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Streptomyces–Alginate Beads Formula Promote Maize Plant Growth and Modify the Rhizosphere Microbiome

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Abstract

The present study aimed to formulate *Streptomyces* with alginate as a plant growth promoter and determine its effect on the microbiome of maize rhizosphere. Five *Streptomyces*–alginate beads formulas were produced, namely ARJ14, ARJ16, ARJ28, ARJ32, and ARJ34 formulas using the extrusion technique. The formula morphology was analyzed using scanning electron microscope, and *Streptomyces* viability was tested using the total plate count method. Illumina sequencing was used to investigate rhizosphere microbiome composition. Alpha and Beta diversity analyses were used to determine the effects of the *Streptomyces*–alginate formulas on the maize rhizosphere microbiome. The ARJ28 formula had the lowest water content and the best *Streptomyces* viability after storage at room temperature for 10 weeks. The growth of maize treated with ARJ28 formula was better and significantly different from that of the positive and negative controls 49 days after planting. Specifically, the stem diameter, fresh weight, and dry weight were 1.32 ± 0.02 cm, 71.67 ± 12.58 g, and 9.57 ± 1.07 g, respectively. The rhizosphere from maize treated with ARJ28 formula contained a higher proportion of Acidobacteria, Chloroflexi, Crenarchaeota, Myxococcota, Patescibacteria, and Verrumicrobiota, as well as *Candidatus-Nitrosotalea*, *Sphingomonas*, and *Bradyrhizobium* genera compared with those in the rhizosphere from ARJ34 formula–treated maize and the controls. Treatment with the ARJ28 formula also resulted in a higher proportion of Actinobacteria in rhizospheres compared with that in rhizospheres of ARJ34 formula–treated maize and negative control. Thus, the ARJ28 formula increased the growth of maize and affected the composition of the maize rhizosphere microbiome.

Keywords: alginate, formulation, microbiome, plant-growth promoter, Streptomyces

1. Introduction

Maize is a cereal food crop that belongs to the Poaceae family. It contains various beneficial phytochemical compounds (Rouf Shah *et al.,* 2016) and is a multifunctional commodity used as food, feed, fuel, and industrial raw materials (Panikkai *et al.,* 2017). These important aspects cause higher demand for maize, and one of the tactics implemented to increase maize production is through fertilization. Farmers widely use chemical fertilizers for their low cost and accessibility. Unfortunately, when used for a long time and in high doses, chemical fertilizers destroy the soil's physical and chemical structure, rendering it less fertile (Magdalena and Sumarni 2013). Additionally, frequent use of chemical fertilizers increases the soil density and decreases soil porosity, resulting in soil resistance to plant root penetration (Massah and Azadegan 2016).

The utilization of Plant–Growth Promoter Rhizobacteria (PGPR) allows for reduced chemical fertilizer usage. PGPR are microbes that either directly or indirectly stimulate plant growth, overcome environmental stress, and simultaneously exert a bioremediation function (Prasad *et al.,* 2017). PGPR may comprise a single isolate strain or microbe consortium with many beneficial properties for plants (Jha and Saraf 2012; Alori *et al*., 2017; Ahmad *et al.,* 2016). Using PGPR as a biological fertilizer can also increase the activity and diversity of the rhizosphere microbiome, stimulate the secretion of chemical compounds that prevent the growth of pathogens and increase the soil organic content (Liu *et al.,* 2021).

Streptomyces bacteria have been well-studied as effective plant–growth promoters. *Streptomyces* bacteria stimulate plant growth directly by producing growth hormones (Hortsmann *et al.,* 2020; Wahyudi *et al.,* 2019; Niu *et al.,* 2022), contributing to phosphate solubilization, and fixing free nitrogen (Kaur *et al.,* 2013; Wahyudi *et al.,* 2019). However, *Streptomyces* bacteria usage in plants faces many obstacles. Several environmental factors, such as soil type, microbial interactions, and structures on the land, are limiting the use of biofertilizers (Singh 2018).The unprotected inoculated bacteria must compete with the often better-adapted native microflora and withstand predation by soil micro fauna, which may rapidly cause the PGPR population to decline (Bashan 2016). Various formulation techniques such as using liquid formulation (Jha and Saraf 2012), peat with formulated soil amendment (Fitriatin *et al.,* 2021), charcoal (Mäder *et al.,* 2011), clay pellets (Schoebitz *et al.,* 2014), and alginate (Bashan *et al.,* 2012), have been applied to ensure that microbes can survive and colonize the rhizosphere.

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Alginate is the material of choice to encapsulate microorganisms because it is biodegradable and protects bacteria from environmental stress. Additionally, bacterial encapsulation with alginate allows to maintain the optimal concentrations of bacteria for a longer period with the slow-release mechanism (Bashan *et al.*, 2014). However, the application of *Streptomyces*–alginate formulas to maize culture and its effect on maize rhizosphere microbiome remain to be investigated. Therefore, the present study was conducted to determine the formula of *Streptomyces* that can stimulate the growth of maize plants and its effect on the maize rhizosphere microbiome.

2. Materials and Methods

2.1. Culture and Cultivation

Streptomyces bacteria isolated from maize plantation soil samples in East Nusa Tenggara, Indonesia, for previous research were used (Wahyudi *et al.*, 2019). Five isolates (ARJ14, ARJ16, ARJ28, ARJ32, and ARJ34) identified as *Streptomyces* in a previous study using the GenBank database (Table 1) (Deviani, C., IPB University, unpublished observations) were rejuvenated on molasses–yeast extract solid medium (composition: 10 g molasses, 3 g yeast extract, 1 L sterile distilled water, 2% agar) and incubated for 7–14 days. For cultivation, three solid culture plugs were taken, put into molasses–yeast extract liquid medium and stored at ±27°C for 10 days in a shaker (Sari *et al.*, 2021).

