method (Aoshima et al., 2006). A 1.0 g of soil was mixed with 8.0 mL of eDNA buffer and 1 mL of 20% sodium dodecyl sulfate (SDS) solution. The suspension was stirred at 1,500 rpm for 20 min, followed by centrifugation at 6,000 × g for 10 min. The supernatant was mixed with chloroform-isoamyl alcohol (24:1 (v/v), and centrifuged at 18,000 × g for 10 min. Then, 500 µl supernatant was taken as the crude nucleic acid in a new tube. 300 µl of isopropanol was added, stirred gently, and centrifuged at 14,000 rpm for 20 min. The liquid was discarded gently, 1 ml of pre-cooled 70% ethanol was added, and centrifuged at 14,000 rpm for 5 minutes. The liquid was gently discarded and dried for about 30 min under a vacuum. The remaining was dissolved in 50 µl of 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The eDNA was quantified based on the band's intensity after 1% agarose gel electrophoresis using KODAK 1 D 3.6 Image Analysis Software (Eastman Kodak Company, CT, USA).

PCR Amplification

The environmental DNA (eDNA) was extracted by following the eDNA extraction method described by Aoshima et al. (2006). The 16S rRNA bacterial gene was amplified using DGGE primers forward (5'-CGCCC GCCGC GCCCC GCGCC CGTCC CGCCG CCCCC GCCCG CCTAC GGGAG GCAGC AG-3') and reverse (5'-CCGTC AATTC CTTTG AGTTT-3'). The amplification PCR was taken in a 25 µL PCR mixture containing 0.1 ng/µL of DNA template, 0.1 U rTaq DNA polymerase, 2.5 µL of 10× buffer, 2.5 µL of 2 mM dNTPs, and 0.5 µL of 10 mM of each primer. DNA polymerase, dNTPs, and PCR buffer were purchased from Toyobo (Toyobo Co. LTD, Osaka, Japan), while all primers were synthesized by Sigma-Aldrich (Sigma-Aldrich Co. LLC, Tokyo, Japan). The condition of PCR is initial denaturation at 95°C for 1 min, followed by 40 cycles of denaturation at 95°C for 1 min, primer annealing at 55°C for 30 seconds, and extension at 72°C for 1 min, and then final extension at 72°C for 5 min.

DGGE Analysis

The amplified 16S rRNA bacterial genes were used for the Denaturing Gradient Gel Electrophoresis (DGGE) analysis. DGGE was performed using a D Code System (BioRad Laboratories Inc., California, USA). The 20 µL of PCR product was loaded into 40% (w/v) polyacrylamide gel with a denaturant gradient of 27.5% - 67.5%. The gel was run in 1× TAE buffer (40 mM Tris (pH 7.6), 20 mM acetic acid, and 1 mM EDTA) at a constant voltage of 70 V at 60°C for 15 h. Thus, the gel was stained using SYBR Gold for 30 min and then rinsed with distilled water. Cluster analysis of the DGGE band pattern was conducted using the FPquest Bioinformatics Software (BioRad Laboratories Inc., California, USA).

Statistical Analysis

Statistical analysis was conducted to compare the bacterial density between treatment (soil inoculated with bacteria) and control (soil without bacterial inoculation) using Analysis of Variance (ANOVA). ANOVA was chosen for its suitability in determining whether there are any statistically significant differences in bacterial density between the treatment and control groups. Additionally, Multivariate Analysis of Variance (MANOVA) was employed to assess the variations in soil properties. MANOVA was deemed appropriate as it allows for the simultaneous analysis of multiple dependent variables, enabling the investigation of potential differences in various nutrient components between treatment and control groups. These statistical analyses were performed using SPSS version 29 (IBM Corp., Armonk, NY, USA) with a significance level set at $\alpha = 0.05$ to determine the statistical significance of the observed differences. Before analysis, assumptions of normality and homogeneity of variances were assessed, and, if necessary, appropriate transformations were applied to meet these assumptions.

