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Saline soil bioremediation using *Muntingia calabura* extracts documents the first potential occurrence of halotolerant bacteria molecularly related to R. *marisflavi* and *C. firmus* in Tarlac, Philippines

Roman Rafael B. Manucdoc*[1], Robert V. Marcos[1] and Geraldine G. Villanueva[2]

[1]Office of University Research Development, Tarlac State University, Tarlac City, Philippines
[2]Center for Natural Products Research, Tarlac State University, Tarlac City, Philippines

*Correspondence e-mail: manucdoc.romanrafaelb@gmail.com

Abstract

Land salinization negatively affects soil quality and crop productivity. This can be countered through bioremediation of salt-affected soil using microorganisms, which can be further enhanced through biostimulation. In the present study, the biostimulatory effect of Muntingia calabura fruit and leaf extracts as determined by soil salinity and bacterial load was explored. Representative bacterial colonies were also characterized via full-length 16S rRNA (~1500 bp) analysis through DNA barcoding. The results showed that the soil with fruit and leaf extracts (SS + FE and SS + LE, respectively) demonstrated lower electrical conductivity (EC) values than those of the control (applied with distilled water). Meanwhile, the bacterial load in all the treatments gradually decreased, possibly due to the salt stress and the extracts' antimicrobial properties. Molecular characterization revealed that the surviving species were closely related to strains of halotolerant bacteria *Rossellomorea marisflavi* with 100.00% percent identity and *Cytobacillus firmus* (98.97% and 98.84%), documenting the first potential occurrence of these taxa in Tarlac, Philippines. The study suggests that the extracts, while lowering total bacterial load, might have served as a growth-supporting nutrient source for the halotolerant species, corroborating the improved EC reduction. Further investigations on the multifaceted bioremediation mechanisms using the extracts are strongly recommended.

Keywords: Salinity bioremediation; Biostimulation; Green waste utilization; Soil amendment; Electrical conductivity; Indigenous bacteria.

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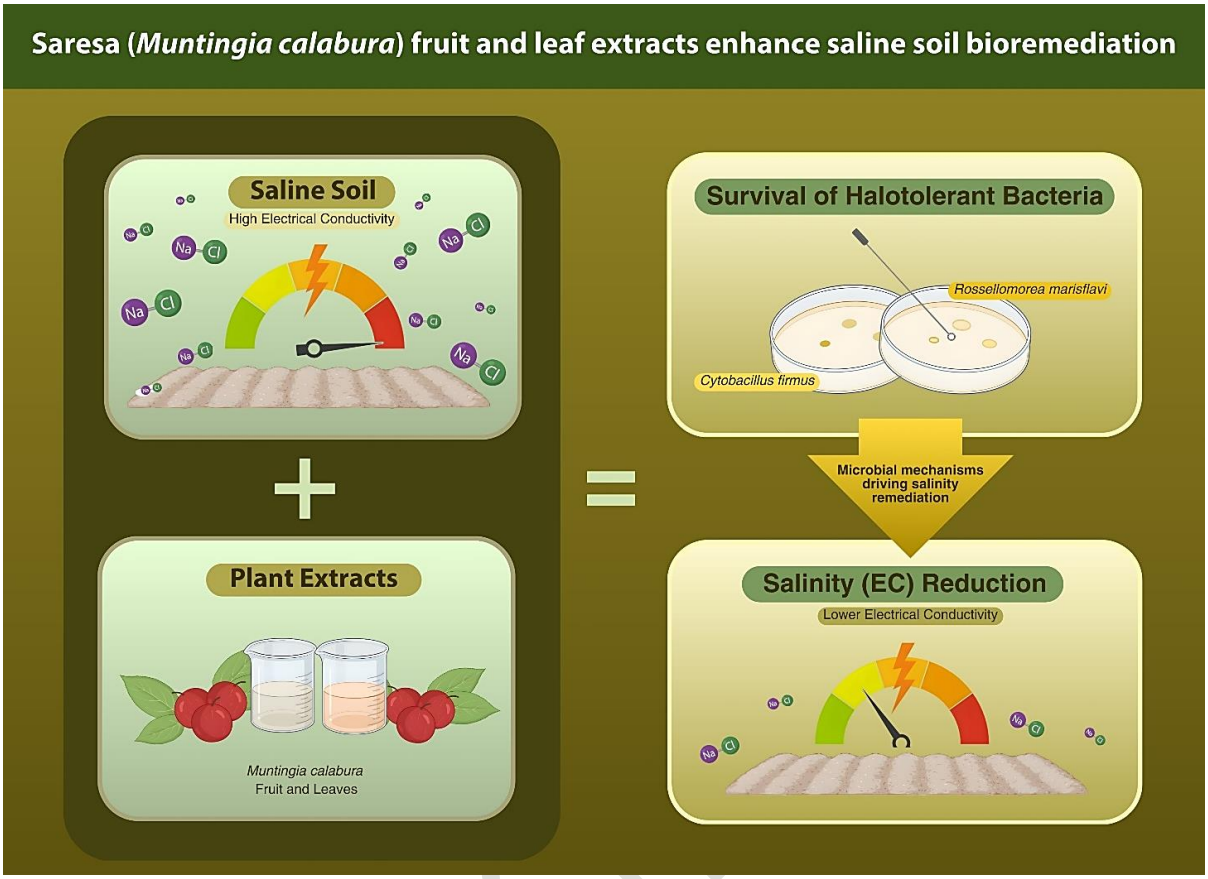
Graphical abstract



