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Biochemical Traits and Biological Assessment of Indigenous Biofilm-Forming Rhizophosphate Bacteria Isolated from Salinity and Acidity Stressed Soils to Enhance Maize Growth

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ABSTRACT

Soil acidity and salinity stress significantly affect nutrient availability and uptake, as well as the growth, development, and yield of maize plants. This research aimed to screen, characterize, and assess the ability of selected indigenous biofilm-forming rhizophosphate bacteria (BFRB) to produce growth factor and promote maize growth. Soil samples were collected from both acid ecosystems (AE) and saline ecosystems (SE). Thirty isolates of rhizophosphate bacteria were obtained based on the clear zone around colonies on selective Pikovskaya agar media. Subsequently, a qualitative biofilm test was conducted, resulting in the identification of 9 isolates of biofilm-forming rhizophosphate bacteria. Biochemical test was performed to asses phosphate solubility index (PSI) and production of indole acetic acid (IAA) and biological assays was performed to measure the effect of selected BFRB on maize growth characteristics. The experiment was arranged in a randomized block design with 10 treatments (control and 9 isolates of BFRB) and 3 replications. The findings revealed that all isolate has avaibility to membentuk biofilm dan mampu melarutkan P. three potential isolates of BFRB from both AE and SE significantly enhanced maize growth. Isolates PS-03, PN-05, and PC-04 from saline soils, as well as isolates NA-01, NG-04, and NF-04 from acid land, exhibited notable increases in root length, plant height, and dry weight compared to the control. For instance, isolates PS-03, PN-05, and PC-04 from saline soils increased maize height by 2160.87% and root length by 392.64%, and they also increased the dry weight of the plants by 20.50%. Similarly, isolates NA-01, NG-04, and NF-04 from acid soil increased maize height by 673.82% and root length by 220.29%, and they increased the dry weight of the plants by 12.57%. These findings suggest that these BFRB isolates hold significant potential for developing rhizophosphate biofilm biofertilizers to enhance maize productivity in marginal soils. Additionally, each of these bacteria produced IPF and IAA, which contributed to the significant increases in plant height and root length, and formed biofilms that were present on the roots of the maize plants. Therefore, field trials are necessary to utilize these biophosphate fertilizers to improve fertilization efficiency and maize productivity in saline and acidic ecosystems.

Keywords: biofilm rhizophospahate biofertilizers, BFRB, saline soils, marginal soils, acid soils, salinity.

INTRODUCTION

Marginal lands, characterized by poor soil quality, limited access to water resources, and harsh environmental conditions, pose significant challenges to agricultural productivity worldwide, including maize cultivation. The expansion of marginal lands is particularly pronounced in Indonesia, where the area of marginal land continues to increase annually. Marginal land in Indonesia is associated with high environmental risks and imposes limitations on

agricultural activities, as it typically exhibits low productivity and offers minimal economic returns (BPS, 2023). The proliferation of marginal land in the country can be attributed to various factors, including land conversion, extensive use of inorganic fertilizers leading to soil degradation, recurrent droughts, and other unsustainable agricultural practices. Marginal lands, by definition, lack the potential for profitable agricultural activities due to their poor soil characteristics and inadequate nutrient availability for crops. These soils often exhibit acidity, alkalinity, shallow depth, coarse texture, or other deficiencies that hinder agricultural productivity [Yaser et al., 2023)]. Consequently, the degradation of marginal lands significantly impacts agricultural output, leading to reduced crop yields and economic losses for farmers.

Marginal lands encompass diverse environments, including acid land and saline land, each presenting unique challenges for agricultural development. Acid land and saline land are prominent examples of marginal lands characterized by distinct soil properties and environmental conditions. Acid land is characterized by low soil pH and cation exchange capacity (CEC), along with high levels of aluminum (Al), iron (Fe), and manganese (Mn). These conditions result in nutrient deficiencies, particularly in nitrogen (N), phosphorus (P), and potassium (K) (Fitriatin et al., 2021). Conversely, saline land is plagued by osmotic stress and imbalances in nutrient uptake, posing significant obstacles to crop cultivation (Dato et al., 2023). Addressing these challenges requires the adoption of environmentally friendly fertilization practices aimed at enhancing soil health and promoting sustainable agriculture. Organic fertilizers, renowned for their environmentally friendly nature, offer a promising solution by increasing CEC, organic carbon content, and pH levels, while improving the efficiency of nutrient uptake compared to conventional inorganic fertilizers (Mustamu et al., 2023). Given the urgent need to optimize fertilizer application to meet crop nutrient requirements, the integration of suitable fertilization strategies becomes imperative for sustainable agricultural practices in marginal lands.