RESULTS AND DISCUSSION

Effect of Microbial Inoculant on Bacterial Activity

Environmental DNA (eDNA) of bacteria in the low-fertilized agricultural soil was collected and then observed on gel electrophoresis gel (Figure 1). Soils subjected to the analysis in this study were the low-fertilized soil inoculated with *B. subtilis*, the low-fertilized soil inoculated with *R. erythropolis*, and the low-fertilized soil that was not inoculated with bacteria (used as controls). Hence, the greater the number of bacteria in the soil, the more eDNA will be obtained. In addition, the more eDNA collected, the brighter the light intensity of the band on the electrophoresis gel. The results of the analysis of bacterial populations during soil incubation using the eDNA method are shown in Figure 2. The eDNA method calculates the number of bacteria to evaluate the number of soil microorganisms by calculating the amount of DNA in the soil sample (Aoshima et al., 2006). Observations for 28 days of incubation showed that the total bacterial number in soil treatment with *R. erythropolis* increased to 5.2×10^8 cells/g-soil, while treatment with *B. subtilis* increased to 3.4×10^8 cells/g-soil. On the other hand, the total bacterial number in control gradually decreased to an undetectable level ($\leq 1 \times 10^6$ cells/g-soil) on day 28.

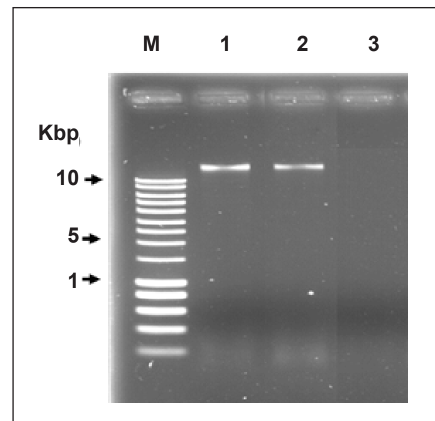

Figure 1. Analysis of the eDNA extracted after 28 days of incubation from soil inoculated with *R. erythropolis*. (Lane 1), *B. subtilis* (Lane 2), without bacterial inoculation (Lane 3). M is the lane of the marker.

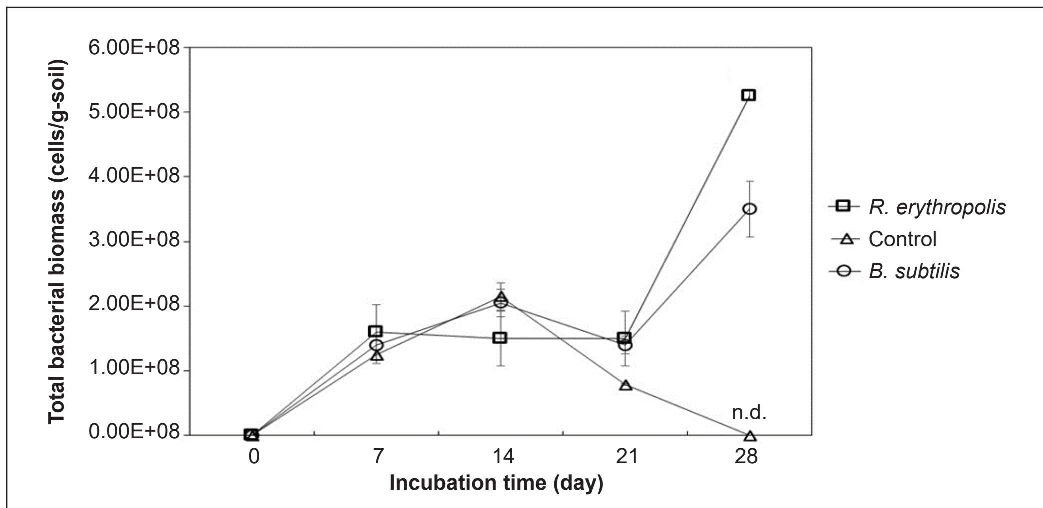

Figure 2. Effect of bacterial inoculant on the bacterial number in the soil during incubation

The bacterial densities of soils treated with bacterial inoculation (*R. erythropolis* and *B. subtilis*) were compared to that of the control (without bacterial inoculation) using Analysis of Variance (ANOVA) (Table 2). The results indicate significant differences in bacterial density between each treatment and the control ($\alpha < 0.05$). The most pronounced difference was observed on day 28. These findings suggest that bacterial inoculation significantly influences bacterial density in the soil. This bacterial density forms the foundation for supporting the nutrient cycling driven by microbial activities, thus facilitating the development of microbiota-based ecological agricultural systems.