1. Introduction

The Philippines, as an agricultural country, highly depends on cultivating various crops for livelihood, economic stability, and food security. With the vast land areas used in farming rice, corn, sugarcane, and other essential crops, maintaining healthy soil quality is vital to maximize agricultural productivity. In particular, different environmental factors affect not only soil health and quality, but also the growth performance and yield of any plant/crop it anchors. Soil salinity, defined as the salt and/or mineral content of the soil, is one of these influencing factors that, when at high levels, becomes detrimental to the biological processes vital to the crop growth and development (Kamran *et al.* 2019).

Defined as an increase in the salinity of the soil, land salinization covers over 118 countries with more than 424 million hectares of topsoil and 833 million hectares of subsoil being salt-affected (Food and Agriculture Organization, 2025). This process can be induced by climate change, the massive introduction of intensive farming-associated irrigation, and the frequent use of groundwater, as well as low-quality water, for irrigation (Machado & Serralheiro, 2017). External stress due to high soil salinity impacts plant growth through osmotic shock and ionic toxicity, which both interfere with photosynthesis, cellular metabolism, and uptake of water and nutrients (Safdar *et al.* 2019). As cited by Shrivastava and Kumar (2015), more than 50% of the arable land is estimated to be salinized by the year 2050 (Jamil *et al.* 2011).

In the Philippines, the Bureau of Soils and Water Management (2020) continuously works to address soil salinity problem in the country. According to their consolidated technical reports, at least 17 provinces have areas with slightly saline to very severely saline conditions, characterized by high electrical conductivity. Meanwhile, there are studies showing that some groundwater sources in Tarlac, relatively utilized for irrigation, displayed high electrical conductivity (Franquera *et al.* 2019; Inson *et al.* 2021). With the soil salinity challenges not only in the country, but also globally, it is imperative to provide strategies for land preservation, ensuring maximum agricultural productivity.

Biostimulation is an ideal strategy to desalinate salt-affected soil. It refers to the addition of nutrients to enhance the bioremediation ability of microorganisms, boosting the removal and/or degradation of soil pollutants in the process. In biostimulation, the microorganisms are stimulated through substrate modification (Adams *et al.* 2015). According to the study of Shahi *et al.* (2016), both the pollutant degradation capability and growth of the bacterial community in soil were enhanced after the addition of total organic carbon. Moreover, the reviews of Cherif-Silini *et al.* (2021), Mokrani *et al.* (2022), Terzaghi *et al.* (2022), and Gupta *et al.* (2024) all emphasize the

utilization of microorganisms as a promising strategy in soil desalination. Furthermore, the application of halophilic and halotolerant microorganisms can aid in the long-term production of crops under salt-stressed conditions (Kumawat *et al.*, 2022). Hence, exploiting the desalinating ability of these microorganisms and boosting it through biostimulation is an advantageous and eco-friendly approach.

Muntingia calabura, locally known as "aratilis" or "saresa", is a plant present in tropical areas and is common in the Philippines. Its fruits and leaves are easily detached from the tree, generating green waste. Typically, these fruits and leaves are of no use to the landowners who have *M. calabura* trees in their premises and simply discard them. Langsdorf *et al.* (2021) emphasized that green waste, when properly utilized, can be converted into functional materials. Therefore, to make use of these fruits and leaves, the biostimulatory effects of their extracts towards the microbial activity of naturally present bacteria in soil were evaluated to promote salinity reduction. Similarly, studies have already reported the bioremediation of pollutants with the amendments of plant materials, including wheat straw (Cai *et al.* 2023), corn stalk (Gideon *et al.* 2025), rice straw (Zhang *et al.* 2025), and sugarcane residual waste (Umar *et al.* 2022). Moreover, *M. calabura* leaves and fruits contain sugars, organic compounds (Pereira *et al.* 2018; Zolkeflee *et al.* 2021), high moisture, fiber, crude fiber, as well as magnesium, sodium, potassium, phosphorus, and iron (Peter *et al.* 2020). All of these chemical constituents of *M. calabura* leaves and fruits can be favorable factors for the growth of indigenous bacteria, thereby increasing their bioremediation potential.

In this context, the study investigated the biostimulatory effects of *M. calabura* fruit and leaf extracts on saline soil by evaluating salinity reduction through electrical conductivity; changes in soil quality parameters (pH and dissolved oxygen), bacterial load, and the molecular characterization of bacterial isolates to determine whether halotolerant species thrive, potentially contributing to salt reduction.

2. Materials and methods

2.1. *Extraction of M. calabura fruits and leaves*

Detached *M. calabura* fruits and leaves were collected from around Tarlac State University – Lucinda Campus, Tarlac City, Tarlac, Philippines. After collection, they were washed under running water to remove dirt and other particles. Surface sterilization was carried out by dipping the fruits and leaves in 70% ethanol for 5 minutes, followed by rinsing with sterile distilled water. Separately, fruits and leaves were boiled in sterile distilled water (1:2 w/v, 50 g of fruits/leaves to 100 mL distilled water) for 5 minutes. The resulting solution was filtered and dispensed into sterilized storage bottles. The extracts were then stored at refrigerated conditions until the laboratory analyses.

