Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Application of Rhizobacteria and NPK for Growth and Productivity of Sweet Corn (*Zea mays* L.)

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ABSTRACT
Soil fertility is one of the limiting factors for sweet corn crops. To overcome it, the wise use of fertilizer is a must to maintain long soil sustainability. Our previous study showed that a rhizobacteria consortium had a significant positive effect on crop growth and productivity; even the chemical NPK performed better. But a long and continuous chemical NPK used has been reported negatively impacting soil and the environment. Thus, combining the application of biofertilizer and chemical NPK could be a practical option. This study aims to isolate bacteria from the sweet corn crop rhizosphere and use them as biofertilizer agents in dual application with NPK. The applied NPK doses were 25%, 50%, and 75% of the recommended dose, while mixed rhizobacteria was 16% of the total volume suspension containing 1.5% molasse. It found five pure rhizobacteria isolates, R1, R2, R3, R4, and R5, which could solubilize phosphate and potassium. It coincided as *Burkholderia cepacian*, *Pantoea dispersa*, *Enterobacter cloacae*, *P. dispersa*, and *P. cypridii,* respectively, based on the 16s RNA gene similarity. Application of mixed rhizobacteria dual with 50% and 75% of NPK showed apparent support for crop growth and productivity. Those supports were significantly similar to those performed after 100% NPK (control). This meant that by reducing chemical NPK and adding rhizobacteria, the growth and productivity of sweet corn crops could be maintained well as if 100% chemical NPK was applied.

Keywords: *Chemical NPK, Growth, Productivity, Rhizobacteria, Sweet Corn.*

1. INTRODUCTION

Sweet corn (Zea mays L.) is an economically important crop in Indonesia since it has multiple functions: food, feed, and industrial raw materials [1]. One of its limiting factors is soil fertility [2, 3]. To maintain and increase soil fertility, chemical fabricated fertilizers are still the primary choice [2, 4], although continuous and prolonged use affects the opposite: fast reducing soil fertility and damaging the environment [5]. Some chemical fertilizers contained heavy metals (for instance, cadmium and chromium) that later not be absorbed by crops will be accumulated in soil [6] and led to severe soil acidification, nutritional imbalance, and deterioration of the rhizosphere micro-ecological environment [7]. Chemical fertilizers are mostly bonded to soil minerals, forming structures unavailable for crops. Therefore, microbes-based fertilizer or biofertilizer is an option [4] since microbes produce many extracellular exudates that enrich soil nutrients [8]. Soil microbes play an important role in dissolving organic compounds for crops and positively affect soil fertility [7].

The previous research [9] successfully isolated phosphate and potassium solubilizing rhizobacteria from sugarcane plantation areas that could produce IAA phytohormones. According to [10], those rhizobacteria, after biochemical characters assignments, belong to the genus Bacillus, Pseudomonas, and Azotobacter, which were also reported belonged to the Plant Growth Promoting Rhizobacteria (PGPR) group [11]. Those rhizobacteria were further applied, and they positively supported the growth and productivity of sweet corn crops, even without any chemical fertilizer [12]. It also mentioned that chemical NPK still performs better than

rhizobacteria [12]. The role of chemical NPK, also clarified by [5, 13], cannot be replaced by any single biofertilizer application.

A mixture application of biofertilizer and chemical fertilizer was an agronomically efficient and effective alternative [14, 15]. It had been proven by [14] on food crops (paddy, corn, and potatoes) as they reduced up to 50% the use of chemical fertilizers. The resulting research of [15] showed that after reducing NPK up to 25% and adding biofertilizer, the growth and yield of maize were as good as the application of chemical NPK following the 100% recommended dose. Anyhow reducing used chemical fertilizers in agriculture definitive minimize soil and ecosystem damages.