This analysis underscores the efficacy of bacterial inoculation in influencing soil microbial communities, potentially enhancing soil fertility and ecosystem functioning. The observed increase in bacterial density corroborates previous studies indicating the role of bacterial inoculation in augmenting soil microbial populations. Additionally, the temporal dynamics of bacterial density, as evidenced by the significant difference on day 28, suggest

Table 2
The results of ANOVA analysis for the bacterial densities of soil dependent variable: Response

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	567665866666666750.000[a]	14	40547561904761912.000	66.096	**.000**
Intercept	668416133333331970.000	1	668416133333331970.000	1089.572	**.000**
Treatment	68788266666666648.000	2	34394133333333324.000	56.065	**.000**
Day	269791200000000416.000	4	67447800000000104.000	109.945	**.000**
Treatment * Day	229086400000000256.000	8	28635800000000032.000	46.679	**.000**
Error	9202000000000000.000	15	613466666666666.600		
Total	1245284000000000000.000	30			
Corrected Total	576867866666666750.000	29			

a progressive establishment and proliferation of the inoculated bacterial strains within the soil environment. These findings contribute to our understanding of microbiota-mediated nutrient cycling mechanisms, highlighting the potential of microbial-based approaches in sustainable agriculture practices. Further investigations into the long-term effects of bacterial inoculation on soil microbial communities and ecosystem functioning are warranted to elucidate the full scope of its implications for agricultural sustainability and productivity.

Low bacterial numbers are among the main characteristics of low soil fertility (Adhikari et al., 2014). Various efforts have been made to increase the fertility of this type of soil for the benefit of agriculture. In the development of organic agriculture, efforts are often made to add organic materials such as rice straw and inoculate bacteria (Ramadass & Thiagarajan, 2017). Adding bacterial inoculants can help create conditions that can trigger the growth of indigenous bacteria (Hawrot et al., 2016; Santos et al., 2019) and increase the circulation of nutrients in the soil (Figure 6). Increasing agricultural soil fertility due to adding organic matter will accompany increased bacteria (Li et al., 2021). This study shows that adding inoculations of *R. erythropolis* and *B. subtilis* can increase the number of bacteria in low-fertilized soil until it meets the standard of the number of bacteria in fertile soil. The bacterial biomass values were divided into the following six groups: 1) shallow ($<1.0 \times 10^8$ cells/g); 2) very low (1.0×10^8 to 2.9×10^8 cells/g); 3) low (3.0×10^8 to 4.4×10^8 cells/g); 4) medium (4.5×10^8 to 5.9×10^8 cells/g); 5) high (6.0×10^8 to 9.9×10^8 cells/g); and 6) very high ($\geq 10.0 \times 10^8$ cells/g) (Adhikari et al., 2014).

In the case of control soil, the addition of bacteria continued until day 21 (increased to ca. 2.0×10^7 cells/g-soil), then continued to decrease. The increase in bacteria may occur because rice straw becomes a source of nutrients for the bacteria. However, the available nutrients can only support the bacteria until the 21st day. After that, the bacterial community in the soil cannot utilize the remaining nutrients, resulting in a decrease in the bacteria population. The bacterial community in the soil can be affected by changes in nutrient availability, which can result in a decrease in the bacterial population. Nutrient limitations, such as decreased phosphorus availability, can limit microbial growth and community structure (François et al., 2021). It is related to the fact that the total phosphorous decreased during control treatment compared to soil treatment with inoculant bacteria (Figure 6). Moreover, the remaining chemical substances in the soil can negatively affect microbes that cannot utilize these remaining nutrients (Nayak et al., 2018). The highest total number of bacteria in this study was shown by the soil treatment using *R. erythropolis.* Treatment using *B. subtilis* inoculation also showed higher total bacteria values than the control but lower than *R. erythropolis.*

The soil used in this study is the former soil of a tobacco field in Miyazaki prefecture, Japan. In this tobacco farming activity, chemicals and pesticides containing chloropicrin are used (Ota, 2013). These agrochemicals can interfere with the growth of indigenous

bacteria and make this soil low in fertility. Adding inoculated bacteria such as *R. erythropilis* and *B. subtilis* can positively impact the degradation of these agrochemicals (Choi et al., 2021; Prashar & Shah, 2016). Once the number of agrochemicals can be reduced, more indigenous bacteria can grow in the soil. *R. erythropilis* is a type of bacteria that can live in contaminated soil and is often used in bioremediation activities (Wolińska et al., 2016). This species is also very likely to degrade agrochemicals better than *B. subtilis,* resulting in a higher total number of bacteria than soil inoculated with *B. subtilis.*

The results of the total bacteria number analysis indicate that adding bacterial inoculants can stimulate the growth of bacteria, impacting soil fertility levels. Hence, applying bacterial inoculants enhances the activity of indigenous soil bacteria (Babalola & Glick, 2012). Moreover, the PCR-DGGE method was used in this study to compare the bacterial community structure in the soils.