Table 1. Identification of the five isolates used in the present study using the GenBank database

No.	Isolate	Homology	Query Cover (%)	E-value	Identity (%)	Accession Number
1.	ARJ14	*Streptomyces asenjonii* strain KNN 35	79%	0.0	87.31%	NR152642.1
2.	ARJ16	*Streptomyces cellulosae* strain MF11	100%	0.0	99.84%	MT2114275.1
3.	ARJ28	*Streptomyces cellulosae* strain F7-7(2)	100%	0.0	100.00%	KR023970.1
4.	ARJ32	*Streptomyces tritolerans* strain YFP6	100%	0,0	100.00%	MG334130.1
5.	ARJ34	*Streptomyces olivaceus* strain NRRl-B-3009	100%	0,0	100.00%	MT543222.1

2.2. Alginate Bead Production

A 2% alginate solution was prepared by dissolving 2 g of powdered sodium alginate (Himedia Laboratories, Mumbai, India) into 100 mL of sterile distilled water. The solution was stirred until homogeneous and sterilized using an autoclave for 15 min at 121°C with a pressure of 1 atm. Alginate bead formulation followed that of Shrivastava *et al.* (2008) with modifications. Briefly, 20 mL of *Streptomyces* inoculant suspension dissolved in 60 mL of 2% sodium alginate (1:3 v÷v). Then, 1.5% (w/v) skim milk was added and the mixture was vortexed. Then, the mixture was pulled up into a 1-mL syringe and extruded through a 26G" needle into stirred 500 mL of 0.1 M calcium chloride at 40 rpm for 30 min. The beads were filtered and washed using sterile distilled water three times. Then, they were dried in a Petri dish using filter paper and placed inside a laminar air flow for 48 h at ±38°C. The filter paper was replaced twice. After that, the formula was stored in a sealed Petri dish, and silica gel was added to it (Bashan *et al.*, 2002). The morphological observations of the formula were conducted in the Central Forensic Laboratory of Indonesian National Police (Pusat Laboratorium Forensik/Puslabfor Polri, Sentul, Indonesia) using a Carl Zeiss EVO MA10 Scanning Electron Microscope (SEM, Carl Zeiss AG, Jena, Germany) with 250×, 1000×, 2000×, and 3000× magnifications. The water content of each formula was calculated using the Association of Official Analytical Collaboration (AOAC) equation (1) (Caputi, 1995) as follows:

$$\text{Water content/moisture (\%)} = \frac{W1-W2}{W1} \times 100 \quad (1)$$

where: W1 = weight of the sample before drying (g)

W2 = weight of the sample after drying (g)

2.3. Analysis of Streptomyces Viability in the Formula

Viability analysis was performed as described by Kim *et al.* (2016) with modifications. One gram of alginate bead formula was subjected to serial dilution. The first dilution consisted in transferring 1 g of the *Streptomyces*–alginate beads formula into a 40 mL conical tube containing 10 mL phosphate salt/PBS buffer solution and vortexing for 2 h to dissolve the alginate. The mixture was then shaken for 24 hat room temperature. After that, a series of seven consecutive dilutions was conducted, each consisting of adding 1 mL of the mixture with 9 mL of 0,85% sodium chloride solution. The colony number was determined using the total plate count method on the molasses-yeast extract solid medium after 24 h incubation.

2.4. Application of the Streptomyces–Alginate beads formula

Streptomyces–alginate formula applied at a greenhouse scale. Approximately 1 g of formula was added to each maize seed as a seed coating. The BISI-2 variety of maize was used. Maize seeds were successively soaked in sterile distilled water for 6 h, dried, transferred into a 0.5% lecithin solution, and mixed with the alginate bead formula. The maize seeds were planted in polybags containing latosol soil, which had been cleaned and sifted to a depth of ±5 cm. Each polybag contained 5 kg of soil, which had been mixed with basic N, P, K fertilizer at a dose of 250 kg urea/ha, 100 kg SP36/ha, and 100 kg KCl/ha. The polybags were 15 × 30 cm in size, and each polybag contained three maize seeds. Fourteen days after planting (14 DAP), the maize with the best growth was maintained, whereas the other two were eliminated.

This study used a one-factor randomized block design (RBD), namely, five *Streptomyces*–alginate formulas (ARJ14, ARJ16, ARJ28, ARJ32, and ARJ34 formulas) with two controls. For negative control, maize was exposed to no biological fertilizer, and maize treated with

a commercial biological fertilizer was used as a positive control. The commercial biological fertilizer consisted of a consortium of bacteria *Pseudomonas* sp., *Azospirillum* sp., *Bacillus* sp., and *Streptomyces* sp. Five replications of each treatment and control were performed. Maize growth data were collected up to 49 days after planting (49 DAP). Growth data included the number of leaves, plant height, and stem diameter. Additionally, measurements of fresh and dry weight were also taken after the plants were harvested.