In addressing the challenges posed by acid land and saline land, the utilization of biofilm-forming rhizophosphate bacteria emerges as a promising solution to enhance soil health and promote plant productivity. Rhizophosphate bacteria, a type of phosphate-solubilizing bacteria commonly isolated from the rhizosphere, represents a novel technology in biological fertilizer development. These bacteria hold the potential to form biofilms around plant roots, thereby establishing microbial communities that play a vital role in nutrient cycling and plant-microbe interactions. Biofilms, characterized by their intricate network of microorganisms encased in extracellular polymeric substances (EPS), offer several advantages for soil and plant health. Biofilms are microbial communities that cover roots and attached each other and produce extracellular polymeric substance (Zaki et al., 2020).

This research undertakes the isolation of bacteria from various marginal lands with the aim of producing biofilms that are expected to support growth maize.

MATERIAL AND METHODS

Study area

Composite soil samples are taken from upper layer (0–20 cm) several marginal land area. Saline soil samples are taken from Pangandaran, Jawa Barat (Fig. 1) and acid soil samples (Fig. 2) are taken from Nusa Tenggara Barat. From each of these plots, five vegetation samples were taken with the details shown in Table 1 and 2.

Soil samples were taken from the rhizosphere and their properties were subsequently analyzed. pH was measured using a glass electrode (Jackson, 1958), organic carbon content was determined using the Kjeldahl method (Kjeldahl, 1883), and cation exchange capacity (CEC) was analyzed using distillation methods (ISRIC, 2002). Phosphorus availability was measured using the Olsen method for soils with pH ≥ 5.5 (Olsen, 2000) and the Bray method for soils with pH < 5.5 (Bray, 2000). Soil texture was measured using the pipette method, which separates sand, clay, and silt (ISRIC, 2002).

Table 1 includes five soil sample from saline stressed locations around coordinates 07°37’29.1”S, 108°49’00.3”E, with varying vegetation such as *Oryza sativa*, *Eleocharis dulcis*, *Nymphee sp*., *Monochoria vaginalis*, and *Cyperus iria*. Organic carbon content ranges from 2.98% to 3.48%, soil pH from 5.6 to 6.3, cation exchange capacity from 15.48 to 19.42 cmol (+) kg^{-1}, available