Bacterial Community Structure

PCR-DGGE analysis was performed for eDNA samples collected from soils (i.e., inoculated with *R. erythropolis*, inoculated with *B. subtilis*, and control) of the beginning (day 0) and the end of incubation (day 28). The PCR amplification of the eDNA shows that the fragment bands were 600 bp (Figure 3). Then, the results of the DGGE observations indicated that the structures of the bacterial diversity were shown in Figure 4. The bands in the DGGE gel may indicate the main species of bacteria in the soils (Vendan et al*.,* 2012). The structural communities of bacteria in the three soils sampled in this study were compared through the Cluster Analysis of the DGGE results (Figure 5). The dendrogram analysis results showed that the bacterial communities in the soil inoculated with bacteria at the beginning of the incubation period differed from that of the non-inoculated (control) (Figure 5A). In the case of inoculated soils, the difference in bacterial community is most likely due to

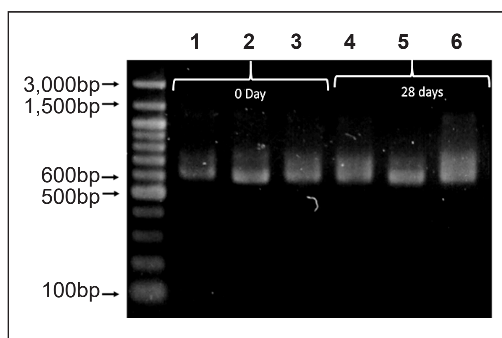

Figure 3. 16S rRNA amplified by PCR separated on 1.5% agarose gel (1, 4 = Control; 2, 5 = *R. erythropolis*; 3, 6 = *B. subtilis)*

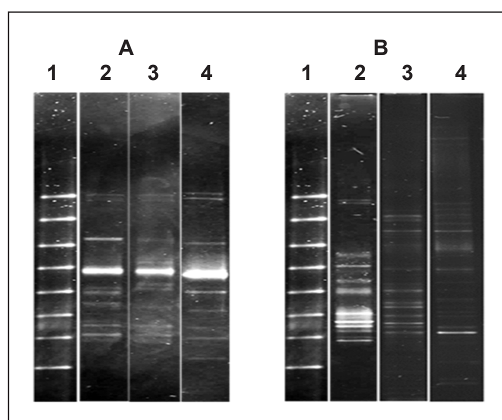

Figure 4. DGGE analysis of 16S rRNA fragments obtained from soils before (A) and after incubation (B) (Lane 1: DGGE marker, Lane 2*: R. erythropolis*, Lane 3: *B. subtilis*, Lane 4: Control)

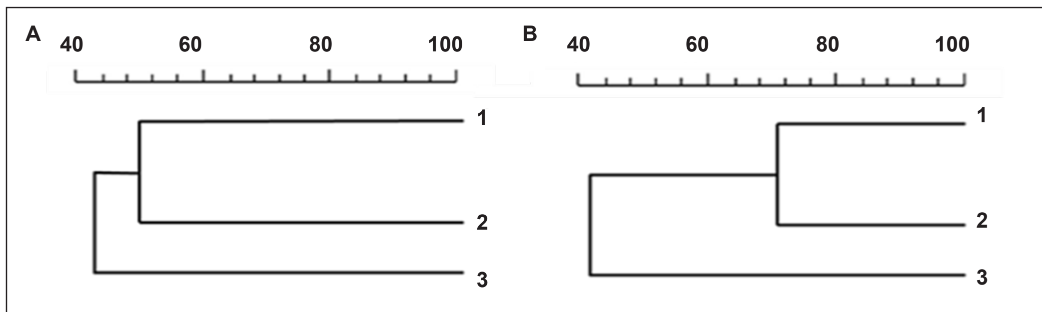

Figure 5. Cluster analysis of 16S rRNA obtained from soils before (A) and after incubation (B) (Lane 1: DGGE marker, Lane 2: *R. erythropolis*, Lane 3: *B. subtilis*, Lane 4: Control)

the different types of bacteria added (i.e., *R. erythropolis* dan *B. subtilis*). The dendrogram of the bacterial community structure analysis after 28 days of incubation is shown in Figure 5B. This analysis showed that the bacterial community in the bacterial-inoculated soil differed from the uninoculated soil.