2.2. *Pot experiment*

Soil samples from around a man-made lagoon (Figure 1) located on the same campus were collected and sieved. The soil was mixed with autoclaved loam soil (1:1 w/w, 50 g each) inside sterile storage bottles. As a pilot research, the setups were then spiked with a 50 mM NaCl solution to simulate a critical ecological tipping point for salinity-induced stress in plants, as described by Shrivastava & Kumar (2015) and Zhou *et al.* (2024). Afterwards, the fruit and leaf extracts were also mixed into the soil mixture using spatulas. The setups were then stored in a room with a temperature and illumination of approximately 27°C and 31 lux, respectively. **Table 1** summarizes the experimental groups, with DW + DW and SS + DW serving as the baseline and negative control groups, respectively. Three (3) replicates were prepared for each treatment.

Figure 1. Man-made lagoon in Tarlac State University – Lucinda Campus

2.3. *Physicochemical and microbiological analyses*

Soil samples from the biostimulation setups were obtained, mixed, and homogenized in sterile distilled water in a 1:5 w/v ratio (Ismayilov *et al.* 2021; Kargas *et al.* 2022). The soil extracts were then transported to the laboratory for physicochemical analyses. Using a multi-parameter meter, the electrical conductivity (EC), as well as the dissolved oxygen (DO) and pH of the extracts, were measured.

Another soil sample sets were transferred and mixed into test tubes containing 0.9% NaCl solution up to 10^{-6} serial dilutions. Aliquots from these dilutions were spread-plated using Plate Count Agar (PCA) enriched with nystatin (to inhibit fungal growth), and NaCl adjusted to 50 mM (to simulate the saline conditions of the origin soil). The plates were then incubated at 30°C for 6 days. After incubation, colony formations were observed and counted.

Table 1. Composition of the biostimulation setups

Treatment	Components
DW + DW	Soil + 20 mL distilled water + 10 mL distilled water
SS + DW	Soil + 20 mL salt solution + 10 mL distilled water
SS + FE	Soil + 20 mL salt solution + 10 mL fruit extract
SS + LE	Soil + 20 mL salt solution + 10 mL leaf extract

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract

2.4. *Isolation and molecular characterization of bacterial colonies*

To further understand the effect of the extracts on the bacterial activity, the most abundant bacterial colonies from the PCA plates were isolated. Three colonies were randomly selected and subcultured onto Nutrient Agar (NA) plates, which were also supplemented with NaCl and nystatin. For initial characterization, biochemical tests were done to determine whether the colonies produce catalase, oxidase, coagulase, and indole.

For the molecular characterization, the bacterial cultures were submitted to Apical Scientific, Selangor, Malaysia, through the Noveaulab Asia Corporation, Quezon City, Metro Manila, Philippines. Bacterial DNA Barcoding was employed to analyze the full-length of 16S rRNA (~1500 bp). The processes involved were (1) extraction of the genomic DNA, (2) PCR amplification and purification, and (3) bidirectional sequencing of the PCR products. Sequence similarities were assessed to determine the top 10 matches, and phylogenetic trees were subsequently generated.

2.5. *Data analysis*

The research utilized a completely randomized design to lay out the four (4) treatments with three (3) replicates. One-way analysis of variance (ANOVA) and Tukey Post-Hoc Test at 5% level of significance were used to analyze and compare the data per treatment in terms of the physicochemical and microbiological parameters. Meanwhile, the computational tool Basic Local Alignment Search Tool (BLAST) was used for analyzing sequence similarity for molecular characterization. The top ten (10) matching sequences were obtained from the National Center for Biotechnology Information (NCBI) database.

3. Results and discussion

3.1. *Salinity of the soil samples as determined by their electrical conductivity*

The presence of NaCl in soil increases its ability to conduct electricity, leading to a higher electrical conductivity (EC). In the present pilot research, soil samples spiked with 50 mM NaCl solution were utilized to simulate the transition from productive to unsustainable agricultural land (Shrivastava & Kumar 2015; Zhou *et al.* 2024), where microbial and/or chemical interventions can be economically impactful. As displayed in Figure 2, the soil samples with *M. calabura* fruit and leaf extracts exhibited lower ECs compared to those of the control SS + DW, with SS + LE demonstrating the best reduction effect. The latter was able to decrease the EC of the saline soil down to 484.56 µS/cm by Day 14, while SS + FE had 517.44 µS/cm; both values were lower than that of the control SS + DW (555.67 µS/cm). Moreover, as shown in **Table 2**, no significant difference was observed among SS + DW, SS +FE, and SS + LE on Day 7, suggesting a temporary parity in their EC reduction performances. Nevertheless, there was a significant difference between the control SS + DW and SS + LE on Days 1 and 14, supporting the leading effectiveness of the leaf extract in reducing EC during these testing points.