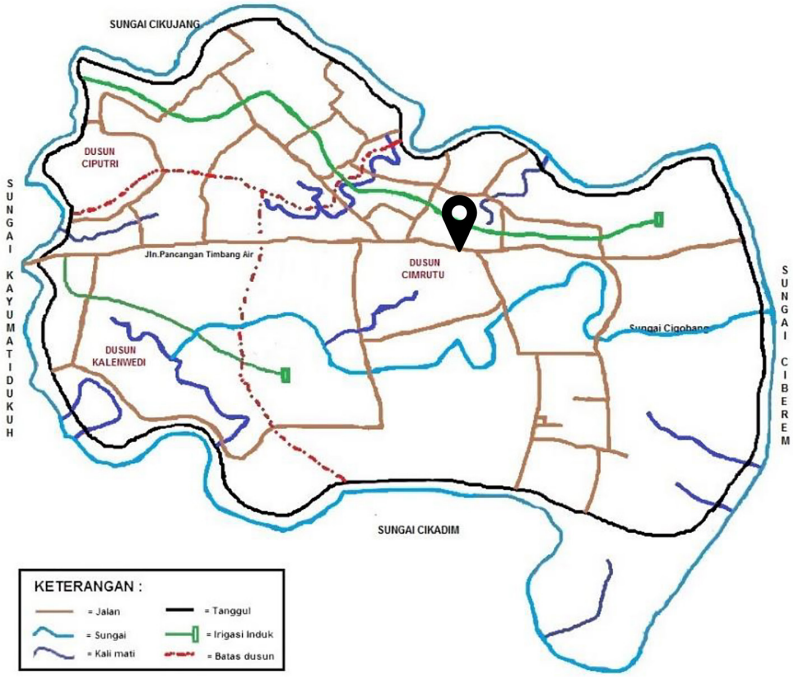

Figure 1. Location of saline ecosystem

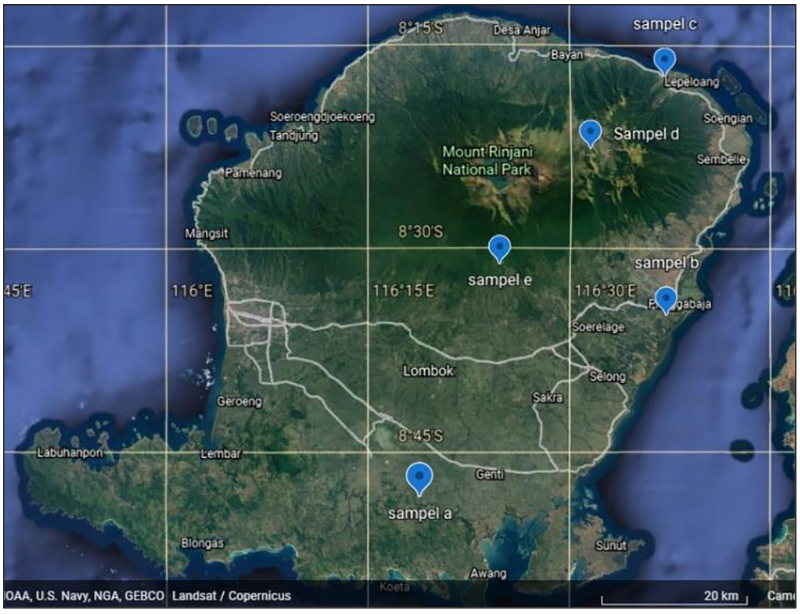

Figure 2. Location of acid ecosystem

Table 1. Sampling site, vegetation and soil properties saline stressed ecosystem

Coordinate	Vegetation	Soil properties
07°37'29.3"S 108°49'00.3"E	*Oryza sativa*	Organic Carbon = 3.06%; pH = 5.6; CEC = 18.88 cmol(+)/kg; Available P = 5.32 ppm; texture = silty loam
07°37'29.6"S 108°49'00.1"E	*Eleocharis dulcis*	Organic Carbon = 2.98%; pH = 6.2; CEC = 16.77 cmol(+)/kg; Available P = 5.12 ppm; texture = silty loam
07°37'29.1"S 108°48'56.3"E	*Oryza sativa with Nymphee sp.*	Organic Carbon = 3.48%; pH = 6.3; CEC = 19.42 cmol(+)/kg; Available P = 5.44 ppm; texture = silty loam
07°37'29.1"S 108° 48'56.3"E	*Monochoria vaginalis*	Organic Carbon = 3.02 %; pH = 5.8; CEC = 15.48 cmol(+)/kg; Available P = 5.02 ppm; texture = silty loam
07°37'29.1"S 108° 48'56.3"E	*Cyperus iria.*	Organic Carbon = 3.02 %; pH = 5.9; CEC = 18.88 cmol(+)/kg; Available P = 5.22 ppm; texture = silty loam

Table 2. Sampling site, vegetation and soil properties on acid stressed ecosystem

Coordinate	Vegetation	Soil properties
S 80 48' 29,82" E 1160 18' 52,81"	*Oryza sativa*	Organic Carbon = 0.63 %; pH = 5.6; CEC = 37.96 cmol(+)/kg; Available P = 5.22 ppm; texture = silty loam
S 80 35' 6,91" E 1160 37' 14,45"	*Zea Mays*	Organic Carbon = 0.63 %; pH = 5.7; CEC = 17.35 cmol(+)/kg; Available P = 5.72 ppm; texture = sandy loam
S 80 17' 31,98" E 1160 37' 7,02"	*Anarcadium occidentale*	Organic Carbon = 0.63 %; pH = 5.4; CEC = 19.54 cmol(+)/kg; Available P = 5.34 ppm; texture = sandy loam
S 80 22' 46,4" E 1160 31' 28,4"	*Graminae*	Organic Carbon = 0.63 %; pH = 5.2; CEC = 23.35 cmol(+)/kg; Available P = 3.28 ppm; texture = sandy loam
S 80 31' 20,83" E 1160 24' 46,8"	Tropical forest	Organic Carbon = 0.63 %; pH = 5.4; CEC = 35.8 cmol(+)/kg; Available P = 4.28 ppm; texture = sandy loam

phosphorus from 5.02 to 5.44 ppm, and all samples have a silty loam texture.

Table 2 includes five soil sampling from acid stressed locations around coordinates S 80 48' 29.82" to S 80 31' 20.83" and E 1160 18' 52.81" to E 1160 24' 46.8", with various vegetation such as *Oryza sativa*, *Zea Mays*, *Anarcadium occidentale*, Graminae, and tropical forest. Organic carbon content ranges from 2.21% to 2.92%, soil pH from 5.2 to 5.7, cation exchange capacity from 17.35 to 37.96 cmol (+) kg^{-1}, available phosphorus from 3.28 to 5.72 ppm, and mostly sandy loam soil texture except for one sample with a silty loam texture.

These results indicate that despite variations in vegetation and slight differences in soil chemical properties, all locations are conducive to the growth of phosphate-solubilizing bacteria. This is evidenced by the high organic carbon content, near-neutral pH, and sufficient available phosphorus, with minor variations in cation exchange capacity and phosphorus potentially affecting the diversity and activity of bacteria at each location.

Preparation and rhizoposphate isolation

Bacteria (BPF) were isolated from five different types of vegetation, as described in Tables 1 and 2. From each type of vegetation, three isolates showing clear zones on Pikovskaya media were isolated. Pikovaskaya medium, consisting of $C_6H_{12}O_6$, $Ca_3(PO_4)_2$, $(NH_4)_2SO_4$, NaCl, $MgSO_4 \cdot 7H_2O$, KCl, MnO_2, $FeSO_4 \cdot 7H_2O$, agar, and H_2O, is used to isolate phosphate-solubilizing microorganisms (Atlas, 2010). Total of 30 selected isolates were obtained from various types of vegetation. A total of 30 rhizophosphate bacteria isolates were obtained based on the presence of clear zones around colonies grown on selective Pikovskaya agar media.