2.5. Maize Rhizosphere Sampling and DNA Extraction

The rhizosphere microbiome community was analyzed for the maize plants treated with the *Streptomyces*–alginate formula with highest and lowest growth, positive control, and negative control. Rhizosphere soil samples were taken following the method described by Lakshmanan *et al.* (2017) with modifications. Maize plants from each treatment polybag were removed and shaken so that a thin layer of soil remained on the root surface. The roots of the maize plant were cut into 5-cm-long pieces using sterile scissors and transferred into a 50-mL conical tube containing 25 mL PBS. The root pieces subjected to the same treatment were combined and centrifuged (15 min, 6000 × g, 4°C) using a VWR 600R Centrifuge (VWR International, LLC., Pennsylvania, USA). The supernatant was discarded, and 5 g of the pellet were subjected to microbiome DNA extraction using a ZymoBIOMICS^{TM} DNA Mini Kit (Zymo Research Corp., Irvine, USA) according to the manufacturer's instructions. DNA quality was checked using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA).

2.6. Illumina Sequencing and Analysis

The isolated metagenomic DNA was submitted to Beijing Novogene Technology Company, Ltd. for 16S rRNA gene sequencing. The sample concentration was first checked using 1%–agarose gel electrophoresis, and samples were dissolved to a final concentration of 1 ng/µL using sterile distilled water. DNA was amplified by polymerase chain reaction (PCR) using the primers 341F (5'-CCTAYGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with specific adapters targeting the V3–V4 region of the 16S rRNA gene. PCR reactions were performed using Phusion High-Fidelity PCR Master Mix (New England Biolabs, Masshachusetts, USA). The gene library was sequenced using Illumina Novaseq 6500 PE250 to produce two-way reads (paired-ends) of 250-bp sequences. The two-way reading data were combined using FLASH software (version 1.2.7, http://ccb.jhu.edu/software/FLASH) to produce raw tags, which were then selected using QIIME (version 1.7.0, http://qiime.org /index. html). The tags were compared with the SILVA138 database (https://www.arb-silva.de/) using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). Sequences were analyzed using Uparse software (version 7.0.1090, http://drive5.com/uparse/). Representative operational taxonomic unit (OTU) sequence phylogenetic relationships and taxonomic distribution were analyzed using MUSCLE (version 3.8.31, http://www.drive5.com/muscle/). Alpha diversity was calculated using QIIME and displayed with the R program. Principal component analysis (PCA) was conducted using the FactoMineR and ggplot2 packages in the R program (version 2.15.3). Unweighted Pair-Group Method with Arithmetic Means (UPGMA) clustering and beta diversity analysis was performed using QIIME software.

2.7. Statistical Analysis

The maize growth parameters were analyzed using a one-way analysis of the variance (ANOVA). If there was a significant effect of the treatment, Duncan's test (DMRT) with $\alpha = 0.05$ was used. Analyses were performed using IBM SPSS Statistics for Windows version 24.0 (IBM, Armonk, New York, United States).

3. Results

3.1. Streptomyces–Alginate Beads Formulation

Five isolates of *Streptomyces* (ARJ14, ARJ16, ARJ28, ARJ32, and ARJ34) were successfully formulated using an alginate carrier (Figure 1). Each formula had a water content of 27.3% (ARJ14 formula), 26.4% (ARJ16), 25.6% (ARJ28 formula), 26.5% (ARJ32 formula), and 26.2% (ARJ34 formula). *Streptomyces*–alginate bead formulas were 500–1000 m in diameter and slightly round or oval and had a glossy smooth surface and yellow–brown color.

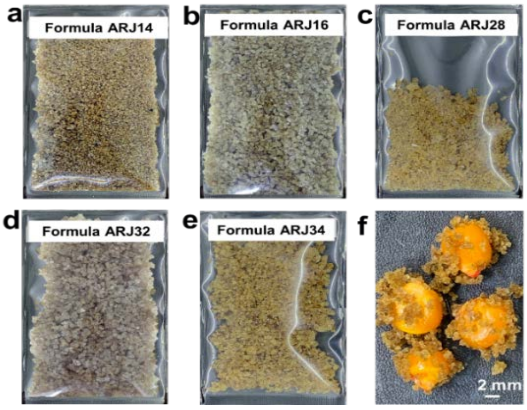

Figure 1. Morphology of *Streptomyces*–alginate bead formula composed of *Streptomyces* ARJ14 (a), ARJ16 (b), ARJ28 (c), ARJ32(d), and ARJ34 (e). Image of formula attached on the maize seed surface (f).

Streptomyces cells were immobilized by the alginate bead matrix. SEM observations confirmed the *Streptomyces* colonies on the surface of the alginate beads (Figure 2).

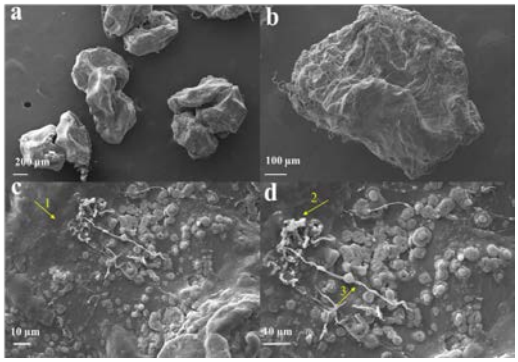

Figure 2. Morphology of the *Streptomyces*–alginate formula analyzed by scanning electron microscopy using (a) 80×, (b) 250×, (c) 2000×, and (d) 3000× magnifications. Arrow 1 indicates

the formula surface, arrow 2 shows *Streptomyces* colonies, and arrow 3 indicates *Streptomyces* mycelium.