The occurrence may be preliminarily explained with the organic compounds potentially present in the extracts used in SS + FE and SS + LE. Through the process of chelation, the compounds may have bound with the soluble salt ions, mobilizing them in the process (Xiao *et al.* 2022; Anderson *et al.* 2023). Moreover, soil bacteria might have also taken part in the remediation through the production of organic acids and chelating agents (Ribeiro *et al.* 2020), as well as ion sequestration and the consequent EC reduction (Hernández-Canseco *et al.* 2022).

The aforementioned concepts may also be linked with the EC decrease observed in the control SS + DW, as soil naturally has present microorganisms and organic compounds (Lehmann & Kleber 2015; Paul 2016; Kleber *et al.* 2021). However, a fluctuation took place in this treatment with a slightly increased EC by Day 14, which may be due to the minerals that took longer to dissolve. These minerals might have broken down and released weathering products, elevating the concentration of dissolved ions in the process (Rieder *et al.* 2024); hence, the small increment demonstrated in SS + DW. Furthermore, the consistent increase of EC in the control DW + DW (non-saline soil) may also be due to the breakdown of minerals over time. This further substantiates the effectiveness of *M. calabura* extracts, as the setup in the treated groups seemingly defied this occurrence, evident in the continued lowering of the EC values in SS + FE and SS + LE by Day 14.

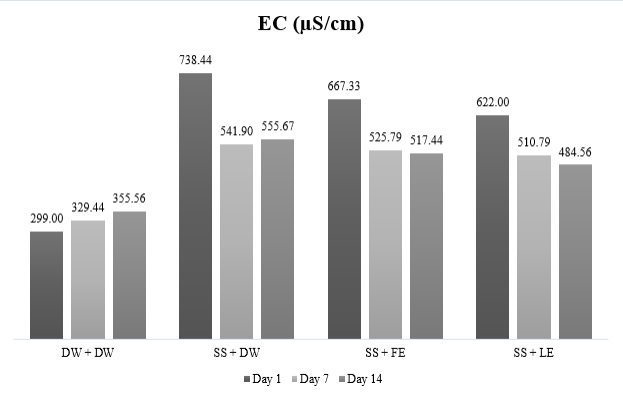


Figure 2. Electrical conductivity of the soil sample from different treatments

3.2. *Influence of M. calabura fruit and leaf extracts on oxygen availability and pH*

To further determine the quality of the soil after 14 days, the dissolved oxygen (DO) and pH of the soil extract samples were tested. **Table 3** summarizes the DO and pH values of the soil samples. Despite the differences among the means, all of the treatments tested showed no significant difference from one another in both parameters. Taking these findings into consideration, SS + FE and SS + LE, both of which were not significantly different from the control DW + DW (non-saline soil), were able to aid the remediation of saline soil without compromising its DO and pH.

Table 2. Salinity of the soil sample extracts as described by their electrical conductivity

Treatment	Electrical Conductivity (µS/cm)		
	Day 1	Day 7	Day 14
DW + DW	299.00	329.44	355.56
SS + DW	738.44	541.90^{ab}	555.67^{a}
SS + FE	667.33^{a}	525.79^{ac}	517.44^{ab}
SS + LE	622.00^{a}	510.79^{bc}	484.56^{b}

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the same superscripts, within a column, have no significant difference (p > 0.05).

Table 3. Dissolved oxygen and pH of the soil samples by Day 14

Treatment	Dissolved Oxygen (mg/L)	pH
DW + DW	7.10^{a}	7.81^{a}
SS + DW	6.73^{a}	7.72^{a}
SS + FE	5.56^{a}	7.71^{a}
SS + LE	6.38^{a}	7.65^{a}

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the same superscripts, within a column, have no significant difference (p > 0.05).

The two parameters, supplemental to the reduced EC, can provide insight as to whether the soil can still be potentially viable for plant cultivation and microbial growth after the remediation process. Oxygen availability affects the growth and metabolism of plants, particularly during seed germination (Ray *et al.* 2016); influences microbial processes involved in nutrient cycling and soil fertility (Li *et al.* 2021); and aids in the root function and nutrient uptake (Li *et al.* 2021). Meanwhile, pH impacts nutrient cycling, plant nutrition, soil remediation, and microbial activity in soil (Neina, 2019; Wang *et al.* 2019).

3.3. *Bacterial load of soil samples*

The quantification of colony-forming units (CFUs) from the samples provided insights on how the extracts influenced the activity of the microbial community indigenous in the soil as determined by the total culturable bacteria. Figure 3 presents all the bacterial loads of the soil samples from the treatments, demonstrating a gradual decrease across the three time points. It is also quite noticeable that the treated groups, SS + FE and SS + LE, had the lowest bacterial load on Day 1 (both 7.20 log CFU/g), Day 7 (6.75 log CFU/g and 6.64 log CFU/g, respectively), and Day 14 (6.49 log CFU/g and 6.51 log CFU/g). Moreover, as shown in **Table 4**, statistical analysis revealed that the baseline control DW + DW showed no significant difference compared to the other treatments on Day 1. While these three treatments exhibited intermediate similarities, a significant difference was observed between SS + DW and SS + LE. Meanwhile, no significant difference was demonstrated between the two control treatments by Day 7, as well as the two experimental groups. Furthermore, all of the treatments tested no significant difference from one another at the last testing point.

