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Distinctive Bacteria Consortium of Rhizosphere and Surface Soil from Semi-Organic Potato Cultivation in Temanggung, Indonesia

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Abstract

Potato cultivation in the middle land is an alternative solution to avoid pathogens. Although it reduces potato quantity and requires more chemical fertilizers to increase production that potentially damages the environment. Therefore, this study aims to characterize contaminations and indigenous microbes from semi-organic potato cultivation centers in Central Java. Soil samples from the semi-organic potato cultivation center of Kledung, Central, were analyzed for metal contamination using the atomic absorption spectrophotometry method and metagenomic soil bacterial diversity profiles. Specifically targeted genes of distinct regions 16SV3-V5. OTUs were identified using Uparse v7.0.1001, while Beta diversity analysis used UniFrac. All these indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). The results showed that semi-organic cultivations had been polluted with Pb > Cd > Cr > As, and carbamate residue was detected in the rhizosphere and surface soil. Manure and compost application alongside NPK fertilizer increases N input, decreasing surface soil bacteria. The surface soil (with a depth of ≤ 5 cm) has a lower index of species richness, diversity, and relative abundance than the rhizosphere layer (depth > 5 cm). Firmicutes, Proteobacteria, and Actinobacterium dominate soil-level bacteria. In comparison, the rhizosphere soil has a higher bacteria diversity dominated by N-tethering rhizobia from the phylum Proteobacteria. The potato root attracts rhizobacteria consortium that support plant growth. A further study should be conducted to describe how the bacterial consortium formed, rhizobia migration from surface to rhizosphere soil, and its interaction with the potato plants.

Keywords: Biomodulator, Firmicutes, Heavy metals, Nitrogen-fixing bacteria, Proteobacteria, Rhizobacteria

Introduction

Potato (*Solanum tuberosum*) is one of the primary nutritious food commodities widely consumed and used as an alternative carbohydrate source for food diversification programs [1]. Central Java Province is the second largest potato producer in Indonesia, with production reaching 307.6 tons in 2020 [2]. In current cultivation, potato is planted in the highlands, above 1,000 m.a.s.l, which humid condition also triggers the emergence of wilt disease caused by *Ralstonia solanacearum* infection [3,4] or *Fusarium* sp [5,6] and late blight caused by *Phytophthora infestans* (Mönt.) de Bary [7]. Pathogenic infections are the main factor in potato crop losses and decreased tuber production of highland potato cultivation in Indonesia [8]. In case, *P. infestans* infection is widespread and epidemic in 14 potato cultivation districts in Central Java. Humid mountain climate conditions increase the infection risk and make it harder to be controlled [9], since the pathogenicity is high and heterothallic [10].

The pathogens infection is sharply lowering potato productivity by up to 100 % [11,12]. Today, the farmers mostly apply excessive organic-chemical pesticides and fertilizers as the main strategy to control the disease. On the other hand, the extensification program is developed by planting the potato in low-medium land to avoid pathogens. However, it reduces potato quantity and requires more chemical fertilizers to increase the production that potentially more damages the environment [13]. According to the recommended dosages, farmers are often less concerned about using pesticides safely [14,15]. In potato cultivation, it was found that the massive use of chemical pesticides tends to increase every year [16], thus potentially triggering the emergence of new-resistant strains of *P. infestans* pathogens [17]. In addition, chemical fungicides can devastate the biodiversity of beneficial microorganisms for potato crops [18,19], increasing heavy metal pollution and pesticide residue in soil that causes health problems [20]. Therefore, it is necessary to develop biomodulators and biocontrol based on indigenous microorganisms that are environmentally friendly in agriculture.

Bio-controlled bacterial and fungal antagonists have now been widely used to reduce chemical fertilizer and pesticide use. Some types of local microorganisms, such as *Trichoderma* sp. [21,22]. *Bacillus,* and *Penicillium* species, effectively inhibit *Fusarium* sp. [23], and *P. infestans* infection [24], [25]. Studying indigenous highland bacteria diversity and understanding their adaptation against pollutant is necessary to replicate their nature in different habitat characteristic. Furthermore, healthy potato rhizobacteria potentially developed as pathogens biocontrol as well as biomodulators to promote plant growth [26], including in Central Java as a potato production center [27]. Therefore, this study aims to map a consortium of rhizosphere bacteria from potato farming to increase commodity productivity and resilience.

Materials and methods

This study was an exploratory observational study to profile semi-organic agricultural soil bacteria in a potato cultivation center in Kledung Village, Temanggung, Central Java, Indonesia (−7.336315, 110.032428). The farmers manage semi-organic potato cultivation by applying organic-chemical fertilizers and cultivation pattern as explained in **Figure 1**, where every plot was managed for 10 row of potato seeds (or ± 6 m length).

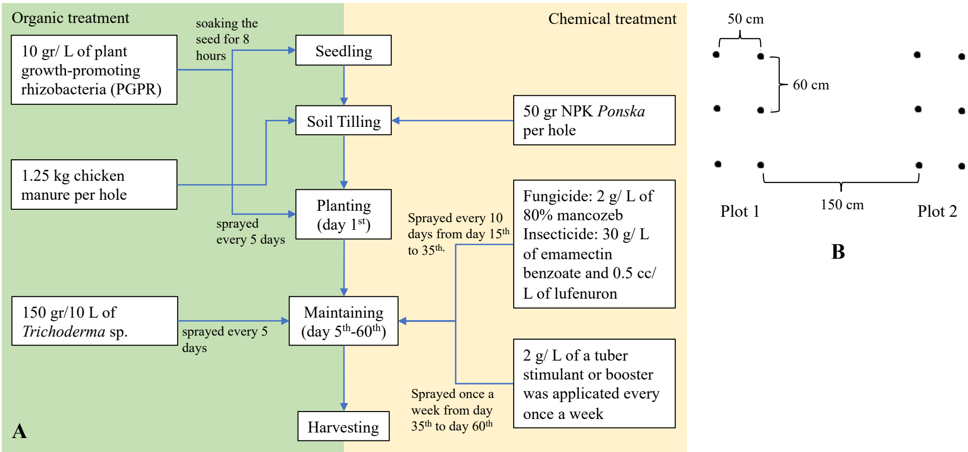

Figure 1 Semi-organic fertilization management (A) and cultivation plot size pattern (B) conducted by the farmers in Kledung village, Temanggung.
Note: The black dot refers to planting holes.