3.2. Streptomyces Viability in the Formula

The viability of the *Streptomyces* in the *Streptomyces*–alginate bead formula was determined using the total plate count method over ten weeks of storage at ±27°C. *Streptomyces* ARJ28 showed the best viability. *Streptomyces* ARJ28 viability in the formula was up to 5.1 x 10^7 cfu/g in the 10th week, whereas the lowest viability (6.2 x 10^5 cfu/g) was recorded for *Streptomyces* ARJ16. The concentration of cells attached to the maize seedlings was 1.4 x 10^7 to 2.0 x 10^7 cfu/g.

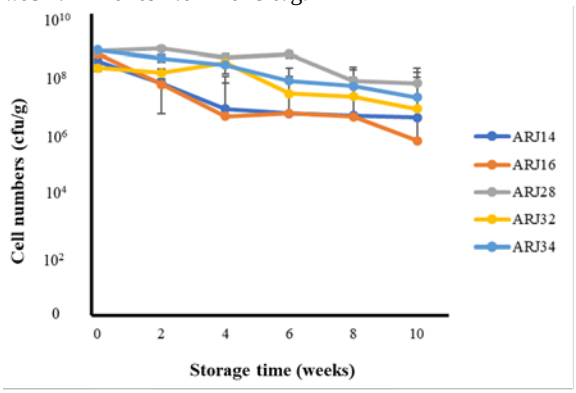

Figure 3. Viability of *Streptomyces* in *Streptomyces*–alginate bead formula quantified using the total plate count method on molasses–yeast extract medium (24 h, ±27°C).

3.3. Effects of Streptomyces–Alginate Bead Formula on Maize

Table 2 shows that the growth of maize inoculated with the *Streptomyces*–alginate bead formula was better than that of the negative control based on the five growth parameters analyzed. At 49 days after planting, maize plants treated with the ARJ28 formula had an average plant height of 117.73 cm, which was significantly greater than that of the negative control (92.03 cm) but not significantly different from that of the positive control and maize subjected to other treatments. Maize plants treated with the ARJ14 formula had the highest average number of leaves (10.67 leaves). This was not significantly different from the number of leaves in the positive control and maize treated with other formulas, whereas the negative control had significantly fewer leaves (7.67 leaves). The average stem diameter was greater in all maize treated with the *Streptomyces*–alginate formulas compared with that of the positive and negative controls. The stalk average diameter of maize plants treated with the ARJ28 formula was the greatest (1.32 cm) and significantly different from that of the negative and positive controls (0.91 and 0.99 cm, respectively). However, there was no significant difference among treatments. The maize plants treated with the ARJ28 formula had the highest average fresh weight (71.7 g), which was significantly different from that of the negative control (20.00 g), positive control (43.33 g), and ARJ34 formula-treated maize plants (51.67 g). Maize plants treated with the ARJ28 formula had the highest average dry weight (9.57 g), which was significantly different from that of the negative control (2.53 g), positive control (4.9 g), and the ARJ34 formula-treated plants (6.7 g).

Table 2. Effects of the *Streptomyces*–alginate bead formula on maize growth

Formula	Plant Height (cm)*	Number of Leaves*	Stem Diameter (cm)*	Upper Plant Body Fresh Weight (g)*	Upper Plant Body Dry Weight (g)*
Negative control	$92,03^{a}$ ± 5,64	$7,67^{a}$ ± 0,57	$0,91^{a}$ ± 0,14	$20,00^{a}$ ± 5,00	$2,53^{a}$ ± 0,45
Positive control	$113,47^{b}$ ± 8,20	$10,00^{b}$ ± 0,00	$0,99^{a}$ ± 0,07	$43,33^{b}$ ± 7,63	$4,90^{ab}$ ± 0,50
ARJ 14	$115,37^{b}$ ± 3,74	$10,67^{b}$ ± 1,15	$1,22^{b}$ ± 0,08	$65,00^{cd}$ ± 5,00	$7,53^{cd}$ ± 2,47
ARJ 16	$113,20^{b}$ ± 4,35	$10,33^{b}$ ± 0,57	$1,18^{b}$ ± 0,12	$68,33^{cd}$ ± 18,92	$8,13^{cd}$ ± 0,96
ARJ 28	$117,73^{b}$ ± 5,90	$10,33^{b}$ ± 0,57	$1,32^{b}$ ± 0,02	$71,67^{d}$ ± 12,58	$9,57^{d}$ ± 1,07
ARJ 32	$115,33^{b}$ ± 2,80	$10,00^{b}$ ± 0,00	$1,20^{b}$ ± 0,04	$63,33^{cd}$ ± 2,88	$8,73^{cd}$ ± 0,55
ARJ 34	$113,97^{b}$ ± 3,91	$9,67^{b}$ ± 0,57	$1,19^{b}$ ± 0,06	$51,67^{bc}$ ± 10,40	$6,37^{bc}$ ± 2,17

Note: *Values are presented as means ± standard errors.

^{a,b,c,d} Different superscript letters indicate significant differences among treatments (column) with P<0.05.

3.4. Maize Rhizosphere Microbiome Analysis

3.4.1. Alpha Diversity

Streptomyces ARJ28 and ARJ34 formulas induced the highest and lowest growth of maize plants, respectively. Therefore, rhizosphere samples of plants treated with ARJ28 and ARJ34 formulas were analyzed and compared with the samples of the positive and negative controls. Table 3 shows the results of the alpha diversity analysis of four rhizosphere samples performed using Uparse and MUSCLE software. The Shannon index was the highest for rhizospheres of the positive control. It was lower for rhizospheres of maize treated with the ARJ28 formula, even lower for rhizospheres of ARJ34 formula-treated maize, and reached the lowest value for the negative control. Thus, the rhizosphere community relative abundance was increased by the inoculation of the ARJ28 and ARJ34 formulas and conventional biofertilizer. Additionally, the range of the rank abundance distribution curve on the horizontal axis was greater for the positive control sample (Figure 4). The Chao1 estimator was the highest for the rhizosphere samples of the positive control and decreased progressively for the samples of maize treated with the ARJ28 formula and the negative control, to reach the lowest value for the rhizospheres of ARJ34 formula-treated maize. These results indicated that the richness of the microbiome community in the rhizospheres of ARJ34 formula-treated maize was lower than that in

rhizospheres of ARJ28 formula-treated maize and both control samples. Thus, there are likely changes in the proportion of the microbiome community.