Salinity, being a major soil stressor (Zhang *et al.* 2019), might cause the bacterial community to decrease over time, in addition to the declining nutrient availability in the substrates. As for the treated groups, the occurrence might be due to the natural antimicrobial properties of *M. calabura* fruits and leaves, known to contain bioactives including phenols, tannins, alkaloids, and flavonoids, among others (Ansori *et al.* 2021; Ariffin *et al.* 2022), which all exhibit inhibitory effects against various microorganisms (Arora *et al.* 2024). Along with this inhibitory activity, the lower values of the treated groups can also be rooted in the salinity stress itself (Zhang *et al.* 2021), as well as the influence of pH on microbial activity (Naz *et al.* 2022), making the soil environment slightly less favorable for the bacterial community over time. Nevertheless, the treated groups still managed to exhibit the leading EC reduction (**Figure 2 and Table 2**). The phenomenon might have taken place due to the diminishing of general bacteria, while paving the way for the thriving and survival of halophilic and/or halotolerant bacteria that could tolerate, and ultimately, help remediate the saline soil environment.

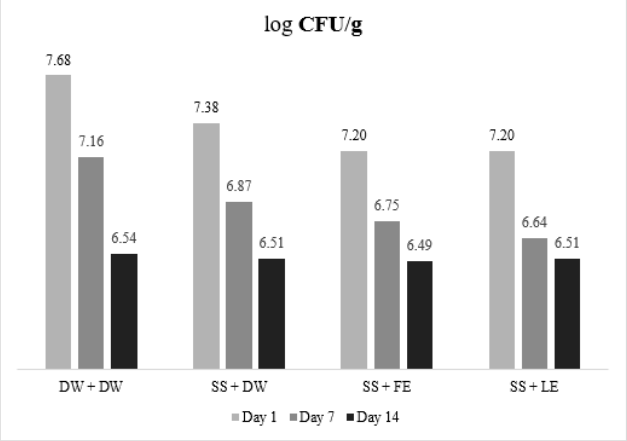


Figure 3. Bacterial load of soil samples from different treatments

3.4. *Molecular characterization of the bacterial colonies*

To characterize the culturable members of the microbial community, representative colonies were selected from each treatment group through biochemical characterization. While this targeted selection provides a taxonomic snapshot of the bacterial isolates capable of thriving under the soil conditions, it was intended as an exploratory identification rather than a comprehensive profiling of the microbial community.

Table 4. Bacterial load of the soil samples expressed in colony-forming units per gram (CFU/g)

Treatment	log CFU/g		
	Day 1	Day 7	Day 14
DW + DW	7.68^{abc}	7.16^{a}	6.54^{a}
SS + DW	7.38^{ad}	6.87^{a}	6.51^{a}
SS + FE	7.20^{bde}	6.75^{b}	6.49^{a}
SS + LE	7.20^{ce}	6.64^{b}	6.51^{a}

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; Means with the same superscripts, within a column, have no significant difference ($p > 0.05$).

Table 5. Biochemical test results of the isolated colonies from each treatment

Treatment	Soil isolates per treatment	Biochemical tests			
		Catalase	Oxidase	Coagulase	Indole
DW + DW	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + DW	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + FE	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-
SS + LE	Colony 1	+	-	-	-
	Colony 2	+	-	-	-
	Colony 3	+	-	-	-

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract; (+) = Detected; (-) = Not detected

Prior to molecular characterization, three (3) colonies from each treatment were selected and subcultured for biochemical tests. As summarized in **Table 5**, all colonies tested positive for catalase. Meanwhile, oxidase, coagulase, and indole production were not detected on the colonies. Considering the results of the biochemical tests, one (1) representative colony from each treatment was subjected to bacterial DNA barcoding for the full-length 16S rRNA (~1500 bp) analysis.

Table 6 highlights the top 10 sequence matches of the bacterial cultures subjected to DNA barcoding. For DW + DW, it was revealed that strains with 100.00% percent identity (PI) belong to *Bacillus cereus*. Colonies from both SS + DW and SS + FE were revealed to have 100.00% PI with the strains of *Rossellomorea marisflavi*. Lastly, the culture from SS + LE had the highest matches with *Cytobacillus firmus* strains (98.97% and 98.84%). The relatedness of these species and/or strains, along with other sequence matches of the bacterial colonies, is summarized using phylogenetic trees (**Figure 4**).