The sampling collection was conducted during dry season in August 2022, with average daily temperature is 15 - 25 °C, humidity around 66.30 %, and rainfall volume up to 150 mm/10 days. The Soil type is regosol, sandy loam texture and pale brown color, with a pH of 5.74. Samples were taken from the surface soil layer to a depth of 5 cm, and rhizospheres (the narrow region of soil or substrate that is directly influenced by roots) with a depth of 6 - 20 cm from productive potato cultivation ready for harvest (75 days of sowing period). Each surface soil and rhizosphere sample were collected aseptically by a mixed method with 5 replication sampling, from the 4 sides, and the middle of the field. The soil sample was then mixed until homogeneous. A total of 100 g of soil was put into a dark bottle, placed in a cool box, and stored in a −80 °C freezer for 48 h before the metagenomic analysis process. Then, as many as 30 g soil was prepared in dark bottle and stored in room temperature before used for heavy metal, and carbamate residues analysis.

Analysis of carbamate residues

A total of 20 g of each soil sample plus 50 mL of acetone and a dichloromethane ratio of 1:1 (v:v). The sample solution was then homogenized using a shaker for 8 h and then passed into a column containing florisil and sodium sulfate (1:1). After that, it was evaporated for 15 min and re-dissolved with methanol of 25 mL.

The determination of carbamate contamination concentration was carried out using the High-Performance Liquid Chromatography (HPLC) Waters 2695 tool device with an analysis column C18, 4.6×250 mm^2, column temperature 42 °C, flow rate 1.0 mL/min, injection volume 400 µL, post-column reactor rate 0.3 mL/min, fluorescence detector (Waters 2475) at an excitation wavelength of 330 nm and emission of 465 nm. Analysis of carbamate residues, including carbofuran and carfosulfan, is performed using the Pickering Cat. No column 0840250.

Standard solutions were prepared with concentrations of 1 g/mL each of carbofuran (Dr. Ehrenstorfer, Germany), buthylphenylmethyl carbamate (BPMC) (Accu Standard, USA), 2-(1-methylethyl)phenyl methylcarbamate (MIPC) (Accu Standard, USA) up to 1 g/mL. The solution was stored using dark-colored bottles in a refrigerator at −20 °C.

Heavy metal analysis

A total of 0.5 g of mashed soil samples were filtered using 100 mesh sized-filter and put into a Kjeldahl flask, then added with 5 mL of HNO_3 and 0.5 mL of $HClO_4$. The solution was homogenized and allowed to stand overnight at room temperature. Digestion was carried out using a block digestor at an initial temperature of 100 °C and raised to 200 °C until 0.5 mL of the solution remained. The solution was cooled and diluted with aqua dest to a volume of precisely 50 mL and homogenized using a stirrer and filtered using Whatman 41 filter paper until clear and continued with a vapor generation accessory-atomic absorption spectroscopy (VGA-AAS) tool for arsenic metals (As) and AAS for lead (Pb), cadmium (Cd), and chrome (Cr). Selection of Pb, Cd, Cr, and As, as these metals are most commonly found in environmental and agricultural pollution [28].

Extraction of genome DNA and measurement

Total genome DNA from samples was extracted using the cationic-anionic surfactant cetyltrimethylammonium bromide/surfactant sodium dodecyl sulfate (CTAB/SDS) method following Minas *et al.* (2011) and Kouakou *et al.* (2022) protocol [29]. DNA concentration and purity were monitored on 1 % agarose gel. According to the concentration, DNA was diluted to 1 ng/µL using sterile water. Specifically targeted *16SrRNA* genes of distinct regions 16SV4/16SV3/16SV3-V4/16SV4-V5 were amplified using a specific primer (e.g., 16S V4: 515F-806R) with the barcode. All PCR reactions were carried outwith Phusion®High-Fidelity PCR Master Mix (New England Biolabs).

The amplicon detection was carried out using the mixture of 1X loading buffer (contained SYB green) with PCR productsand operated electrophoresis on 2 % agarose gel. Samples with the bright main strip between 400 - 450 bp were chosen for further experiments. PCR products were mixed in equidensity ratios and then purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Library preparation and sequencing

Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Pre-Kit for Illumina, following the manufacturer recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2,100 system. Finally, the library was sequenced on an Illumina platform, and 250 bp paired-end reads were generated.

Data analysis: Paired-end reads assembly and quality control

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Then, paired-end reads were merged using FLASH V1.2.7, (http://ccb.jhu.edu/software/FLASH/) [31], a high-speed and accurate analysis tool,which was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags. Quality filtering on the raw tags was performed under specific filtering conditions toobtain high-quality clean tags [32], according to the QIIME(V1.7.0, http://qiime.org/index.html) quality-controlled process [33]. The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using the UCHIME algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) [34]. The sequences were removed [35], to detect chimaera sequences, and the Effective Tags were finally obtained.

OTU cluster and species annotation

Sequences analysis was performed by Uparse software (Uparse v7.0.1001， http://drive5.com/uparse) [36]. Sequences with $\geq$ 97 % similarity were assigned to the same OTUs. The representative sequence for each OTU was screened for further annotation. For each representative sequence, the GreenGene Database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) [37], was used based on the RDP classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier) algorithm to annotate taxonomic information [38].