Table 3. Alpha diversity analysis of the rhizosphere samples

Sample	Shannon
ARJ28	7,196
ARJ34	6,847
Positive Control	7,402
Negative Control	6,721

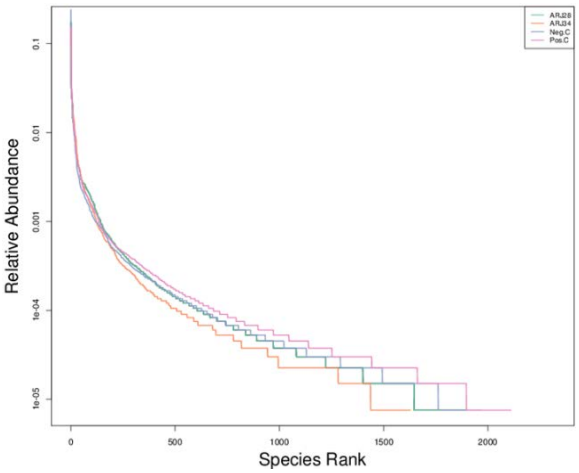

Figure 4. The rank abundance distribution curve of the microbiome community from the rhizosphere samples of maize treated with ARJ28 or ARJ34 formula and of the negative and positive controls.

3.4.2. Beta Diversity of the Bacterial Community in the Maize Rhizospheres

The principal coordinate analysis (PCoA) performed using QIIME software showed a clear separation of the rhizosphere microbiomes of maize treated with the ARJ28 and ARJ34 formulas from those of the positive and negative controls (Figure 5). The rhizosphere microbiomes of the positive and negative controls were clustered, indicating that both microbiomes were quite similar. Additionally, the rhizosphere microbiome of maize treated with formula ARJ28 and that of maize exposed to formula ARJ34 were located in different quadrants and separated by a considerable distance, indicating that there were differences between both microbiomes (Figures 5).

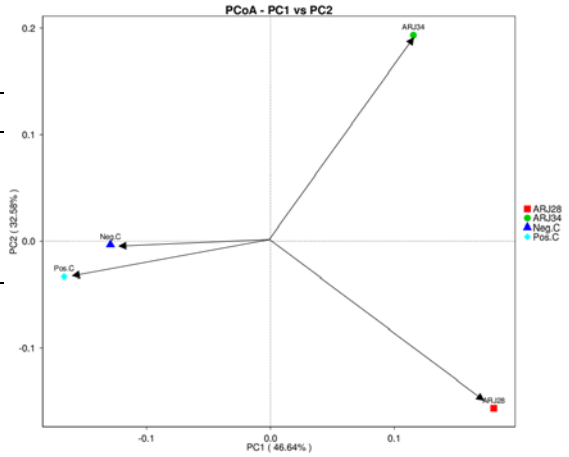

Figure 5. PCoA of the rhizosphere microbiomes of maize treated with ARJ28 or ARJ34 formula, negative control (Neg. C), and positive control (Pos. C). The rhizosphere microbiomes from the positive and negative controls differed from those of maize treated with ARJ28 and ARJ34 formulas.

3.4.3. Taxonomic Distribution of Bacterial Communities between Rhizosphere Samples

The distribution of bacterial community in the maize rhizospheres was determined using the SILVA138 database. The OTU analysis performed using the QIIIME software (version 1.7.0) successfully identified 18 phyla from the Bacteria domain and one phylum from the Archaebacteria domain. There were 10 phyla with more than 1% relative abundance: Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidota, Firmicutes, Crenarchaeota, Myxococcota, Patescibacteria, and Verrucomicrobiota (Figure 6). Clusterization of the maize rhizosphere microbiome communities using UPGMA revealed two clusters, namely the treatment cluster (treated with ARJ28 and ARJ34 formulas) and the control cluster (positive and negative controls).

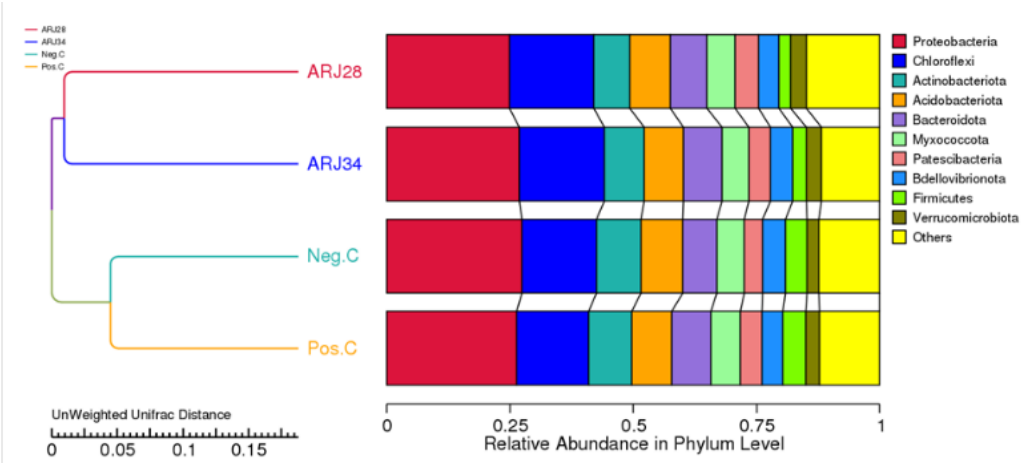

Figure 6. Clusters of bacterial communities in the rhizospheres of maize plants treated with ARJ28 and ARJ34 formulas and that of the positive (Pos. C) and negative (Neg. C) controls.