Table 6. Sequence matches of the bacterial colony isolated from each treatment

Treatment	Aligned Region	Sequence Length	Homologous Sequences (Percent Identity)	Sequence Length (Linear rRNA)	NCBI Accession Number
DW + DW	1486 bp	1488 bp	*Bacillus cereus* ATCC 14579 (100.00%)	1512 bp	NR_074540.1
			Bacillus cereus strain CCM 2010 (100.00%)	1535 bp	NR_115714.1
			Bacillus proteolyticus strain MCCC 1A00365 (99.93%)	1509 bp	NR_157735.1
			Bacillus albus strain MCCC 1A02146 (99.93%)	1509 bp	NR_157729.1
			Bacillus sanguinis strain BML-BC004 (99.93%)	1555 bp	NR_175555.1

			Bacillus wiedmannii strain FSL W8-0169 (99.87%)	1540 bp	NR_152692.1
			Bacillus paramycoides strain MCCC 1A04098 (99.87%)	1509 bp	NR_157734.1
			Bacillus cereus strain IAM 12605 (100.00%)	1486 bp	NR_115526.1
			Bacillus paranthracis strain MCCC 1A00395 (99.80%)	1509 bp	NR_157728.1
			Bacillus fungorum strain 17-SMS-01 (99.80%)	1576 bp	NR_170494.1
SS + DW	1489 bp	1491 bp	*Rossellomorea marisflavi* strain TF-11 (100.00%)	1506 bp	NR_025240.1
			Rossellomorea marisflavi strain TF-11 (100.00%)	1466 bp	NR_118437.1
			Bacillus haikouensis strain C-89 (98.51%)	1473 bp	NR_148273.1
			Rossellomorea aquimaris strain TF-12 (98.25%)	1507 bp	NR_025241.1
			Rossellomorea arthrocnemi strain EAR8 (98.44%)	1474 bp	NR_181775.1
			Heyndrickxia acidicola strain 105-2 (97.32%)	1548 bp	NR_041942.1
			Mangrovibacillus cuniculi strain R1DC41 (97.18%)	1549 bp	NR_181118.1
			Bacillus salis strain ES3 (96.64%)	1515 bp	NR_179406.1
			Falsibacillus albus strain GY 10110 (96.51%)	1533 bp	NR_171509.1
			Heyndrickxia shackletonii strain LMG 18435 (96.36%)	1503 bp	NR_025373.1
SS + FE	1487 bp	1489 bp	*Rossellomorea marisflavi* strain TF-11 (100.00%)	1506 bp	NR_025240.1
			Rossellomorea marisflavi strain TF-11 (100.00%)	1466 bp	NR_118437.1
			Bacillus haikouensis strain C-89 (98.51%)	1473 bp	NR_148273.1
			Rossellomorea aquimaris strain TF-12 (98.25%)	1507 bp	NR_025241.1
			Rossellomorea arthrocnemi strain EAR8 (98.44%)	1474 bp	NR_181775.1

			Heyndrickxia acidicola strain 105-2 (97.31%)	1548 bp	NR_041942.1
			Mangrovibacillus cuniculi strain R1DC41 (97.17%)	1549 bp	NR_181118.1
			Bacillus salis strain ES3 (96.64%)	1515 bp	NR_179406.1
			Falsibacillus albus strain GY 10110 (96.51%)	1533 bp	NR_171509.1
			Heyndrickxia shackletonii strain LMG 18435 (96.36%)	1503 bp	NR_025373.1
SS + LE	1461 bp	1463 bp	*Cytobacillus firmus* strain NBRC 15306 (98.97%)	1477 bp	NR_112635.1
			Cytobacillus firmus strain IAM 12464 (98.84%)	1483 bp	NR_025842.1
			Cytobacillus gottheilii strain WCC 4585 (97.74%)	1512 bp	NR_108491.1
			Cytobacillus oceanisediminis strain H2 (99.57%)	1393 bp	NR_117285.1
			Cytobacillus depressus strain BZ1 (97.52%)	1459 bp	NR_146034.1
			Robertmurraya dakarensis strain Marseille-P3515 (97.25%)	1472 bp	NR_147382.1
			Mesobacillus harenae strain Y40 (97.13%)	1550 bp	NR_178928.1
			Mesobacillus subterraneus strain COOI3B (97.13%)	1539 bp	NR_104749.1
			Cytobacillus massiliigabonensis strain Marseille-P2639 (96.93%)	1514 bp	NR_179554.1
			Mesobacillus thioparans strain BMP-1 (97.12%)	1472 bp	NR_043762.1

Note: DW = distilled water; SS = salt solution; FE = fruit extract; LE = leaf extract

While DNA barcoding enabled characterization of the aforementioned halotolerant species, the bacterial load observed across treatments only reflects the total culturable bacteria. Nevertheless, the isolation of *B. cereus* from DW + DW (non-saline soil) is discernible, given that the bacterium is ubiquitous in soil environments (Hassan *et al.* 2018; Kulkova *et al.* 2023). Meanwhile, *R. marisflavi* is a bacterium that is saline resistant, as highlighted in the studies of Li *et al.* (2024), Bai *et al.* (2024), and Ali *et al.* (2025). Likewise, *C. firmus* is also resistant to salt stress (Gao *et al.* 2024; Chu *et al.* 2025), corroborating its dominance in SS + LE, and its consequent isolation.