To study the phylogenetic relationship of different OTUs, and the difference of the dominant species in different samples, multiple sequence alignments were conducted using the MUSCLE software Version

3.8.31 (http://www.drive5.com/muscle/) [39]. OTUs abundance information was normalized using a standard sequence number corresponding to the sample with the least sequences. Next, alpha and beta diversity analyses were performed based on this output normalized data.

Alpha and beta diversity

Alpha diversity is applied in analyzing the complexity of species diversity for a sample through 6 indices, including Observed-species, Chao1 estimator (http://www.mothur.org/wiki/Chao); Shannon (http://www.mothur.org/wiki/Shannon); Simpson (http://www.mothur.org/wiki/Simpson); ACE estimator (http://www.mothur.org/wiki/Ace); Good-coverage (http://www.mothur.org/wiki/Coverage). All these indices in our samples were calculated with QIIME (Version1.7.0) and displayed with R software (Version 2.15.3).

Beta diversity analysis was used to evaluate differences of samples in species complexity; beta diversity on both weighted and unweighted UniFrac was calculated by QIIME software (Version 1.7.0). Cluster analysis was preceded by principal component analysis (PCA), which wasapplied to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). The unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was performed as a hierarchical clustering method to interpret the distance matrixusing average linkage. It was conducted by QIIME software (Version 1.7.0).

Results and discussion

Based on the AAS analysis results, semi-organic potato cultivation in Kledung, Temanggung Regency, was contaminated by heavy metals, such as Pb, Cd, Cr, and As, in high concentration (**Table 1**). Even though heavy metal concentrations decreased from the surface to the rhizosphere soil.

Table 1 Heavy metal contamination of the soil of semi-organic potato cultivations.

Sample	Heavy Metal (ppm)			
	Pb	**Cd**	**Cr**	**As**
Surface Soil	72.53 ± 1.68	2.13 ± 0.13	14.94 ± 0.12	16.31± 0.285
Rhizosphere	47.31 ± 10.93	1.85 ± 0.01	14.82 ± 0.06	12.64 ± 1.55
Change (%)	34.77 ± 1.80	13.14 ± 0.55	0.80 ± 0.03	22.50 ± 1.53
Threshold*	≤ 50.00	≤ 2.00	≤ 210.00	≤ 10.00

Note: * The maximum allowed heavy metal level for improving agricultural land is based on the Minister of Agriculture of the Republic of Indonesia Regulation No. 70 of 2011.

The content of heavy metal contamination in agricultural surface soil in Kledung, from the highest to the lowest, is Pb > As > Cr > Cd. In addition, heavy metal concentrations also decrease along with the depth of the soil layer, where the highest concentrations of metals are found in the surface soil layer. The highest rate of change in metal concentrations occurs in Pb metal and is followed by As, and then the lowest is Cd compared to other metals. Decreasing heavy metal concentrations from top soil to the rhizosphere may be caused by bioremediation activity of bacteria and plants, and leaching [40].

Heavy metal comes from the bedrock eroded by chemical and biological reactions, physical weathering [41], and anthropogenic factors such as emissions and industrial fumes [42]. In addition, most potato cultivations in Indonesia still rely on chemical fertilizers, including NPK as the main fertilizer and carbamate as an antifungal. Several studies have shown that the increased application of chemical fertilizers and pesticides contributes to an increase of heavy metals in potato farmland, including Pb, Cd, Cr, and As [8,43]. Furthermore, manure, husk ash, and straw compost as organic fertilizer also contribute to the Pb, Cd, and As contamination [44,45]. It is because the main ingredient of the previous fertilizer has also been contaminated with heavy metals both from the environment and during the cultivation process. Nonetheless, further studies need to be conducted to identify the source of heavy metals to create a safety agriculture system for the community.

Meanwhile, the results of carbamate residue analysis in surface and rhizosphere soil using 4 main parameters, including buthylphenylmethyl carbamate (BPMC) and carbosulfan, were declared undetectable. In contrast, the content of MIPC residues was found at low concentrations (**Table 2**). This shows that carbamate contamination is relatively low due to applying chemical fungicides to prevent *P. infestans* infection.

Table 2 Contamination of pesticide residues on potato cultivations in Kledung District.

Sample	Carbamate pesticides (ppm)			
	Carbofuran	**BPMC**	**MIPC**	**Karbosulfan**
Surface Soil	< LoQ	< LoQ	0.24 ± 0.18	< LoQ
Rhizosphere	< LoQ	< LoQ	0.20 ± 0.14	< LoQ
LoD value	0.0033	0.0015	0.0017	0.0020
LoQ value	0.0150	0.0100	0.0150	0.0100

Description: BPMC = buthylphenylmethyl carbamate; MIPC = 2-(1-methylethyl) phenyl methylcarbamate; LoD = Detection limit is the smallest limit test parameter owned by a tool/instrument. LoQ = Quantization limit (LoQ) is the lowest concentration or number of analytes that can still be determined and meets the criteria of accuracy and precision.

On the other hand, the carbamate contamination seems decrease in each top soil layer. Several research mention that soil bacteria activities may contribute in pesticide bioremediation [46]. The low concentration of carbamate residue is likely related to farmers' habit of using organic fungicides based on antagonistic agents, such as *Trichoderma.* Organic pesticide application suppresses carbamate usage to threaten late blight disease. Several studies have proven that indigenous *Trichoderma* species from potato cultivations are more efficient, effective, and eco-friendly in inhibiting late blight caused by *P. infestans* [27,47].

In addition to heavy metals and carbamates in semi-organic cultivations, the balance of microorganisms when cultivating potato crops also needs to be considered. This is due to the diversity of bacteria and mold, especially in the rhizosphere area, directly to disease resistance, physiological and potato crop production [48]. Based on the metagenomic analysis, microbiota diversity in surface soils up to 5 cm deep significantly differs from soils in the rhizosphere area (**Table 3**). In this study, bacterial mapping was carried out using the 16s RNA marker gene region V3 - V4. The alpha diversity condition of bacteria in the rhizosphere is higher than the surface soil area, indicating bacterial recruitment activity involving the rooting of potato plants.