However, the community structure became more similar to the control compared to the initial incubation. This result indicates that the provision of bacterial inoculation can stimulate the growth of indigenous bacteria. The addition of bacterial inoculation makes soil conditions more suitable for the life of indigenous bacteria, increasing the number of bacteria in the soil (Figure 2). This more favorable soil condition may occur because bacteria such as *R. erythropolis* and *B. subtilis* reduce the concentration of agrochemicals in the soil (Choi et al., 2021; Prashar & Shah, 2016). Bacterial inoculants change the structure of the bacterial diversity, which has an essential role in soil fertility. Applying microbial inoculants may improve the condition of the low-fertilized soils (Trabelsi & Mhamdi, 2013).

Effect of Microbial Inoculant on Soil Properties

This study analyzed soil properties after 28 days of incubation to explore the impact of adding bacterial inoculants to the soil (Figure 6). This analysis showed that adding bacterial inoculants made the total carbon, total phosphorus, and CN ratio values of the soils given bacterial inoculation higher than those of the control. In contrast, pH and Total Nitrogen were lower than the control. The difference in impact occurs in the Total Potassium parameter. When compared with the control, the value of this parameter was higher in the soil that was inoculated with *R. erythropolis* while lower in the addition of *B. subtilis* inoculation.

The soil properties in this study were compared using Multivariate Analysis of Variance (MANOVA). The results, depicted in Figure 6, illustrate variations in the soil samples, denoted by different letters (a, b, c). Similar letters indicate no significant differences between samples, while different letters denote significant differences. The analysis revealed significant differences in soil properties between soil samples with and without bacterial