Furthermore, outlined in **Table 7** are the reported isolation origins of the aforementioned bacterial species, some of which harbor saline environmental conditions. In the present study, the bacterial species observed are most likely indigenous to the soil, which is a known reservoir of diverse microbial communities (Philippot *et al.* 2024). The addition of *M. calabura* extracts, especially in SS + LE, served as a key survival nutrient source that enhanced the growth and detectability of *C. firmus*. On the other hand, although *R. marisflavi* was detected in both the control SS + DW and SS + FE, the *M. calabura* fruit extract, probably through its nutrient and/or biochemical composition,

might have enhanced the remediation capability of the species. This is evident as the EC values demonstrated by SS + FE, like those by SS + LE, were lower than those of SS + DW. Overall, the addition of the extracts might be associated with reduced growth of non-halotolerant bacteria and a lower overall bacterial load. At the same time, the extracts may have served as a nutrient source for halotolerant species, potentially supporting their growth, accounting for the observed lower total bacterial load yet improved EC reduction in the treatments. This nutrient source may comprise sugars, fiber, minerals, and other biochemical constituents present in *M. calabura* fruits and leaves (Krishnaveni & Dhanalakshmi, 2014; Pereira *et al.* 2018; Peter *et al.* 2020; Upadhye *et al.* 2021; Zolkeflee *et al.* 2021). Furthermore, the results may also indicate that *C. firmus*, with the influence of *M. calabura* leaf extract, was the most potent in remediating the saline soil, as evidenced by the lowest ECs observed in SS + LE.

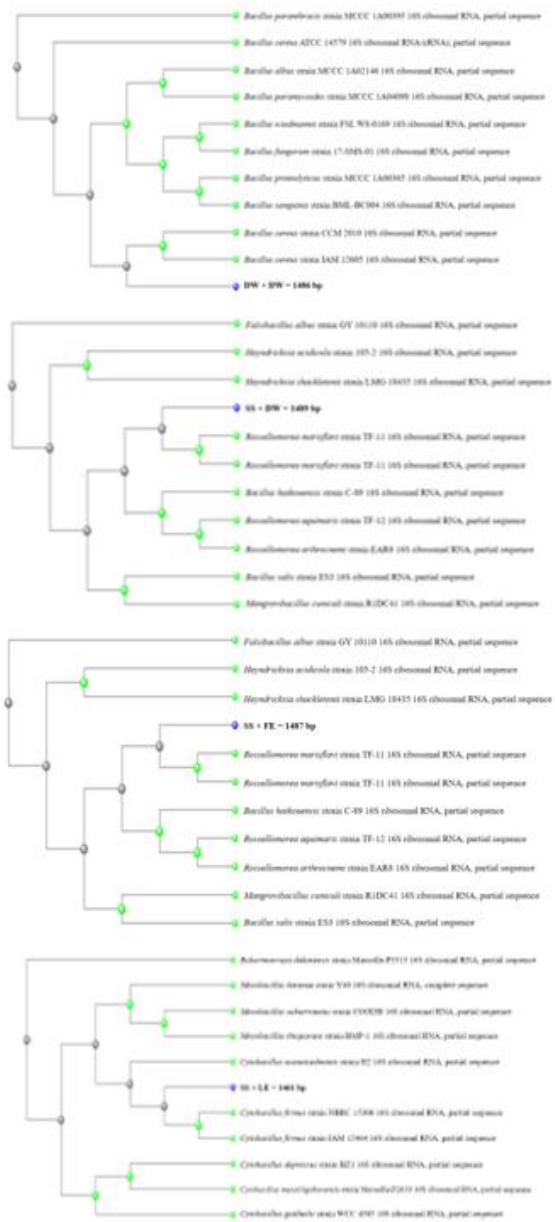


Figure 4. Phylogenetic trees describing the relationships among bacterial species

Fundamentally, the bacterial quantity was not behind the reduction of the ECs, given the gradually decreasing CFUs. Rather, it is possible to deduce that the biological processes and activities of the bacterial species might have played the key role in the soil remediation. There can be a wide range of potential mechanisms behind the saline soil remediation as exhibited by the three bacterial species. Extracellular polymerase substances (EPS) might have been employed by bacteria to bind sodium ions, sequestering them within the matrix (Pawar *et al.* 2013; Choudhary *et al.* 2016; Bhagat *et al.* 2021). Moreover, production of organic acids and chelating agents by the bacteria might have contributed to the salinity reduction (Ribeiro *et al.* 2020). Another mechanism may be through synergism among multi-strain bacterial consortia, which was emphasized by Afzal *et al.* (2023) as a tool for salinity reduction. This was also highlighted in the study of Cui *et al.* (2025), whereby promotion of microbial interactions and symbiotic relationships drives soil quality improvement. Furthermore, the transporters of the bacteria in their structure play a vital role in the remediation by regulating specific ions in their environment. Through the salt-in strategy, which involves the accumulation of ions in the bacterial cytoplasm, the bacteria in the soil samples might have maintained their osmotic balance with the surrounding environment (Neagu & Stancu, 2025). While the presence of halotolerant bacteria likely contributed to the observed bioremediation, as indicated by electrical conductivity reduction, further studies are needed to confirm their mechanisms under the influence of the *M. calabura* fruit and leaf extracts.

On the other hand, the bacterial species were also reported in the studies as endophytes inhabiting plant tissues (**Table 7**). Moreover, the ability of *M. calabura* to accommodate microbial life within its tissues is emphasized in the studies of Bimantara *et al.* (2022) and Simha *et al.* (2025), reporting the isolation of endophytic fungi from the plant. Although the plant extracts might have introduced the bacteria into the soil environment, this cannot be conclusively established yet under the current experimental conditions and setup. Hence, future studies are needed to elucidate the plausibility of the plant as a potential origin for both *R. marisflavi* and *C. firmus*.