Table 3 Recapitulation of sequencing results and alpha diversity index from rhizosphere and surface soil in a potato farm.

Estimator	Rhizosphere	Surface soil
OTUs number	1,155	441
Observed species (richness)	1,105	359
Shannon (*H*)	7.969 ± 0.24	4.084 ± 0.56
Simpson (*D*)	0.991 ± 0.02	0.887 ± 0.02
Chao1	1,268.533 ± 97.29	416.109 ± 61.05
ACE (abundance-base coverage estimator)	1,211.942 ± 101.62	429.171 ± 62.86
Good's coverage	0.997	0.998
PG whole tree	103.341	43.367

Description: Interpretation of Shannon-Wiener index results: $H \geq 3$ values = high diversity and species richness; Simpson index: $D \rightarrow$ value 1 = diversity and high species abundance. Chao1 and ACE show a comparison of species diversity between microhabitats.

The identification results showed that rhizosphere microhabitats have 3 times more abundant OTUs than surface soils, indicated by the observed species (richness) value in Table 3. Furthermore, the Shannon, Simpson, Chao1, and ACE indices show that the microhabitats of the rhizosphere have higher species diversity with a relative abundance between equivalent species than surface soils. This indicates that a consortium of soil bacteria is associated with the potato plant's roots, while introduced bacterial species dominate surface soil.

The number of identified amplicon-sequences calculation showed that rhizosphere samples were inhabited by more bacteria than surface soil (**Figure 2(A)**). A total of 65 species of bacteria are found only in the surface soil layer, while more than 960 species are found in rhizosphere soils. However, at least 389 species-sharing are found in both subsoils (**Figure 2(B)**).

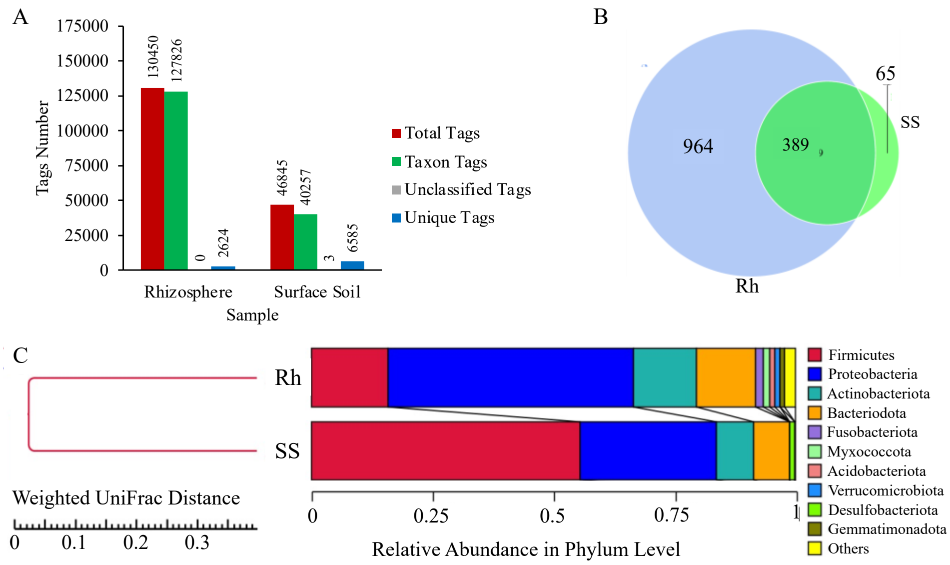

Figure 2 The comparison of bacteria abundance and richness in surface soil (SS) and potato rhizosphere (Rh) microhabitat in semi-organic potato cultivation. Label A = number of identified tags; B = Venn diagram of OTUs for the bacteria community; and C = relative abundance of the bacteria by phylum level.

A comparison of soil bacterial communities shown on Weighted UniFrac Distance shows that the phylum Proteobacteria community dominates more than 50 % of the total bacteria in the rhizosphere soil layer. Furthermore, the 3 bacterial communities dominating rhizosphere soils sequentially are Firmicutes, Bacteriodota, and Actinobacteriota. On the opposite, the composition of the bacterial community in the surface soil is shifting to the Firmicutes quantity, which is high enough to reach more than 55 % of the total bacteria, followed by Proteobacteria, Bacteriodota, and Actinobacteriota (**Figure 2(C)**).

Variations in bacterial communities in the subsoil are strongly influenced by various factors, including light intensity, humidity, temperature, fertilizer type [49], heavy metal contamination, and chemical pesticides [50,51]. Therefore, the species abundance and diversity on surface soils are likely limited by environmental factors rather than interactions between bacterial communities. This fact is based on the findings of the OTUs, where bacteria that populate most surface soil are Firmicutes, especially the *Bacilli* order, which endure more than other bacteria by producing endospores to protect themselves from extreme environments [52]. In semi-organic fields, the first contact of chemical fertilizers and pesticides is the surface soil, which directly changes the soil's pH gradient. In addition, all-day UV light exposure may also affect the formation of endospores in *Bacilli* while limiting the growth of other bacteria.

In the rhizosphere soil, Proteobacteria and Actinobacteriota dominate microhabitat that may be related to the fertilization process. It relates to Dai *et al.* (2018) research, which states that adding the element N through fertilizer correlates to an increase in the number of Proteobacteria and Actinobacteriota [53]. Furthermore, other studies have also shown that manure application for fertilization increases Proteobacteria, Bacteriodota, and Actinobacteriota abundance [54]; however, it declines Firmicutes population.

The rhizosphere has a higher species richness index and relative abundance than surface soils. This shows that the rhizosphere contains balanced environmental factors such as minerals and pH gradients that are maintained the needed nutrients for Proteobacteria growth [55]. The decrease in pH, caused by excessive ion H+ and cation, is one of the main factors causing the loss of soil bacterial diversity. In contrast, the increase in soil bacteria community is driven by the availability of N [53], pH suitability, and changes in the structure of plant communities.