Following the successful studies of [9, 12], this study aimed to isolate bacteria from sweet corn crop soil and use them back as biofertilizers agents in dual application with chemical NPK on the sweet corn crops. The rhizobacteria were taken and applied back from and into the sweet corn rhizosphere. Even PGPR was found with an extensive range of host plant associations [16], but strategies of adaptation of both parties have strongly influenced the interactions, and host specificity may lead to intimate interactions [17].

2. METHODOLOGY

2.1. Isolation and identification of rhizobacteria

The sample was soil from a sweet corn plantation since the isolated rhizobacteria will be applied back to sweet corn crops. Isolation and purification were carried out following a standard protocol [18] onto a selective solid medium of Pikovskaya and Alexandrow to find and select phosphate and potassium solubilizing bacteria directly based on a clear zone around a single colony. Five rhizobacteria colonies that grew and formed clear zones were gradually transferred on the same fresh medium until pure isolates were obtained and coded as R1, R2, R3, R4, and R5.

Those pure isolates were sent to the GriyaSain Company, Indonesia (http://griyasains.com) for processing the 16S rRNA amplification using universal PCR primers of 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Zhang and Kong, 2014). Following standard protocol, the amplified fragments were further sequenced by the 1st BASE Lab Company, Singapore (https://base-asia.com/). The obtained 16S rRNA gene sequences were stored in FASTA and edited format using the Bio Edit program (Hall 1999) and thus compared to related sequences available in GenBank databases of the National Center for Biotechnology Information (NCBI) with the BLASTN (Basic Local Alignment Search Tool) algorithm (www.NCBI.nlm.nih.gov). The highest sequence similarity score was used as a reference to determine the species.

2.2. Suspension of rhizobacteria preparation

Each isolate was fully streaked onto a Petri dish containing 15 ml Nutrient Agar and incubated for 24 hours. Each isolate had ten replicate Petri dishes. Those ten cultures were resuspended into 200 ml sterile 0.85% salt-containing water and reached an OD600nm value of >1.0. The 200ml resuspended cultures were mixed equally (R1, R2, R3, R4, R5 = 1:1:1:1:1) and further diluted into molasse containing water with a ratio of 16% : 1.5% : 82.5% = bacteria : molasse : water (v:v:v). After incubating for 1.5 hours at room temperature, the rhizobacteria suspension was ready to be applied.

2.3. Planting soil and sweet corn crop preparation

About 10 kg commercial planting soil was put into a 40 cm x 40 cm polybag and poured 300 ml rhizobacteria suspension. After 24 hours, 300 ml of rhizobacteria suspension was repoured and incubated for 3x24 hours. Afterward, a 2-leaves young sweet corn crop was planted into it. The first day of young crop planting was indicated as the 0 days after planting (0 DAP). Every day the crop was maintained by watering and weeding, whereas invading insects were physically removed. The sweet corn crop was harvested after 83 DAP.

2.4. Soil bacteria and NPK application

During 0 DAP to 83 DAP, solid chemical NPK was applied on the 7 and 40 DAP following the treatment dose. Meanwhile, 300 ml of bacteria suspension was poured on the 20, 34, 48, and 62 DAP. The treatments were below, and each treatment had three replication crops:

K1 : water (control 1)
K2 : 100% of recommend dose NPK (300 kg/ha) (control 2)
K3 : rhizobacteria suspension only, without NPK (control 3)
P1 : 25% of recommend dose NPK (75 kg/ha) + rhizobacteria suspension
P2 : 50% of recommend dose NPK (150 kg/ha) + rhizobacteria suspension
P3 : 75% of recommend dose NPK (225 kg/ha) + rhizobacteria suspension

2.5. Data analysis

The observed growth parameters were crop height (cm), stem diameter (cm), number of leaves and leaf area (cm2), while the productivity was the length (cm)

and weight (gr) of corn cob, number of seeds, and weight per 100 seeds (gr). The data were collected every seven days, from 0 DAP to 83 DAP which was 0, 13, 20, 27, 34, 41, 48, 55, 62, 69, 78, and 83 DAP. To test the effect of treatments on crops, the collected data were statistically analyzed using a One-Way Anova. If there was a natural effect, a further test was the Least Significant Difference (BNT) at a 5% significance level. The analysis was calculated with the Minitab 18, a free statistical software.