Biochemical

Biofilm test

To determine whether the bacteria produce biofilm, a biofilm assay on a microtiter plate can be performed. First, the bacteria are refreshed on NA media (slant agar) in test tubes and incubated for 24 hours in an incubator. Next, add liquid agar media and dilute it with liquid agar 1:100, then incubate for 24 hours in the incubator. After that, add 100 microliters to the microtiter plate, perform 4–8 repetitions, and incubate for 4–24 hours at 37 °C. Soak the microtiter plate and wash it with distilled water twice carefully. Add 125 microliters of 0.1% crystal violet to the microtiter plate and incubate for 10–15 minutes. Rinse the microtiter plate with distilled water, then invert the microtiter plate overnight until dry (O'Toole, 2011)

Posphate solubility index

To calculate the phosphate solubilization index, first measure the diameter of the bacterial colony growing on the culture medium and the diameter of the clear zone surrounding the bacterial colony, which indicates phosphate solubilization activity (Sharon et al., 2016):

$$Posphate\ Solubilizing\ Index = \frac{Diameter\ of\ the\ clear\ zone\ surrounding\ bacteria}{Diameter\ of\ bacteria}$$

Biological assay

The selected phosphate-solubilizing biofilm bacteria isolate were arranged in a completely randomized design with 10 treatments (1 control and 9 bacteria) and three replications for the bioassay. The bioassay of phosphate-solubilizing bacteria was conducted using maize seedlings by adding a 10% PSB concentration to Murphy liquid medium (10 ml bacterial suspension). The composition

of Murphy's medium is as follows: $CaSO_4$ H_2O, KH_2PO_4 $2H_2O$, $MgSO_4$ $7H_2O$, NaCl, KCl, $ZnCl_2$, $CuSO_4$ $5H_2O$, $FeSO_4$, agar, and aquadest (Atlas, 2010). Maize seedlings were planted in sterilized test tubes measuring 20×300 mm. Subsequently, the roots of maize seedlings were immersed in the Murphy liquid planting medium, while the tops of the plants were supported by sterilized plastic and flexible pipes. The test tubes were then arranged in racks and stored in the greenhouse of the Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. Root length, plant height, and dry weight were measured after 14 days. Photos of the roots were taken using a microscope with 1000x magnification (Fig. 3).

Selection of superior rhizophosphate isolate

The selection of superior isolates is conducted by ranking by scoring method based on biofilm indicators, phosphate solubilizing index, and plant growth. Then, the top three isolates from each site will be tested for indole 3-acetic acid *(*IAA).

Production of IAA selected isolate

To determine the IAA concentration in liquid culture, first perform a standard curve measurement by adding 4 ml of Salkowski reagent (50 ml perchloric acid + 1 ml $FeCl_3$) and measure using a spectrophotometer at a wavelength of 535 nm. Mix 1 ml of inoculum with 4 ml of Salkowski reagent and let it stand at room temperature for 30 minutes. Measure the absorbance using a spectrophotometer at a wavelength of 535 nm, and determine the IAA hormone concentration in each treatment using the standard curve (Patten and Glick, 1996). To observe biofilm formation on root, use a microscope with 1000x magnification. This microscope is used to view the biofilm formed on corn roots. With high magnification, the fine details of the biofilm and its structure on the maize roots can be clearly seen.

Data analysis

The effects of the different treatments on the variables studied were assessed using ANOVA with DSAASTAT (Dipartimento di Scienze Agrarie ed Ambientali Statistic). Mean differences were determined by Duncan's Multiple Range Test, with significance set at $P < 0.05$.

RESULT AND DISCUSSION

Biofilm test

Out of the 30 isolates that were initially isolated, they were then selected based on their ability to form biofilms. From this selection, 18 isolates were found to be capable of producing biofilms, with 9 isolates originating from saline (PS-03; PS-06; PE-01; PN-05; PN-07; PM-03; soils and 9 from acidic soils.

The purple color observed in the biofilm method indicates the isolate's ability to form an extracellular matrix containing polysaccharides. This matrix plays a crucial role in the biofilm's stability and resilience to environmental stress, as well as providing protection to the microorganisms

Figure 3 Bioassay test using maize seedling in murphy liquid medium

within it. The main factor causing purple coloration in biofilms is the production of polysaccharides. Biofilms produce polysaccharides like d-glucan and d-mannan, which provide structure to the matrix. These polysaccharides can exhibit purple or pink hues. Additionally, interaction with dyes like crystal violet contributes to this coloration. Crystal violet is used to observe biofilms and interacts with polysaccharides in the matrix, resulting in detectable purple coloration on microtiter plates. Furthermore, the intensity of the purple color also depends on the concentration of cells and extracellular matrix (EPS) produced by the biofilm. The higher the concentration of cells and EPS, the more intense the purple color appears (O'Toole, 2011)

Posphate solubilizing index

The bacteria that have been tested for their ability to form biofilms are subsequently assessed for their phosphate solubilization index using Pikovskaya media as illustrated in Tables 3. This approach facilitated the differentiation between isolates originating from saline and acid lands, thereby contributing to a comprehensive understanding of microbial dynamics across various soil conditions.