Various studies have also shown that fertilizing high N elements shifts the abundance of bacterial communities from copiotroph towards more dominant oligotrophs, thereby reducing bacteria diversity and soil nutrients contents [56-58]. Therefore, chemical fertilization enriches certain minerals such as N, P, and K for a long time, has an impact on reducing the quality of other soil nutrients, until it needs higher doses to improve field quality that increases production costs to be more expensive [59]. In addition, the layers of soil bacteria abundance are also influenced by soil porosity and the harvesting process simultaneously [60]. High-density soils limit O_2 diffusion, increasing the accumulation of CO_2, leading to anaerobic conditions, and lowering the gradient pH [61]. This is relevant to the findings of this study, where anaerobic bacteria, such as *Fusobacteria,* are found only in rhizosphere soils due to their obligate anaerobic nature.

Based on the findings in this study, phylum Firmicutes, with 3 main orders, Bacilli, Lactobacilales, and Clostridia, dominates bacterial diversity by more than 52 % in the surface layer of topsoil. But its abundance decreases in the rhizosphere layer (**Figure 3**). Mostly, Firmicutes found not to dominate terrestrial ecosystems, although they have a vital role in regulating the diversity of soil bacterial [62,63]. However, in this study, manure-based organic fertilizers can introduce fecal bacteria into the soil dominated by Firmicutes. This significantly changes the structure of the relative abundance of soil bacteria and impacts the quality of agricultural soil fertility [54].

Meanwhile, Firmicutes in the rhizosphere are dominated by *Bacillus* and *Lactobacillus,* which are not found in surface soils. In addition, the composition of Firmicutes is quite interesting to study due to the possible stratification of habitat niches between Bacilli, Lactobacilales, and Clostridia. Bacilli and Clostridia are anaerobic and tend to sink deeper, while Lactobacilales are on the surface because they require O_2 for fermentation. This Lactobacilales family dominantly inhabits rhizosphere, with the main species including *Lactobacillus plantarum, L. murinus, L. lactis, L. paracollinoides,* and *L. reuteri,* and essential contributes in soil fertility. The genus *Lactobacillus* produces short-chain fatty acids, such as butyric, which facilitate the absorption of cations-anions by the roots of potato plants [64]. In addition, the presence of Lactobacilales can inhibit *P. infestans* [65], helps nitrogen fixation, triggers phosphate dissolution, and produces indoleacetic acid in horticultural crops [66,67]. A relatively higher abundance of Lactobacilales community in rhizosphere soils than surface soils are likely triggered by a bunch of biomass and polysaccharides in potato roots. In addition, human pathogenic bacteria from the genus *Escherichia* and *Shigella* were found abundantly, reaching 15 % of the total bacteria on the surface soil, which showed high contamination of intestinal bacteria from fertilization using manure.

Furthermore, potato pathogens such as *Ralstonia* (*Pseudomonas*) are found abundantly in surface soil with a depth of ≤ 5 cm but undetected in the rhizosphere. More specifically, *Ralstonia solanacearum* potentially infects potato plants and causes soil-borne wilt disease through tuber and/or root contact between potato plants, with high pathogenicity and resistance to environmental stress [68,69]. It indicates a potential for a wilt disease outbreak in semi-organic potatoes in Temanggung Regency. However, the presence of *Ralstonia* has decreased drastically and has no significant relative abundance in the rhizosphere (≥ 6 cm). This is likely a form of antibiotic interaction between bacterial communities that affect each other. In this study, the results of the metagenomic analysis also showed that the relative abundance of the genus *Bacillus* reached 2 % of the total bacteria on rhizosphere soils. The 2 main species found in this study are *Bacillus humi* and *B. thuringiensis. Bacillus* sp, in the soil, often acts as a natural limiting factor against the growth of pathogens, such as *Ralstonia* sp and *Fusarium sp*. mold. Other studies have shown that the isolate *Bacillus* spp. B315 can delay the incubation period of wilt disease by up to 7 days with a control effectiveness of 64.9 %.

Bacillus is abundant in the rhizosphere layers due to its anaerobic nature, requiring a humid environment and avoiding sunlight. Various species of Bacillus produce hydrolysis enzymes that can limit the growth of other microorganisms [68], prevent nitrogen loss [70,71], increase the uptake of P and K by the roots [72], and produce promoting growth phytohormone [73]. For example, strains of *B subtilis*, which are abundant in the environment, produce antibiotic lipopeptides, including iturin [74], surfactants [75], and fengycin [76]. Enzymes of the lipopeptide group protect *Fusarium oxysporum* [75], and *Rosellinia necatrix* in horticultural crops [78], by inducing systemic resistance in the host plant or suppressing the growth of pathogenic fungi directly [79-81]. This shows the potential use of indigenous *Bacillus* as a plant regulator and biocontrol of potato pathogens.

Interestingly, although there are similarities in phylum-level composition from the surface and rhizosphere sample soil, the bacteria diversity at the class level seems significantly different. Proteobacteria in the surface soil are composed of the Gammaproteobacteria group only, with low relative abundance. However, it drastically increases the population of the other meaningful orders in the rhizosphere soil.

Gammaproteobacteria and Alphaproteobacteria are the most abundant bacteria that inhabit the rhizosphere and dominate the composition of bacteria up to more than 50 % of the total species.

Gammaproteobacteria play essential roles in bioremediation and have proven to eliminate various types of heavy metal contamination from the soil. The abundance of the Gammaproteobacteria also has become a critical factor in decreasing heavy metal concentrations in the rhizosphere. In addition, high concentrations of Pb, Cd, Cr, or As limit the growth of unresistant bacteria against heavy metals [50], thus affecting the diversity of rhizobia [82]. The dominant species of Gammaproteobacteria found in rhizosphere soils, such as *Pseudoxanthomonas mexicana, Thermomonas* sp., and *Lysobacteria* sp. This result is relevant to Wang *et al.* [48], which state that *Pseudoxanthomonas, Thermomonas,* and *Lysobacteria* meaningfully make up a community of potato rhizosphere bacteria.