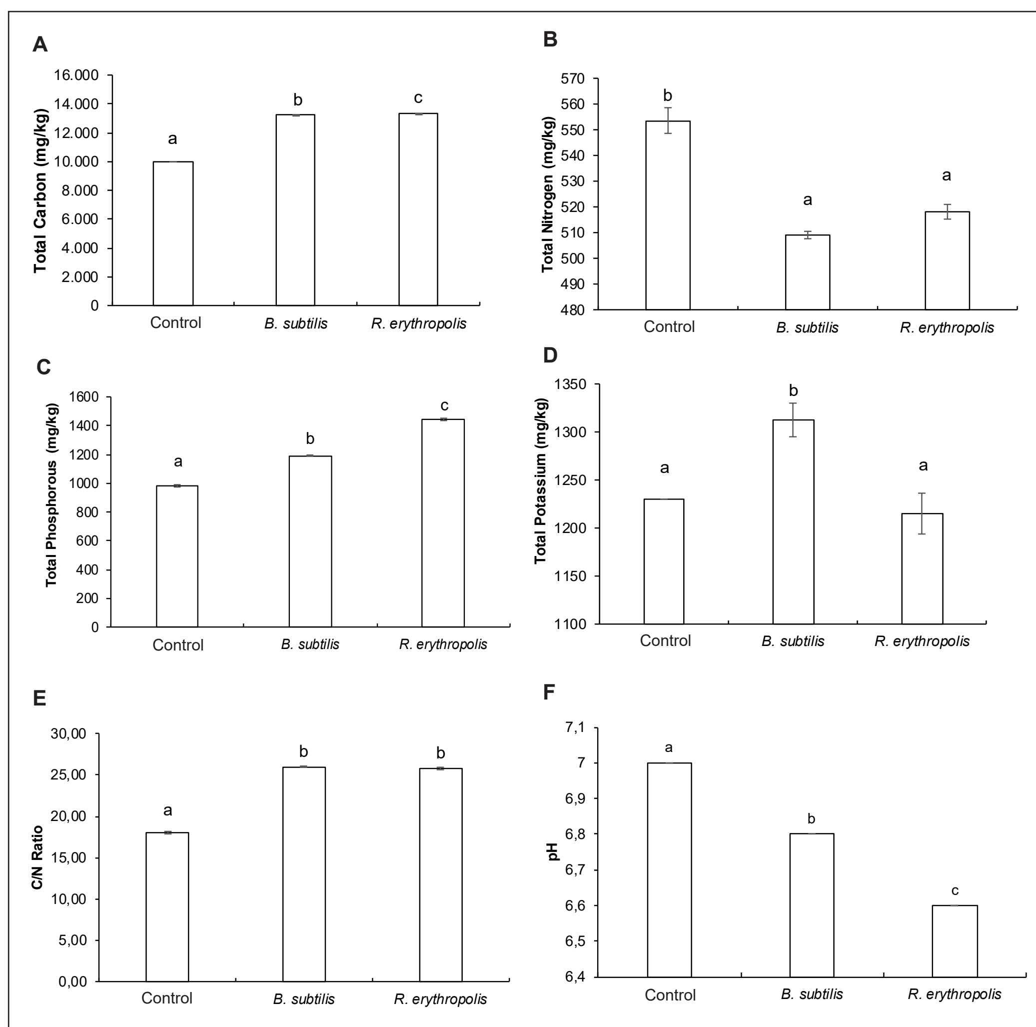

Figure 6. Total carbon (A), total nitrogen (B), total phosphorus (C), total potassium (D), CN ratio (E), and soil pH (F) of the soils on the 28th day

inoculation. It appears that bacterial inoculation stimulates the activity of soil bacteria, resulting in distinct soil conditions. Interestingly, the Total Nitrogen content between soil samples inoculated with *R. erythropolis* and those inoculated with B. *subtilis* did not differ significantly. This finding suggests that, regarding nitrogen conditions in the soil, the type of bacteria inoculated does not exert a significant influence, contrary to other soil properties.

This discrepancy in the response of Total Nitrogen content to bacterial inoculation warrants further investigation. Several factors may contribute to this observation, including the specific metabolic activities of the inoculated bacterial strains, their interactions with indigenous soil microbial communities, and the availability of nitrogen sources in the soil. Further research could focus on elucidating the mechanisms underlying the modulation of

nitrogen dynamics by different bacterial species in soil ecosystems. Additionally, examining the long-term effects of bacterial inoculation on nitrogen cycling processes and soil fertility could provide valuable insights into optimizing microbial-based approaches for sustainable soil management practices.

The decrease in pH in the soil given inoculants is possible because the bacterial community can stabilize soil pH in the ideal range for agriculture (ca. pH 5–7) (Carvalho, 2019). Moreover, the decrease in pH during decomposition is probably due to the production of organic acids, such as lactic acid, due to microbial activity (Qi et al., 2016). Stabilizing the pH indicates that the decomposition process reaches a state of equilibrium where the production and consumption of organic acids are balanced. The difference in the trend in the value of Total Potassium indicates that the activities of *R. erythropolis* and *B. subtilis* are different in recycling potassium from organic matter. Hence, *R. erythropolis* shows a more remarkable ability to recycle potassium from organic substrates (Carvalho, 2019). The results of this study suggested that adding bacteria seems to influence the nutrient cycle in the soil environment (Feirer et al., 2012; Zhang et al., 2016), resulting in a change in soil properties.

CONCLUSION

This investigation delved into the effects of *R. erythropolis* and *B. subtilis* inoculation on the properties of agriculturally fertilized soil. Our findings underscore the potential of bacterial inoculation in stimulating the proliferation of indigenous bacterial populations and enhancing nutrient cycling within the soil matrix. Notably, the inoculation with *R. erythropolis* and *B. subtilis* exerted discernible impacts on the structure of bacterial communities, leading to an augmentation in bacterial abundance towards the threshold indicative of fertile soil. Consequently, our study posits bacterial inoculation as a promising intervention for ameliorating the conditions of low-fertilized soil, particularly following its prior use in agriculture employing agrochemicals. These results hold significant implications for sustainable soil management practices, suggesting a viable strategy to revitalize soil fertility in agricultural contexts. By harnessing the potential of bacterial inoculants, agricultural systems can mitigate the deleterious effects of conventional practices and pave the way toward enhanced soil health and productivity. Further research is warranted to elucidate the mechanistic underpinnings of bacterial-mediated improvements in soil properties and to refine strategies for optimizing the efficacy of bacterial inoculation in diverse agricultural settings.

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