3. RESULT AND DISCUSSION

3.1. Isolated rhizobacteria

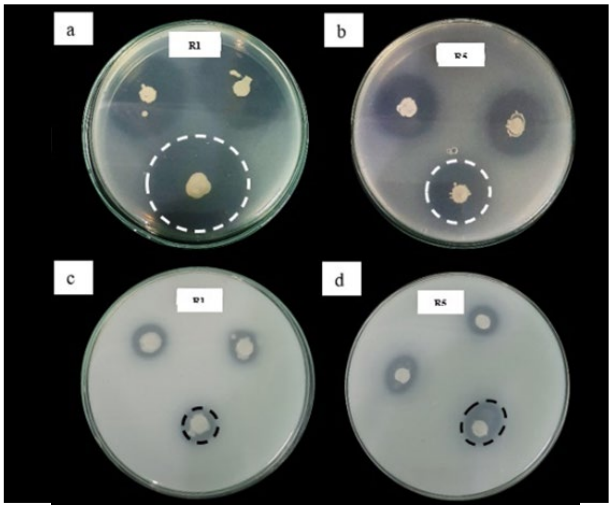


Figure 1. Isolate R1 and R5 performed clear zone around the colony after five incubation days on medium Pikovskaya (a-b) and medium Alexandrow (c-d), indicating that they solubilized inorganic and immobilized phosphate and potassium in the medium.

The R1, R2, R3, R4, and R5 were performing apparent clear zone on the specific medium Pikovskaya and Alexandrow, indicating that they belonged to phosphate and potassium solubilizing bacteria (Figure 1). Based on the 16S rRNA gene, they were closed to *Burkholderia cepacia*, *Pantoea dispersa, Enterobacter cloacae*, *P. dispersa*, and *P. cypridii*, respectively, with similarities up to 100% (Table 1). This result was linear to other studies that those species could dissolve organic phosphate and potassium into inorganic ones [19, 20, 21, 22, 23]. Species *B. cepacia*, *P. dispersa*, and *E. cloacae* were also reported to fix free nitrogen from the atmosphere [24, 25, 26]. Further on, *B. cepacia* could convert soil organic nitrogen compounds into inorganic nitrogen and be available for plants [24], while genus *Pantoea* facilitated nitrogen fixation in plant roots even under nitrogen-limited conditions [27].

Table 1. Species of isolated bacteria based on 16S rRNA sequence similarity

Sample	Species	Similarity (%)	Nucleotide Seq ID*
R1	*Burkholderia cepacia*	100	FJ907187.1
R2	*Pantoea dispersa*	97.99	MF431769.1
R3	*Enterobacter cloacae*	100	MT212231.1
R4	*Pantoea dispersa*	99.66	MT072166.1
R5	*Pantoea cypridii*	99.49	JX556216.1

*www.NCBI.nlm.nih.gov

Thus, N, P, and K as essential macronutrients for plants, those rhizobacteria isolated from sweet corn planting soil (R1, R2, R3, R4, and R5) were prospectus candidates for soil biofertilizer agents. *B. cepacia*, *P. dispersa*, *P. cyperidii,* and *E. cloacae* belonged to Plant Growth Promoting Rhizobacteria (PGPR) [11], potentially living on plant rhizospheres and supporting plant growth by releasing extracellular exudates to their surroundings. *B. cepacia* is also one of the dominant bacteria colonizing the rhizosphere of sweet corn crops