The rhizosphere analysis of maize plants treated with ARJ28 and ARJ34 formulas and that of the positive and negative controls were different regarding the relative abundance of 10 taxa at the phylum level (Figure 7). The Proteobacteria phylum was the most abundant phylum in rhizospheres of maize treated with ARJ28 (61.51%) and ARJ34 (62.51%) formulas and in those of the positive (64.05%) and negative (70.36%) controls. The Acidobacteriota phylum relative abundance was the highest in the rhizospheres of maize treated with ARJ28 formula (11.91%), whereas it was 8.40%, 11.02%, and 9.31% in rhizospheres of ARJ34 formula-treated maize, the positive control, and the negative control, respectively. The Firmicutes phylum was the most abundant in the rhizospheres of maize treated with ARJ34 formula (8.07%), whereas its relative abundance was 0.60%, 1.23%, and 1.66% in rhizospheres of ARJ28 formula-treated maize, the positive control, and the negative control, respectively. The phylum Actinobacteriota relative abundance was the highest in the rhizospheres of the positive control (5.48%) and was 4.76% in rhizospheres of maize treated with ARJ28 formula, 4.19% in rhizosphere of the negative control, and 3.45% in rhizospheres of maize treated with ARJ34 formula. The rhizosphere of maize treated with the ARJ28 formula exhibited the highest relative abundance of Chloroflexi, Crenarchaeota, Myxococcota, Patescibacteria, and Verrumicrobiota, which were 3.96%, 2.49%, 2.21%, 1.51%, and 1.27%, respectively.

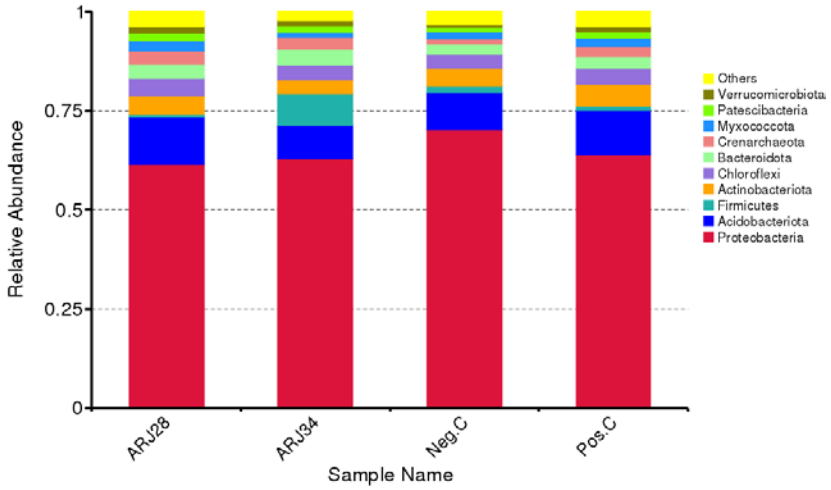

Figure 7. Distribution of the most abundant phyla in the rhizosphere of maize plants treated with ARJ28 and ARJ34 formulas, the negative control (Neg.C), and the positive control (Pos.C).

There were differences in the relative abundance of genera among rhizospheres of maize treated with ARJ28 and ARJ34 formulas, the positive control, and the negative control (Figure 8). *Burkholderia-Caballeronia-Paraburkholderia* was the most abundant genus in rhizospheres of maize treated with the ARJ28 and ARJ34 formulas, positive control, and negative control, with a relative abundance of 19.71%, 18.75%, 18.32%, and 26.83%, respectively. The *Bacillus* genus relative abundance was the lowest in rhizospheres of maize treated with the ARJ28 formula (0.43%), whereas it was 7.81%, 0.77%, and 1.08% in the rhizospheres of maize treated with the ARJ34 formula, positive control, and negative control, respectively. The genus *Dyella* relative abundance was the highest in rhizosphere samples of the negative control (5.85%) and the lowest in the positive control rhizospheres (2.71%). The relative abundances of the genera *Massilia* and *Ralstonia* were the highest in rhizospheres of maize treated with the ARJ34 formula (4.32% and 3.57%, respectively) and the lowest in rhizospheres from the positive controls (2.04%). The relative abundances of the genera *CandidatusNitrosotalea*,

Sphingomonas, and *Bradyrhizobium* were the highest in rhizospheres of maize treated with the ARJ28 formula (3.26%, 2.68%, and 2.33%, respectively). The relative abundances of the genera *Phenylobacter* and *Asticcacaulis* were the highest in the rhizospheres of the positive control (3.05% and 2.78%, respectively). The relative abundance of other bacterial genera was 60.9% in the positive control rhizospheres and 57.72%, 50.15%, and 50.32% in rhizospheres from maize treated with the ARJ28 and ARJ34 formulas and negative control rhizospheres, respectively.