The following Table presents the phosphate solubilizing indices (PSI) of nine superior bacterial isolates obtained from saline lands. The highest PSI value was recorded for isolate PS-03 at 1,56, while the lowest value of 1.25 was shared by isolates PS-06, PN-07, PM-03, and PC-01. Isolates PE-01, PN-05, and PN-06 showed the same PSI value of 1.3; whereas isolate PC-04 exhibited a PSI of 1.44. These data indicate variations in phosphate solubilization ability among the bacterial isolates, which could be beneficial for agricultural applications in saline soils.

The data presents the phosphate solubilizing indices of bacteria isolated from acid lands. Among the isolates, NA-01, NG-04, and NF-04 stand out with PSI values of 1.3, 1.5, and 1.4 respectively, suggesting their potential in phosphate solubilization. These isolates exhibit PSI values at or above the average range observed in the dataset, indicating their significant capacity to solubilize phosphate compounds. Such robust performance could imply their effectiveness in enhancing soil fertility and supporting plant growth in acid environments.

Bacteria with high phosphate solubilizing indices play a crucial role in the growth of maize plants. The increased availability of phosphate, facilitated by these bacteria, enables maize plants to absorb more nutrients and grow better (Fitriatin et al, 2024). Therefore, bacteria with high PSI can be an effective strategy to enhance maize productivity, especially in marginal soils.

Biological assay

After 14 days of planting, measurements were taken for plant height and root length, as presented in Tables 5 and 6.

The results of this study demonstrate significant variations in the effects of different treatments on plant growth parameters, particularly plant height and root length. Treatment PS-03, showing the highest plant height of 5.20 cm and a percentage increase in plant height of 2160.87%, stands out as the best. Similarly, treatments PN-05 and PC-04 demonstrate significant increases in plant height, with percentage increases of 1147.83% and 1434.78%, respectively. From this, it can be concluded that the use of bacteria with high PSIs, as represented by treatments PS-03, PN-05, and PC-04, has the most positive impact on plant

Table 3. The phosphate solubilizing index (PSI) of bacteria isolated from Saline Lands

Saline isolate bacteria		Acid isolate bacteria	
Isolate sample code	PSI	Isolate sample code	Indices (PSI)
PS-03	1.56	NS-03	1.3
PS-06	1.25	NS-05	1.2
PE-01	1.3	NZ-01	1.3
PN-05	1.3	NZ-03	1.3
PN-06	1.3	NA-01	1.4
PN-07	1.25	NG-02	1.3
PM-03	1.25	NG-04	1.5
PC-01	1.25	NF-01	1.3
PC-04	1.44	NF-04	1.6

Table 4. Effect isolate saline stress soilecosystem on plant height and root length of maize seedling after 14 days

Inoculation	Plant height (cm)		Increament plant height (%)	Root length (cm)		Increament root length (%)
Control	0.23	a		1.63	a	
PS-03	5.20	f	2160.87	8.03	f	392.64
PS-06	1.63	bc	608.70	5.67	d	247.85
PE-01	1.80	bc	682.61	5.87	d	260.12
PN-05	2.87	d	1147.83	6.93	e	325.15
PN-06	2.20	c	856.52	5.47	d	235.58
PN-07	2.00	bc	769.57	3.30	bc	102.45
PM-03	1.37	b	495.65	3.87	c	137.42
PC-01	1.97	bc	756.52	2.93	b	79.75
PC-04	3.53	e	1434.78	7.50	ef	360.12

Note: The average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test

Table 5. Effect isolate acid stress soil ecosystem on plant height and root length of maize seedling after 14 days

Treatments	Plant height (cm)		Increament plant height (%)	Root length (cm)		Increament root length (%)
control	2.33	a		2.07	a	
NS-03	4.83	b	107.30	4.70	bcd	127.05
NS-05	4.80	b	106.01	4.32	abcd	108.70
NZ-01	3.00	a	28.76	3.78	abc	82.61
NZ-03	4.83	b	107.30	3.41	ab	64.73
NA-01	18.03	f	673.82	6.63	de	220.29
NG-02	11.63	d	399.14	3.44	ab	66.18
NG-04	16.87	e	624.03	6.15	cd	197.10
NF-01	6.83	c	193.13	4.60	bcd	122.22
NF-04	12.40	d	432.19	9.13	e	341.06

Note: The average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test

Table 6. Ranking results of isolates from saline stress soil using the scoring method