Table 2. An average number of growth parameters at 41 DAP and 83 DAP

Treatment	Height (cm)		Stem Diameter (cm)		Leaf Number		Leaf Area (cm^2)	
	41 DAP	83 DAP	41 DAP	83 DAP	41 DAP	83 DAP	41 DAP	83 DAP
K1	$65,2\pm9,0^{C}$	$126,7\pm36,6^{CD}$	$1,4\pm0,0^{D}$	$1,9\pm0,1^{C}$	$10,3\pm1,2^{B}$	$7,0\pm1,7^{A}$	$353,3\pm127,2^{C}$	$431,8\pm160,1^{C}$
K2	$128,7\pm6,7^{A}$	$183,3\pm12,6^{AB}$	$2,3\pm0,0^{A}$	$2,6\pm0,0^{A}$	$11,3\pm0,6^{AB}$	$8,7\pm2,1^{A}$	$850,3\pm47,6^{A}$	$946,2\pm53,6^{A}$
K3	$45\pm6,1^{D}$	$87,3\pm10,0^{D}$	$0,7\pm0,0^{E}$	$1,2\pm0,1^{D}$	$6,0\pm0,0^{C}$	$7,0\pm1,0^{A}$	$172,3\pm15,8^{D}$	$212,7\pm1,8^{D}$
P1	$102,3\pm7,5^{B}$	$159,5\pm13,1^{BC}$	$1,5\pm0,3^{CD}$	$2,3\pm0,0^{B}$	$11,0\pm1,0^{AB}$	$8,7\pm1,2^{A}$	$646,4\pm57,2^{B}$	$738,8\pm52,5^{B}$
P2	$125,3\pm22,3^{A}$	$186,2\pm34,1^{AB}$	$1,8\pm0,2^{BC}$	$2,4\pm0,2^{AB}$	$11,7\pm1,2^{AB}$	$8,3\pm2,1^{A}$	$682,1\pm17,9^{B}$	$774,4\pm26,2^{B}$
P3	$134\pm4,0^{A}$	$212,0\pm32,1^{A}$	$1,9\pm0,3^{AB}$	$2,6\pm0,0^{A}$	$12,3\pm0,6^{A}$	$7,7\pm0,6^{A}$	$754,1\pm50,9^{AB}$	$862,3\pm66,6^{AB}$

Note: The same letter in the same column shows no significant difference according to the BNT test at a 5% significance

[28]. *P. dispersa* might trigger other natural soil microbial activity, making it suitable for optimizing agro-microecological systems [20].

3.2. The growth and productivity after dual application of rhizobacteria and NPK

The effects of the treatments on the sweet corn crop growth parameters were presented in Table 2 after 41 DAP and 83 DAP. The treatment P2 and P3 put a significantly similar effect with control K2 (100% NPK application) on the crop height, stem diameter, leaf number, and leaf area. This may indicate that even

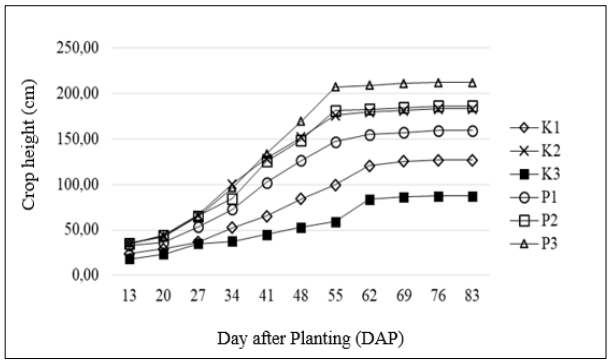


Figure 2. Growth patterns showed a similar pattern but different speed growth.

chemical NPK was less dose applied, but in combination with the isolated rhizobacteria, the crop growth was significantly supported. This was parallel to other studies that mentioned biofertilizer could not replace chemical NPK but might partially be substituted [5, 13, 14, 15]. This partial NPK substitution by rhizobacteria will significantly affect soil and the environment if the dual application is made continuously and consistently since rhizobacteria need time to adapt to biotic and abiotic environmental factors to produce or dissolve the required nutrients plants [29].