Furthermore, *P. mexicana* is a bacterium that contributes significantly to the relative abundance of nitric oxide reductase, which plays a role in converting nitrates into nitrites [83]. In contrast, *Lysobacteria* sp. plays the opposite role [84]. Furthermore, *P. mexicana* has antinematode activity against *Meloidogyne incognita* and its metabolism [85]. These bacteria are also involved in antibiosis activity against pathogens, increasing the absorption of essential minerals for potato plants [86]. Another species that also plays a role in helping potato plants resist pollution is *Comamonas testosterone* because it can act as a bioremediation of heavy metal polycyclic hydrocarbons [87]. The existence of rhizosphere bacteria needs to be maintained in abundance because it has the potential to be a plant growth regulator for potato plant growth.

In the class Alphaproteobacteria, there are 2 main orders; Sphingomonadales and Rhizobiales (**Figure 3(A)**). The main species of the order Sphingomonadales sequentially include *Sphingopyxis alaskensis, Pedobacter boryungensis*, *Sphingobacterium mizutaii*, and *P. ginsengisoli* with a relative abundance of less than 2 %. Rhizobiales, on the other hand, became the most dominant class of bacteria, with a relative abundance reaching more than 20 %. Rhizobiales form endosymbionts with the host and provide an advantage by providing essential nutrients such as N-organics [88]. Several symbiont species that benefit potato plants have been successfully identified, such as *Devosia* sp., D *riboflavina, Aminobacter* sp., *Bosea* sp., *Allorhizobium, Neorhizobium*, *Pararhizobium, and Rhizobium.* These species are only found to compose bacterial communities in parts of the rhizosphere and surface soil layers. The uniqueness of the rhizosphere bacteria consortium then led to distinctive bacteria with a beta diversity score of 0.750 (**Figure 4**)

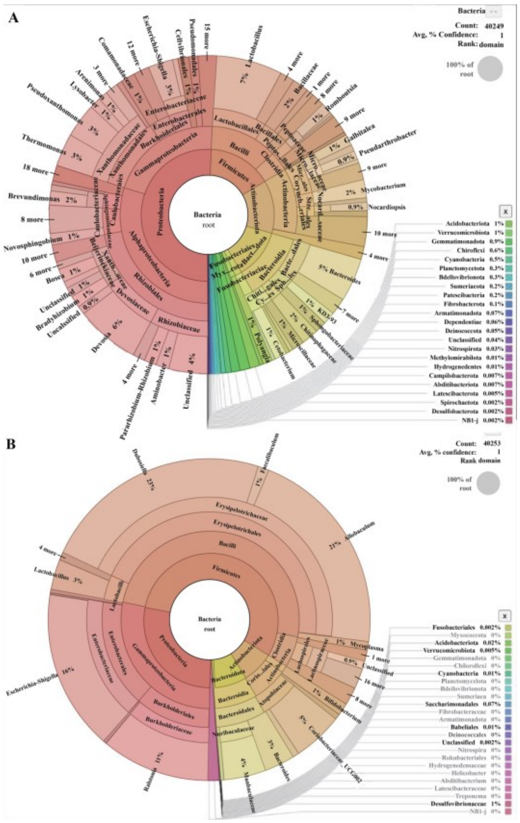

Figure 3 Distinctive composition of bacteria diversity in surface soil and rhizosphere.