Isolate	Indicator					Result	Isolate rank
	Biofilm	PSI	Maize growth				
			Plant heigh	Root length	Dry weight		
PS-03	1	9	9	6	9	34	1
PS-06	1	1	2	4	2	10	9
PE-01	1	5	3	3	5	17	5
PN-05	1	7	7	1	7	23	3
PN-06	1	6	6	8	1	22	4
PN-07	1	2	5	2	6	16	8
PM-03	1	3	1	7	4	16	7
PC-01	1	4	4	5	3	17	6
PC-04	1	8	8	9	8	34	2

growth compared to the control treatment. Furthermore, an analysis of root length also provides crucial insights in this study. The root length data indicates significant variation among different treatments. Treatment PS-03 exhibits the lowest root length at 1.63 cm, while treatment PN-05 displays a root length of 6.93 cm. This represents a substantial increase in root length, indicating

the efficacy of treatment PN-05 in promoting root growth. Additionally, treatments PS-03 and PC-04 also show percentage increases in root length of 392.64% and 360.12%, respectively. Thus, it can be concluded that the use of specific bacteria, particularly those represented by treatments PS-03 and PC-04, has a significant impact on enhancing plant root growth.

The effects of different treatments on plant height and root length were investigated in this study. Notably, Treatment NA-01 exhibited the most significant increase in both plant height and root length, with mean values of 18.03 cm and 6.63 cm, respectively. These results represent impressive increments of 673.82% and 220.29% compared to the control. Additionally, treatments NG-04 and NF-04 also demonstrated substantial increases in both plant height (16.87 and 12.40 cm) and root length (6.15 and 9.13 cm), indicating their potential for promoting maize growth in marginal soils. Conversely, Treatments NZ-01 and NZ-03 showed the lowest plant heights and root lengths, with notable decreases compared to the control. Overall, the findings underscore the importance of selecting appropriate biofilm-forming rhizophosphate bacteria. Particularly, Treatments NA-01, NG-04, and NF-04 show promising results in enhancing maize productivity by improving plant height and root length.

Our study highlights the significant potential of biofilm-forming rhizobacteria with phosphate solubilizing abilities (BFRB) in promoting plant growth and productivity. BFRB play a crucial role in increasing phosphate availability in the soil, an essential nutrient for plant development (Fang et al., 2024). This enhanced availability facilitates improved nutrient uptake by plants, resulting in enhanced growth. Furthermore, the capacity of these bacteria to form biofilms contributes to the sustainability and efficiency of their activities in the soil, further enhancing plant growth.

This underscores the significant potential of bacteria utilization in improving overall plant health and productivity, both in terms of root growth and overall plant growth. These findings highlight the potential of selected bacteria strains to positively influence maize growth parameters in marginal soils. Overall, the varying effects observed among treatments underscore the importance of further research to identify and characterize biofilm-forming rhizophosphate bacteria with the highest potential for enhancing maize productivity in marginal soils.

Therefore, a better understanding of the interaction between rhizophosphate biofilm and plants can aid in the development of more efficient and sustainable agricultural strategies.Drying was conducted using an oven at a temperature of 70 degrees Celsius until a stable dry weight was achieved. Subsequently, this dry weight was measured, and the data obtained is presented in Figure 4 and 5.

In this study, the impact of different treatments on maize dry weight was assessed. Treatment PS-03 showed the highest dry weight, with a mean value of 1.94 g, representing an increase of 20.50% compared to the control. Treatments PN-05 and PC-04 also exhibited notable increases in dry weight, both recording a 19.25% increment compared to the control. Conversely, treatment PS-06 demonstrated the lowest dry weight among all treatments, with a mean value of 1.72 g, indicating a minimal increase of 6.83% compared to the control. Overall, the results suggest that certain treatments, particularly PS-03, PN-05, and PC-04, have the potential to enhance maize productivity by increasing dry weight, highlighting their suitability for improving crop yields in marginal soils.

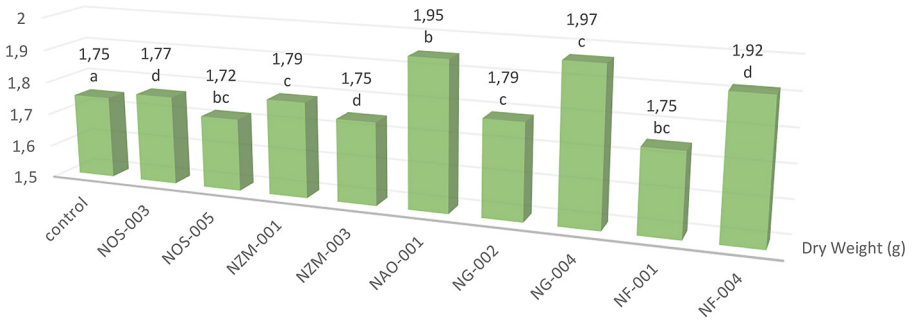

Figure 4. Effect isolate saline ecosystem on dry weight of maize seedling after 14 days. The average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test