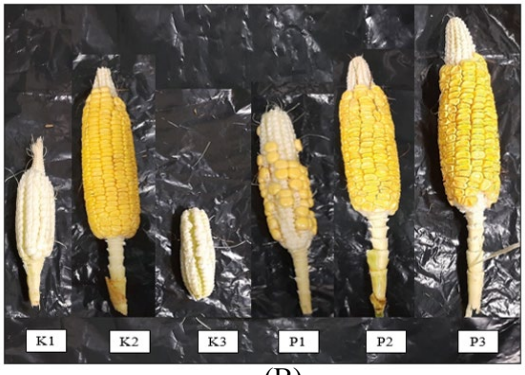
It has been reported that nitrogen is the highest element needed for plants, as it is essential for the formation of chlorophyll, stimulates vegetative growth, especially roots, stems, and leaves, as well as in the formation of proteins, fats, and various other organic compounds [2, 30, 31, 32]. Phosphate found in every living plant cell is essential for growth and plant functions, including energy transfer, photosynthesis, the transformation of sugars and starches, nutrient movement within the plant, and transfer of genetic characteristics from one generation to the next. Potassium is also essential as it increases root growth, maintains turgor, aids in photosynthesis and foods formation, reduces respiration, prevents energy losses, enhances translocation of sugars and starch, builds cellulose, and reduces lodging, and helps retard crop diseases [13, 33, 34, 35].

The stem diameter of three treatments (P1, P2, and P3) after 41 DAP reached a similar size (Table 2), and after 83 DAP, they were at the optimal size of about 2,4 cm. The leaf number was also increasing over the DAP, but after 41 DAP, the leaves were falling. Even the number of leaves decreased, but after 81 DAP, the leaf area increased. It seemed that decreasing leaf number would be balanced by increasing leaf area. Leaf number and leaf area were important for energy gaining through photosynthesis.

The height parameter was fast and noticeable to observe and distinguish the difference among treatments. Figure 2 showed the crops were growing slowly at the beginning until 27 DAP with no significant difference. After 34 DAP, each treatment put a similar pattern but w nith different increasing speeds, and the height being constant after 55 DAP as an indicator they start entering the vegetative phase. The P2 and P3 had no significant difference with control K2 (100% NPK) after 41 DAP to 83 DAP (Table 2). At the end of the observation (83 DAP), P2, P3, and control K2 reached about 186 cm, 212 cm, and 183 cm height, respectively. This might indicate that rhizobacteria significantly provided N, P, K needed by the crop and substituted the less chemical NPK.

The productivity was measured based on corn cob length, weight, and corn seed number. Adding

(A)



(B)

Figure 3. Harvested corn cob length after 83 DAP (A) and different number and arrangement of seeds after skin of cob was wrapped (B). P2 and P3 showed similar performance with control K2; the seeds were neatly and compactly

Table 3. Everage number of productivity parameters at 83 DAP

Treatment	Cob length (cm)	Cob weight (gram)	Seed number	Weight of 100 seeds (gram)
K1	26,8 ^{BC}	110,9 ^{AB}	0 ± 0 ^{B}	0 ± 0 ^{B}
K2	29,5 ^{AB}	187,8 ^{A}	275,5 ± 153,4 ^{A}	29,9 ± 0,2 ^{A}
K3	22,0 ^{C}	43,0 ^{B}	0 ± 0 ^{B}	0 ± 0 ^{B}
P1	29,3 ^{AB}	147,5 ^{A}	78 ± 9,9 ^{AB}	0 ± 0 ^{B}
P2	32,5 ^{AB}	187,9 ^{A}	211 ± 84,9 ^{AB}	26,5 ± 7,7 ^{A}
P3	35,0 ^{A}	212,8 ^{A}	189,5 ± 126,6 ^{AB}	26,5 ± 9,0 ^{A}

The column shows no significant difference according to the BNT test at a 5% significance level.

rhizobacteria significantly affected partial chemical NPK substitution; P2 and P3 showed no significantly different performances than control K2 (Table 3). At 83 DAP, the average corn cobs lengths for P2, P3, and K2 were 32.50 cm, 35 cm, and 29.50 cm, respectively, while the average weight was 187,85 gram, 212,57 gram, and 187,75 gram.