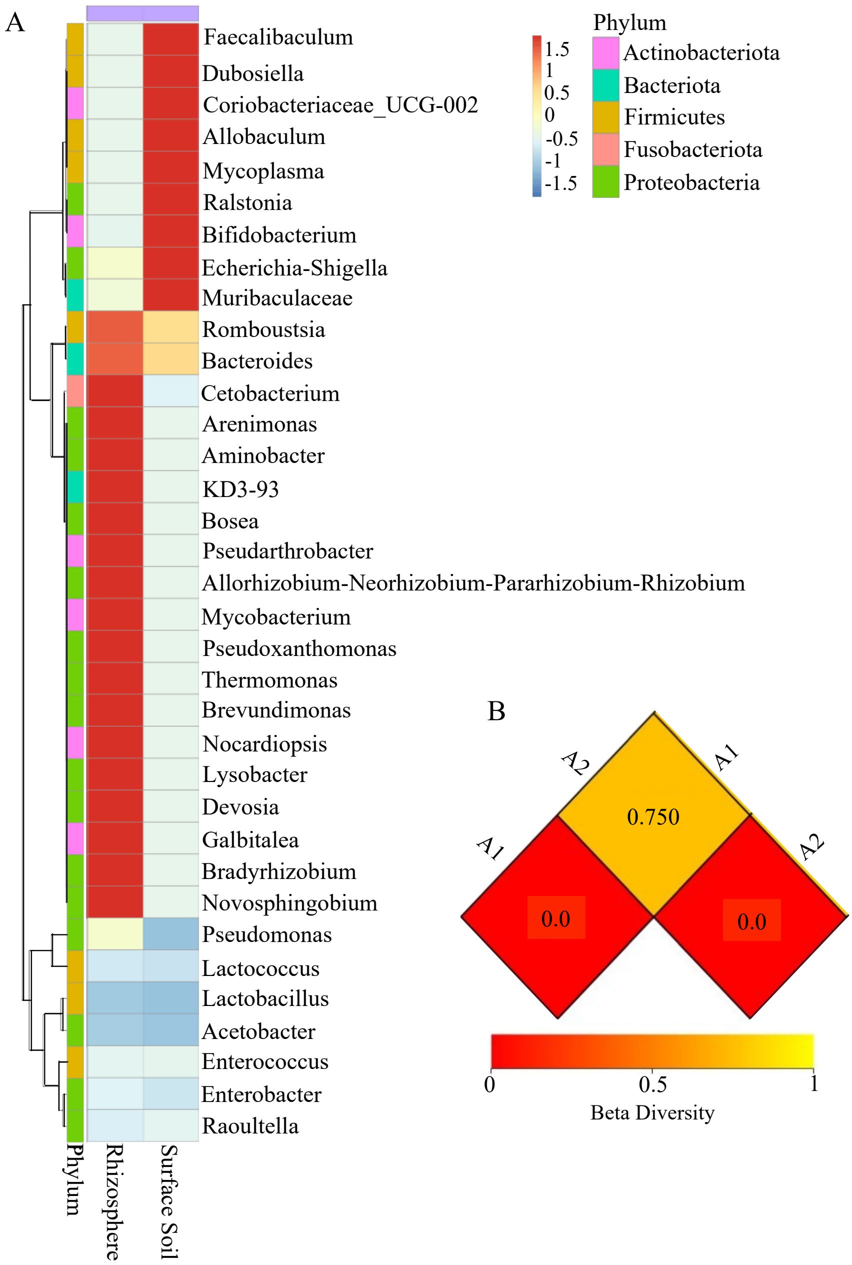

Figure 4 species in each subsoil based on genus diversity (A) and beta diversity close to a value of 1 or very different (B).

By their abundance, *Devosia* sp and *D. riboflavina* became the most dominant species of the class Rhizobiales (**Figure 5**). Previous studies have also explained that *Devosia* dominates various layers of agricultural soil. They have bioremediation potential by degrading different aromatic and xenobiotic compounds such as benzoates, p-hydroxybenzoates, biphenyls, catechols, and chlorinated aromatic compounds [89]. In addition, *Devosia* has an essential role in the fixation of NO from the environment to help the availability of nitrogen in the soil [90].

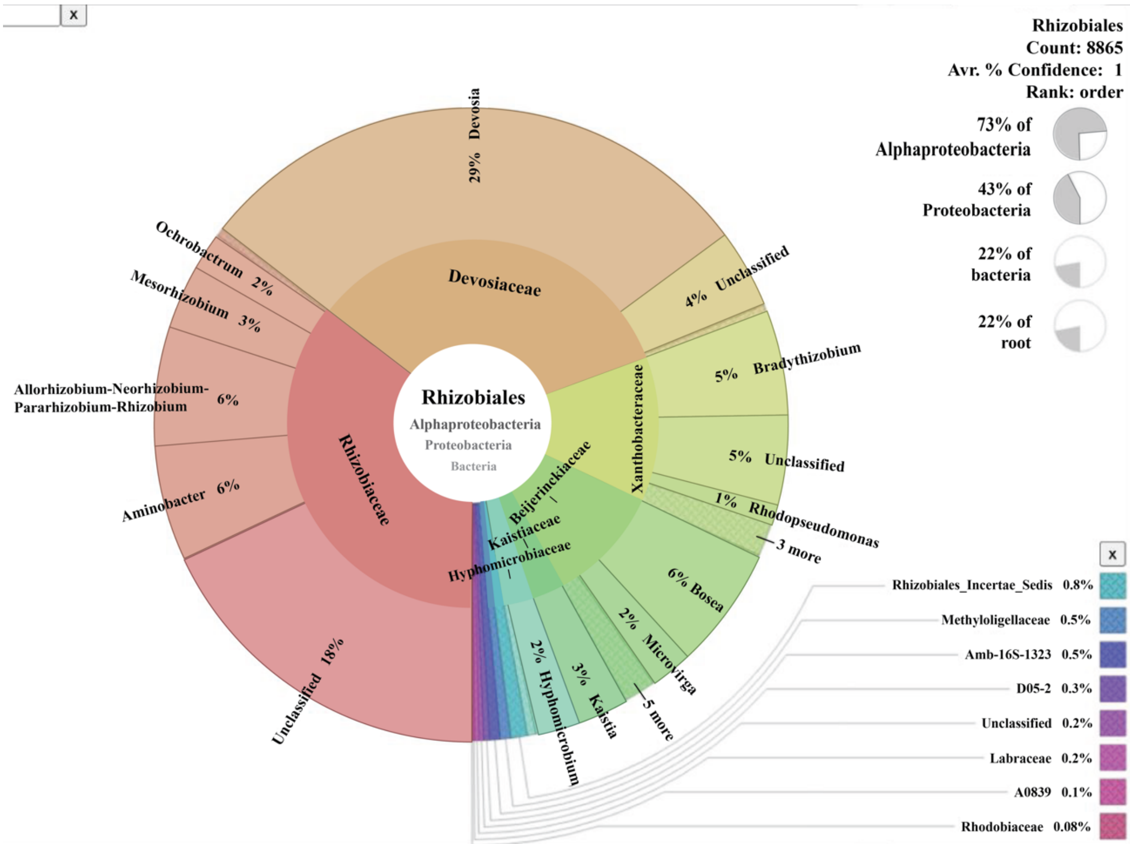

Figure 5 Composition and relative abundance of Rhizobiales bacteria that play a role in N fixation in semi-organic potato farming.

This study also succeeded in documenting the presence of significant rhizobia, which has an essential role in ensuring the availability of macro elements (N, P, and K) and trace elements in the soil, such as *Aminobacteria* sp. *Bradyrhizobium elkanii,* and *Mesorhizobium* sp. Furthermore, as many as ± 6 % of rhizobia from Rhizobiales are the genus *Allorhizobium, Neorhizobium, Pararhizobium,* and *Rhizobium,* which have been widely known as regulators of the availability of N, P, and K, in soil. Rhizobia is a gram-negative bacterium that forms an endosymbiotic nitrogen-fixing association with plant roots [89]. In general, rhizobia interacts in mutualistic associations with Leguminaceae plants, promoting the formation of special structures on the roots, called nodules, in which nitrogenous fixation processes occur [90]. The nodule bacteria inside differentiate into bacteroids, which can convert atmospheric nitrogen into ammonium, which is then transported to plant cells and metabolized [93]. In this study, rhizobia were also found massively in potato rooting, showing the bacterial group's flexibility in symbiosis with other plant species. The present of diverse bacteria in rhizosphere may also influenced by plant exudate that produced by the potato root, including phytohormone or essential nutrition needed by bacteria [94,95]. However, further studies should be conduct in describing how the interaction between potato plants and rhizobacteria and how the consortium of rhizosphere bacteria formed.