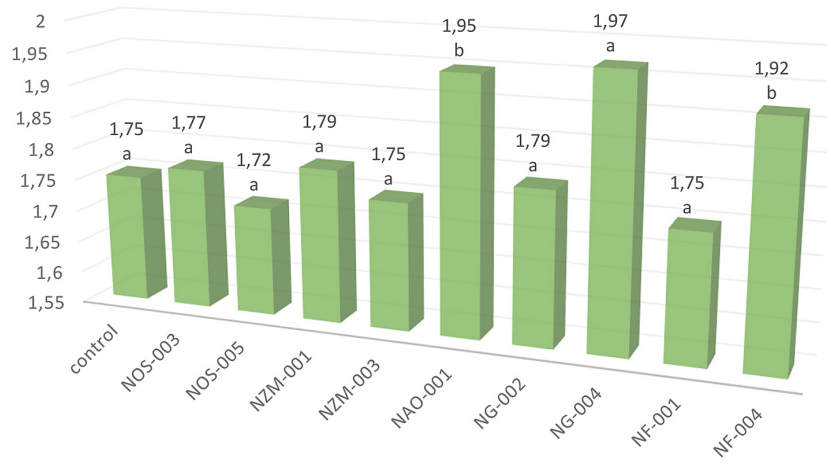

Figure 5. Effect isolate acid ecosystem on plant height and root length of maize seedling after 14 days. The average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test

The effects of various treatments on maize dry weight were investigated in this study. Notably, Treatments NG-04 and NA-01 showed the most substantial increases in dry weight, with mean values of 1.97 g and 1.95 g, respectively. These findings depict increments of 12.57% and 11.43% compared to the control. Additionally, Treatments NS-03, NZ-01, and NF-04 also displayed significant enhancements in dry weight, with increments ranging from 1.14% to 9.71%. Conversely, Treatments NS-05, NZ-03, NG-02, and NF-01 exhibited minimal changes in dry weight compared to the control. Overall, the results suggest that specific biofilm-forming rhizophosphate bacteria, particularly those employed in Treatments NA-01 and NG-04, hold potential for improving maize productivity by increasing dry weight in marginal soils. There is a correlation between BFRB and plant growth. Plants equipped with bacteria possessing high BFRB tend to acquire more phosphate nutrients from the soil environment (Luo et al., 2023). As a result, these plants exhibit better growth and yield greater dry weight. Conversely, plants that lack an adequate supply of phosphate may experience hindrances in their growth and dry weight production.

Selection of superior rhizophosphate isolate

After conducting tests for biofilm production capability, phosphate solubilization index, and biological assay, the bacteria were then ranked using the scoring method as shown in Table 7.

Based on the scoring method, three superior isolates were identified from saline soil: PS-03, PC-04, and PN-05. From acidic soil, the superior isolates were NF-04, NA-01, and NG-04. These

Table 7. Ranking results of isolates from acid stress soil using the scoring method

Isolate	Indicator					Result
	Biofilm	PSI	Maize growth			
			Plant heigh	Root length	Dry weight	
NS-03	1	2	3	6	4	6
NS-05	1	1	2	4	1	9
NZ-01	1	3	1	3	5	7
NZ-03	1	4	4	1	2	8
NA-01	1	7	9	8	8	2
NG-02	1	5	6	2	6	5
NG-04	1	8	8	7	9	3
NF-01	1	6	5	5	3	4
NF-04	1	9	7	9	7	1

isolates achieved the highest scores in biofilm formation, phosphate solubilization index, and plant growth. The plant growth assessment included plant height, root length, and dry weight.

Indole acetic acid and biofilm on maize root

Auxin is a plant hormone that plays a crucial role in regulating plant growth and development. Some phosphate-solubilizing bacteria have the unique ability to produce auxin, in addition to converting insoluble phosphate in the soil into a form that plants can absorb. The auxin produced by these bacteria stimulates root growth and enhances nutrient uptake efficiency (Park et al., 2021). This combination of auxin production and phosphate solubilization by the bacteria not only increases the availability of essential nutrients for maize but also promotes faster growth and higher yields, making it a key strategy for boosting agricultural productivity. Table 8 shows the ability of the selected bacteria to produce IAA.

The data on Indole Acetic Acid (IAA) obtained from bacterial isolates in saline environments show variability in their hormone production capabilities. Isolate PS-03 exhibited high IAA production with an average value of 4.611 ± 0.043, while isolates PN-05 and PC-04 also showed relatively high production levels with 4.913 ± 0.022 and 3.819 ± 0.018, respectively. Conversely, isolate NA-01 from acidic environments showed lower IAA production with a value of 2.712 ± 0.009. Isolates NG-04 and NF-04 from acidic environments demonstrated relatively high IAA production with values of 4.722 ± 0.044 and 3.211 ± 0.014, respectively. Classification of isolate capabilities in IAA production can be determined based on their average IAA production values, where isolates above 4.5 can be considered highly capable, those between 3.0 and 4.5 moderately capable, and those below 3.0 lowly capable (Spaepen et al., 2007; Glick, 2014). Statistical analysis using a t-test indicated that the differences in IAA production between isolates from saline and acidic environments were statistically significant ($p < 0.05$), affirming the influence of the environment on bacterial hormone production capabilities.