Further on, after wrapping the skin corn, the seeds neatly and compactly arranged for P2, P3, and K2 (Figure 3). This result supported mentioned NPK was significantly important for the vegetative stage. Biofertilizer was reported effective when combined with other organic or inorganic fertilizers [2]. Without other organic or inorganic fertilizers, the application of biofertilizer will have the same effect as no fertilization at all.

This study used a commercial planting soil for growth medium without identifying existing indigenous microorganisms and NPK content. It was under purpose since this study was also figuring out the effect of adding isolated rhizobacteria to the indigenous microorganism in supporting crop growth and productivity. As predicted, Table 2, Figure 2, Table 3, and Figure 3 showed that without chemical NPK, the indigenous microorganisms (control K1) did not support the optimal development and failed to support the vegetative phase in seed production. Meaning that commercial planting soil was NPK poor, and the existing indigenous microorganisms could not provide them.

Further on, the growth and productivity of the control K3 (the crop was treated with the rhizobacteria only and no chemical NPK) were even less than that of indigenous microorganisms (control K1). It indicated that NPK was still insufficient to support the crop if only rhizobacteria were applied, although the added rhizobacteria were proven to solubilize phosphate and potassium (Figure 1).

Interestingly, after adding the rhizobacteria (control K3), the crop growth and productivity decreased compared to those control K1. This might show an antagonistic symbiosis between indigenous microbes and rhizobacteria, even though the rhizobacteria were from the sweet corn rhizosphere. It seemed contradictory to [12] that the added rhizobacteria isolated from the sugarcane rhizosphere supported better sweet corn crop growth than indigenous soil microorganisms. Even statistically, the support was significantly different from that supported by a 100% chemical NPK, but it proved that the sugarcane-rhizobacteria supported the sweet corn cob with neat and compact seed arrangements [12].

This contradiction might refer to microbial interaction between the indigenous microorganisms and added rhizobacteria and their effect on crop growth (microbes-plant interactions). It has been well known that microbial interaction may release many kinds of extracellular organic compounds, at which the organic compounds may interfere the plant growth and vice versa. Therefore another study needs to be performed to find out these interactions between sweet corn rhizobacteria, indigenous microorganisms, and sweet corn rhizosphere. To develop an efficient and effective biofertilizer, an analysis of the interactions between plants and their microbial communities in the rhizosphere was also important to find out the balance nutrients supporting both plants-microbes [36].

Soil bacteria were successfully isolated and identified as *Burkholderia cepacia*, *Pantoea dispersa*, *P. cyperidii,* and *Enterobacter cloacae*, and they were able to solubilize phosphate and potassium. Dual application of rhizobacteria and less chemical NPK was significantly proven, supporting the growth and productivity of sweet corn crops. Other studies need to be performed to figure out the interaction between indigenous and added rhizobacteria and between plants and microbes.

AUTHOR CONTRIBUTIONS

M.S., N.D.K., N.H.A. and E.Z. designed the study. M.S., N.D.K., and N.H.A designed the experiments. S.K.S. performed all experiments. M.S. and S.K.S. wrote the manuscript.

ACKNOWLEDGMENTS

We thank the Institut Teknologi Sepuluh Nopember (ITS) Surabaya for the grant under the scheme of Kolaborasi Pusat ITS 2021. This work was one part of three works of the main umbrella project titled Agriculture Growth Promoting Inoculant (AGPI) as a solution for increasing soil fertility of submarginal land.

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