Furtthermore, to the best of the authors' literature search and collection, the study documents the first potential occurrence of the latter two taxa in Tarlac, a province located in Central Luzon, Philippines. The keywords "*Rossellomorea marisflavi* + Central Luzon + Tarlac + Philippines" and "*Cytobacillus firmus* + Central Luzon + Tarlac + Philippines" were used to search published documents in PubMed, Google Scholar, and Scopus. Gutierrez *et al.* (2025) already reported the isolation and identification of *C. firmus* in La Union, Philippines. However, the aforementioned province is located in the Ilocos Region, which is geographically distinct and apart from Central Luzon. Meanwhile, to date, no reports of *R. marisflavi* in Tarlac, Philippines have been identified, supporting the novelty of the present finding. Nonetheless, this study advances scientific understanding of the

microbial diversity within different environmental settings in the country.

4. Conclusion

In the present study, SS + FE and SS + LE demonstrated EC values lower than those of the control SS + DW. By Day 14, the treatments, including DW + DW (non-saline soil), showed no significant difference among one another in terms of DO and pH. This indicates the effectiveness of *M. calabura* extracts in reducing EC, particularly in SS + LE, without compromising the other two physicochemical parameters. While the 14-day pot experiment spiked with 50 mM NaCl solution provided valuable initial insights into the biostimulatory effects of the extracts, future studies using longer time points and higher salt concentrations can further build on these findings to fully capture sustained effects.

Table 7. Reported origins of the closest related species of the bacterial isolates.

Closest related species	Origin and site of isolation
Bacillus cereus	Mongo sprouts, silage, and soil samples in the Philippines (Venturina et al. 2016)
	Rice paddy fields in the Philippines (Bańares et al. 2019)
	Muddy sediments of mangrove swamps in Thailand (Chantarasiri, 2015)
	Halophytic weed Cenchrus ciliaris growing in Khewra Salt Range of Pakistan (Hassan et al. 2018)
Rossellomorea marisflavi	Rice grown in acid sulfate soils in Vietnam (Lam et al. 2024)
	Hindon River (in proximity to Gautam Buddha Nagar in Uttar Pradesh of India) (Kumar et al. 2025)
	Rhizosphere of Zea mays L. (cultivar Xianyu 335) grown in Beijing, China (Li et al. 2025)
	Rhizosphere soil samples from maize and vegetable farms at the Centre for Agricultural Technology and Entrepreneur Studies (CATES), Bells University of Technology, Ota, Ogun State, Nigeria (Ogunsola et al. 2025)
Cytobacillus firmus	Rhizosphere of pepper (Capsicum annuum L.) from the greenhouse of Kyungpook National University (Republic of Korea) (Jung et al. 2023)
	Hot springs located in the southern region of Saudi Arabia (Al-Harthy et al. 2024)
	Soil samples contaminated with heavy metals from disposal sites in Pantnagar, Uttarakhand, India (Ghosh et al. 2025)
	Chromium slag dump in Xinxiang, Henan Province, China (Yin et al. 2025)
	Avicennia marina (Gray Mangrove) in La Union, Philippines (Gutierrez et al. 2025)

Despite the gradual decrease of bacterial load in the treatments, molecular characterization revealed that representative cultures of the surviving species were closely related to two halotolerant bacteria: *R. marisflavi* and *C. firmus*. This may indicate that although the addition of extracts was associated with a lower overall bacterial load, it may have supported the growth of halotolerant bacteria, potentially accounting for the improved saline bioremediation observed in the treatments. While current methods identified only representative isolates, future extensive microbiological and molecular analyses must be conducted to validate microbial community dynamics, specifically the halotolerant species across the treatments. On the other hand, the findings suggest that the addition of the extracts, while resulting to lower total bacterial load, might have served as a nutrient source for the halotolerant species, accounting for the improved saline bioremediation in the treatments. Overall, exploring the multifaceted mechanisms behind the saline bioremediation using *M. calabura* extracts must be further explored. Moreover, to the best of the authors' literature search and collection, the study marks the first documented potential occurrence of the two aforementioned taxa (and specific strains) in Tarlac, Philippines.

Beyond the ecological benefits, the effectiveness of *M. calabura* extracts to reduce EC and promote halotolerant bacteria presents a potentially cost-effective pathway for land reclamation. With the research objectives and findings being aligned with the circular bioeconomy, saline soil bioremediation using the extracts can help in restoring soil productivity, supporting agricultural livelihoods and food security. Policymakers can take part by subsidizing the development and production of plant-derived soil amendments/conditioners as an approach for environmental and agricultural sustainability.

Authors' Contributions

MANUCDOC: Conceptualization, Methodology, Experimentation, Data Analysis and Interpretation, Writing (Original Draft), Writing (Review and Editing), Project Administration, Funding Acquisition; MARCOS: Conceptualization, Funding Acquisition; VILLANUEVA: Methodology, Experimentation, Writing (Review and Editing)

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Conflict of interest

The authors declare no conflict of interest.

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