After 14 days of planting, root photos were taken using a microscope with a 1000x magnification (Fig. 6). Observations revealed structures adhering to the plant roots in the bacteria-treated group. These structures indicate the presence

Table 8. Ability of isolates to produce IAA (ppm)

Saline Isolate		Acid Isolate	
Isolate	IAA	Isolate	IAA
Control	0000		
PS-03	4.611 ± 0.043	NA-01	2.712 ± 0.009
PN-05	4.913 ± 0.022	NG-04	4.722 ± 0.044
PC-04	3.819 ± 0.018	NF-04	3.211 ± 0.014

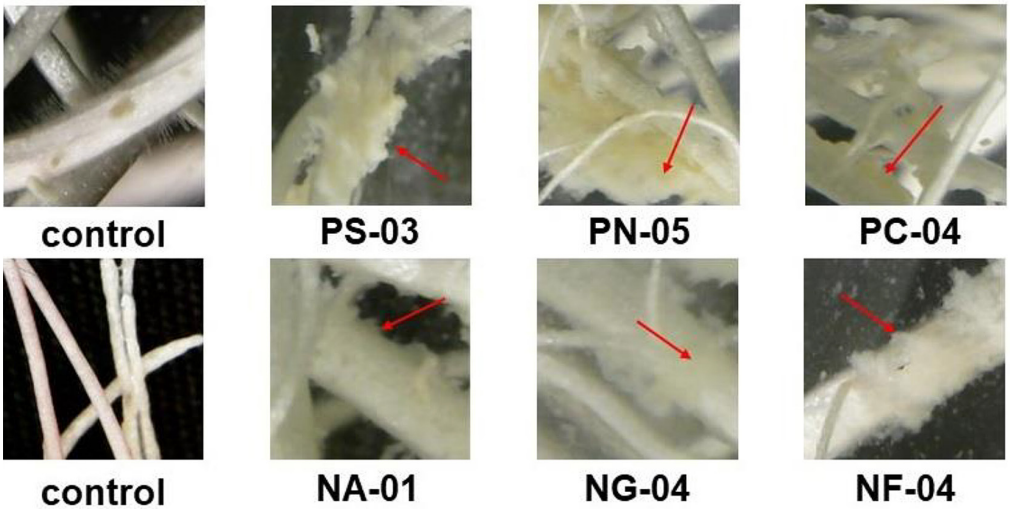

Figure 6. Biofilm on maize roots (shown by red arrow) at 1000x magnification

of biofilm formed by the applied bacteria on the plants. The appearance of this biofilm suggests an interaction between the bacteria and the plant roots during the growth period, likely providing benefits for plant health and growth. This finding highlights the crucial role of bacteria in enhancing plant health and offers potential for the implementation of sustainable agricultural practices in the future.

In this study, the formation of biofilm on plant roots is an intriguing phenomenon worthy of observation. This biofilm comprises various structures adhering to the root surface (Chen et al., 2012). Other studies have also documented variations in the color of biofilm found on root surfaces, which can range from clear, white, to yellow (Zhou and Gao, 2019). The presence of biofilm structures and color variations provides a more comprehensive understanding of the complexity of biofilm formation on plant roots.

The biofilm formed by rhizophosphate bacteria aids in improving the availability of phosphate and other nutrients for plants, thereby stimulating better root growth. With the presence of the biofilm, plants can acquire nutrients more efficiently from their surroundings, leading to increased plant growth. Previous studies have shown that rhizophosphate biofilm can produce growth factors such as indole-3-acetic acid (IAA), known to stimulate plant growth (Haque et al., 2020).

CONCLUSIONS

Based on the test results, from the acidic soil, 3 superior isolates were obtained, namely NA-01, NG-04, and NF-04, capable of solubilizing phosphate up to 1.6, and producing IAA of 4.722 ppm, and enhancing plant growth by 9.71%. Meanwhile, from saline soil, 3 superior isolates were obtained, namely PS-03, PN-05, and PC-04, capable of solubilizing phosphate up to 1.56, and producing IAA of 4.913 ppm, and enhancing plant growth by 19.25%.

These findings indicate that isolates NA-01, NG-04, and NF-04 from acidic soils, as well as PS-03, PN-05, and PC-04 from saline soils, are highly potential to be formulated as bio-phosphate fertilizers to enhance the growth and yield of maize in both saline and acidic land ecosystems. Therefore, field trials are necessary to utilize this bio-phosphate fertilizer to improve fertilization efficiency and corn productivity in saline and acidic ecosystems. If the field trial results show that this rhizophosphate consortium has significant potential to enhance sustainable agriculture, improve degraded soil health, and increase crop yields, it can ultimately enhance food security stability.

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