Indigenous soil rhizobia potential as biomodulator in potato crop

Soil bacteria diversity contributes to the balance of ecosystems, especially in agriculture. Each species of bacteria has an important role that has been specialized. For example, rhizobia help provide nutrients to plants and other bacteria [91,96]. Through the profile of soil bacteria, as presented in **Figure 6**, the characteristics of microorganisms that play an essential role in the growth and productivity of potatoes can be understood and developed as biomodulators and organic pesticides.

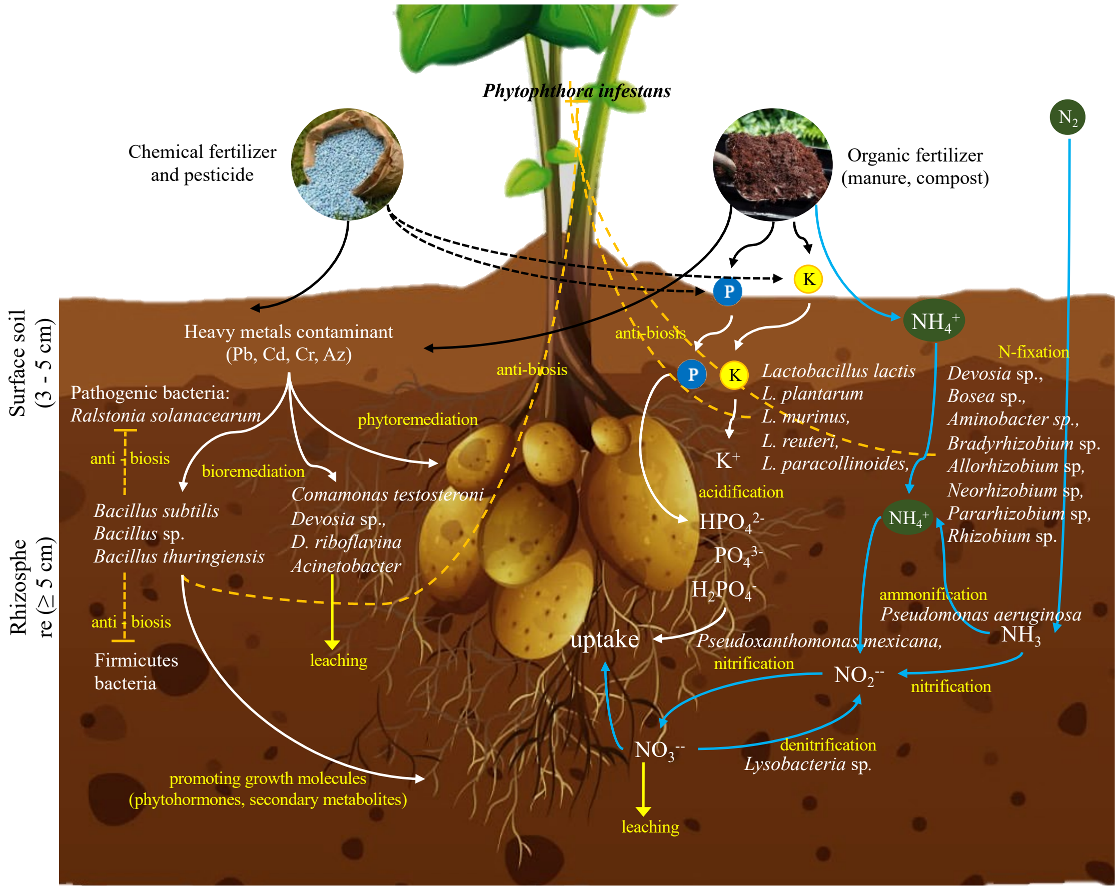

Figure 6 The scheme of interaction between soil bacteria and their role in supporting the growth of potato thorn. A dotted line shows the relationship of antibiosis.

The loss of certain species certainly impacts the entire ecosystem, considering that soil bacteria are volatile and depend on environmental factors and agricultural care. Therefore, based on this study, it is necessary to impose restrictions on chemical fertilizers and pesticides while switching to organic materials to reduce heavy metal contamination and maintain the diversity of soil bacteria. The root of potato plants attracts bacteria consortiums that support plant growth, so there is an interaction between the host and rhizobia.

Conclusions

The combination of organic and chemical fertilizers may increase the excessive nitrogen supply, heavy metal contamination (Pb, Cd, Cr, and As) and carbamate residues, that influences microbiome in the soil. Surface soil is dominated by Firmicutes, with the main species are *Faecalibaculum, Allobaculum, Dubosiella*, and *Mycoplasma*; *Ralstonia* and *Escherichia-Shigella* from Proteobacteria; and Actinobacterium that consisted of Bifidobacterium. In contrast, the rhizosphere is dominated by N-fixating rhizobia from phylum Proteobacteria, while the Firmicutes population is suppressed. Rhizobia community is mainly composed of *Pseudoxanthomonas mexicana, Thermomonas* sp., *Lysobacteria* sp., *Devosia riboflavina*, *Aminobacter* sp., *Bosea* sp., *Allorhizobium, Neorhizobium, Pararhizobium,* and *Rhizobium*. In addition, rhizobacteria literature potentially used as a reference for soil management in the low or medium land by conditioning the rhizosphere to fit potato natural habitat and prevent pathogens in the highlands.

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