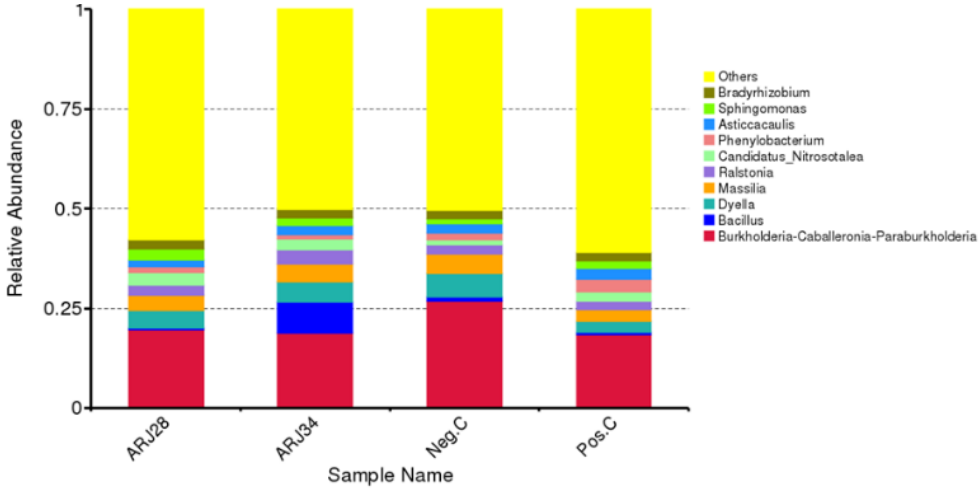

Figure 8. Distribution of bacteria genera in rhizospheres from maize plants treated with ARJ28 and ARJ34 formulas, the negative control, and positive control.

4. Discussion

Here, we successfully formulated five *Streptomyces* isolates using sodium alginate as a carrier. Sodium alginate is a polysaccharide that can be obtained from algae and bacteria. It is environment-friendly, relatively inexpensive to produce, naturally biodegradable, and non-toxic (Puscaselu *et al.*, 2020). *Streptomyces* was encapsulated with alginate using the extrusion method, which consisted in dripping an alginate solution that had been mixed with a *Streptomyces* liquid culture into a calcium chloride solution to generate a reaction between alginate and divalent cations (Malusá *et al.*, 2012). Sodium alginate–divalent cations bonds form a structure that encapsulates bacterial cells and releases these cells slowly over a certain period (Bashan, 2016). Thus, the bacteria are not directly exposed to environmental stress and other microbial contamination (Schoebitz *et al.*, 2013). The diameter of the formula was 400–700 µm, which is categorized as microbeads. Microbeads are large enough to encapsulate some bacteria but too small to attach to seedlings (Bashan *et al.*, 2014). The *Streptomyces*–alginate bead formulas had low water content, the lowest one (25,6%) being in the ARJ28 formula. Therefore, the ARJ28 formula might be better preserved after 10 weeks of storage than the other formulas. Low water content can indeed support microbial survivability in dry formulas for longer storage (Lobo *et al.*, 2019).

The growth of maize treated with the *Streptomyces*–alginate beads formulas was increased at 49 days after planting. Specifically, the plant height, number of leaves, stem diameter, fresh weight, and dry weight were increased by ARJ28 formula treatment compared with those of the negative control. The ARJ28 formula may stimulate the growth of maize plants by excreting plant growth-promoting substances and stimulating the absorption of nutrients important for maize growth. Based on a previous study, *Streptomyces* ARJ28 can produce indole-3-acetic acid (IAA), grow on a nitrogen-free medium, and significantly increase the growth of maize in the Ragdoll test (Wahyudi *et al.*, 2019). *Streptomyces* bacteria are known to produce growth-promoting substances such as IAA (Goudjal *et al.*, 2013), cytokinins, and gibberellins (Olanrewaju and Babalola 2019), dissolve phosphate (Alori *et al.*, 2017), and fixe nitrogen (Dahal *et al.*, 2017). Previous research also found that *Streptomyces* bacteria stimulate maize plant growth as assessed by the plant height, root length, aerial body wet and dry weight, and root fresh weight (Dicko *et al.*, 2018).

The better growth of maize plants treated with the ARJ28 formula might directly result from *Streptomyces* ARJ28. Additionally, the rhizosphere microbiome that is affected by the formula might play a role. Although the ARJ28 formula did not induce the highest microbiome abundance and diversity, the growth of maize treated with the ARJ28 formula was better than that of maize subjected to other treatments or of the positive and negative controls. The ARJ28 formula might attract beneficial microbes in the rhizosphere, resulting in these microbes becoming dominant and in a lower abundance of other microbes. Indeed, PGPR inoculation affects the chemical diversity of root exudates and induces the release of specific compounds involved in the recruitment of beneficial microbes (Kong and Liu, 2022). PGPR inoculation also modifies the functional diversity of the rhizosphere, thereby disrupting plant–soil feedback and modifying the structure of the rhizosphere microbiome (Alzate Zuluaga *et al.*, 2021). In addition, the by-products of PGPR metabolism can be utilized by other rhizosphere microbes as nutrients or energy sources (Kong and Liu, 2022).

The beta diversity analysis showed that *Streptomyces*–alginate bead formulas affected the maize rhizosphere microbiome relative abundance. UPGMA cluster analysis confirmed that the rhizosphere microbiomes of maize treated with the ARJ28 and ARJ34 formulas differ from

those of the positive and negative controls. Particularly, there were differences in the composition and proportion of nine bacteria phyla (Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidota, Firmicutes, Myxococcota, Patescibacteria, and Verrucomicrobiota) and one phylum of Archaebacteria (Crenarchaeota). These results are similar to those of previous studies by Wang *et al.* (2021) and Akinola *et al.* (2021) on maize plant rhizospheres. Differences in the composition and relative abundance of the rhizosphere microbiome community can be influenced by abiotic and biotic factors (Andreote *et al.*, 2014). Microbial inoculation of the soil can affect the activity of native microflora by attaching to the plant roots and competing for space and nutrients released through root exudation. Once the inoculated microbes had enough nutrients and space, they can affect the host plant (Mohanram and Kumar, 2019).

The relative abundance of Actinobacteria in the rhizosphere samples of maize plants treated with the ARJ28 formula was higher than that in the rhizospheres of maize plants treated with the ARJ34 formula and the negative control, but lower than that in the rhizospheres of the positive control. Nevertheless, the inoculation of the ARJ28 formula also increased the relative abundance of Acidobacteria, Chloroflexi, Crenarchaeota, Bacteroidota, Myxococcota, Patescibacteriota, and Verrucomicrobiota. Inoculation of biological fertilizers can enrich, attract, and stimulate the growth of beneficial microbes in plant roots, thereby increasing the availability of nutrients and resistance to pathogenic infections (Dennis *et al.,* 2010). Certain members of Acidobacteria, Chloroflexi, Crenarchaeota, Bacteroidota, Myxococcota, Patescibacteriota, and Verrucomicrobiota are known to promote plant growth through direct or indirect mechanisms. Acidobacteria interact with plants through mechanisms related to auxin production and exhibit growth-promoting effects (Kielak *et al.,* 2016). For example, the growth of tomato and black bean plants increases with the number of Acidobacteria members in the rhizosphere (Kalam *et al.,* 2017). Chloroflexi is a phylum found in a considerable proportion in agricultural soils (Trivedi *et al.,* 2016). It also inhabits other ecosystems and has ecological importance in the habitats of mesophilic, thermophilic, aerobic, anaerobic chemoorganoheterotrophic, and photolithoautotrophic bacteria (Rincón-Molina *et al.,* 2022). The Bacteroidetes phylum is also commonly found on agricultural land, and some members of this phylum produce IAA, dissolve tricalcium phosphate, and break down chitin (Flores-Núñez *et al.,* 2018). The Myxococcota phylum is widely distributed in soil, freshwater, and saltwater and produces a variety of secondary metabolites such as antimicrobial compounds that indirectly act as bioprotectants (Korkar *et al.,* 2022). Crenarchaeota is a phylum from the Archaebacteria domain known to play an essential role in the oxidation of ammonia as an initial step in the nitrification process (Zhou *et al.,* 2015).

At the genus level, the rhizospheres of maize plants treated with the ARJ28 formula contained the highest levels of *CandidatusNitrosotalea*, *Sphingomonas*, and *Bradyrhizobium*, which play a role in stimulating plant growth. *Candidatus Nitrosotalea* is a member of the Archaebacteria domain that can oxidize ammonia, which is essential for the rate of steps in the nitrification process (Maver *et al.,* 2021). *Sphingomonas* is a well-known group of soil bioremediation bacteria and plant-growth promoters in stressed environments (Asaf *et al.,* 2020). *Bradyrhizobium*, which lives freely in the soil and rhizospheres, is involved in carbon metabolism and the degradation of aromatic compounds (Schneijderberg *et al.,* 2018) and fixes nitrogen (Wongdee *et al.,* 2018).

In the present study, the rhizospheres of formula-treated maize plants did not contain a dominant proportion of the *Streptomyces* genus. Even though *Streptomyces* inoculation positively correlated with better maize growth, it was difficult to ensure that the inocula had succeeded in dominating the rhizosphere since the sampling for rhizosphere microbiome analysis was performed in the late vegetative phase. It is important to note that the plant microbiome composition is dynamic and can change throughout the plant life cycle (Edwards *et al.,* 2018). Some microbes may be dominant in the early vegetative phase and be less present in the late developmental stages. The microbial communities can be highly dynamic in the early vegetative phase but start to converge during vegetative growth and become more stable during the reproductive phase (Ferrarezi *et al.,* 2022). In the present study, *Streptomyces* inoculation may promote plant growth during the vegetative stage and may be found in different proportions in each phase. During the vegetative stages, *Streptomyces* may convert plant exudates or macromolecules/supramolecules present in the rhizosphere into a form that can be used by other plant growth-promoting microbes (Sousa and Olivares, 2016). This process may attract other beneficial indigenous microbes. Due to the slow release mediated by alginate encapsulation, the abundance of *Streptomyces* was maintained in the rhizospheres, although not to levels sufficient to dominate the rhizospheres until the end of the vegetative stage.

5. Conclusion

In the present study, the *Streptomyces*–alginate bead ARJ28 formula promoted maize plant growth better than the other treatments and controls. The application of the *Streptomyces*formula increased the relative abundance of Acidobacteria, Chloroflexi, Crenarchaeota, Bacteroidota, Myxococcota, Patescibacteriota, and Verrucomicrobiota as well as the genera *Candidatus Nitrosotalea*, *Sphingomonas*, and *Bradyrhizobium* in rhizospheres. These taxa are known for their plant growth-promoting activity and are thought to play a role in the growth of maize plants. Therefore, the ARJ28 formula might be used as biological fertilizer.

6. Author's Note

The authors declare that there is no conflict of interest regarding this article publication, and the paper was confirmed free of plagiarism.

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