



## Enhancing lettuce (*Lactuca sativa L.*) production with plant growth-promoting rhizobacteria

### Peningkatan produksi selada (*Lactuca sativa L.*) dengan *plant growth-promoting rhizobacteria*

Muzna Ardin Abdul Gafur, Zulkarnain Sangadji, Riskawati Riskawati\*, Felia Imelda Mayor

Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sorong, Sorong 98416, Indonesia

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#### ABSTRACT

Cultivating lettuce (*Lactuca sativa L.*) using plant growth-promoting rhizobacteria (PGPR) in Sorong Regency is uncommon, limiting production despite the high market potential. This study aims to evaluate the effect of PGPR and identify the optimal concentration to improve lettuce growth and yield. The research was conducted in Malawili Village, Sorong Regency, Indonesia, using a randomized block design (RBD) with four treatments: P0 (7.5 mL/L), P1 (10.5 mL/L), P2 (13.5 mL/L), and P3 (16.5 mL/L). Results revealed that PGPR significantly enhanced growth parameters, including plant height, leaf number, fresh biomass weight, biomass weight per plot, fresh weight for consumption, and root length. Among all treatments, 13.5 mL/L (P2) demonstrated the best outcomes, with plant height reaching 14.6 cm, fresh biomass weight of 633.3 g, and root length of 9.6 cm. PGPR application at appropriate concentrations effectively stimulates growth, increases nutrient uptake, and improves yield. These findings underline PGPR's potential to enhance lettuce production sustainably.

#### ABSTRAK

Budidaya selada (*Lactuca sativa L.*) menggunakan *plant growth-promoting rhizobacteria* (PGPR) di Kabupaten Sorong masih jarang dilakukan, sehingga membatasi produksi meskipun memiliki potensi pasar yang tinggi. Penelitian ini bertujuan untuk mengevaluasi pengaruh PGPR dan menentukan konsentrasi optimal untuk meningkatkan pertumbuhan dan hasil selada. Penelitian dilakukan di Desa Malawili, Kabupaten Sorong, Indonesia, menggunakan rancangan acak kelompok (RAK) dengan empat perlakuan: P0 (7.5 mL/L), P1 (10.5 mL/L), P2 (13.5 mL/L), dan P3 (16.5 mL/L). Hasil penelitian menunjukkan bahwa PGPR secara signifikan meningkatkan parameter pertumbuhan seperti tinggi tanaman, jumlah daun, bobot biomassa segar, bobot biomassa per plot, bobot segar konsumsi, dan panjang akar. Konsentrasi 13.5 mL/L (P2) menghasilkan pertumbuhan terbaik, dengan tinggi tanaman mencapai 14.6 cm, bobot biomassa segar 633.3 g, dan panjang akar 9.6 cm. Aplikasi PGPR pada konsentrasi yang tepat terbukti efektif merangsang pertumbuhan, meningkatkan penyerapan nutrisi, dan memperbaiki hasil. Hasil ini menegaskan potensi PGPR dalam meningkatkan produksi selada secara berkelanjutan.

\*Corresponding author

E-mail: [riskawati@um-sorong.ac.id](mailto:riskawati@um-sorong.ac.id)

#### INTRODUCTION

Lettuce (*Lactuca sativa L.*) is a leafy vegetable that originates from temperate regions (Naihati et al., 2018). It is classified as a home garden crop with significant market potential due to its nutritional value. Lettuce is rich in essential nutrients, including vitamins B and C and protein, making it a valuable source of nutrition for communities (Camejo et al., 2020; Kim et al., 2016). Several types of lettuce are cultivated, such as leaf lettuce, head lettuce, stem lettuce, curly lettuce, and

romaine lettuce. Among these, curly lettuce is the most commonly grown variety, recognized for its green, curly leaves and its high nutritional content, particularly vitamins A and C, which are essential for maintaining vision and promoting bone growth (Aini et al., 2010; Lingga, 2010). Curly lettuce is classified as an annual plant that can thrive in cold, humid environments, including both highlands and lowlands (Wibowo & Siddik, 2021). All lettuce types are versatile and can be consumed fresh, in salads, or as side dishes. Lettuce's appeal lies in its short cultivation period, relatively stable price, and broad market demand (Della & Hartatik, 2021).

Despite a reported growth rate of 5.19% in lettuce production from 2018 to 2019, domestic production remains insufficient to meet the annual consumption rate of 35.30 kg per capita. In 2018, lettuce imports reached 21.1 tons, underscoring the need to increase domestic production to bridge the supply-demand gap (Badan Pusat Statistik, 2019). Historical data from 2014 also indicate that the vegetable production target of 91.7% was not met, revealing persistent challenges in meeting community consumption needs (Direktorat Jenderal Hortikultura, 2014).

Lettuce productivity is particularly limited in rural and remote areas, such as Sorong Regency, where it is predominantly cultivated hydroponically. Hydroponic systems involve high costs compared to conventional soil-based cultivation on mineral or dry lands. However, dry-land cultivation remains underutilized due to farmers' limited knowledge of its practices. These suboptimal lands often suffer from low productivity and poor organic matter content, despite their critical role in improving soil fertility and ecosystem function (Rachman et al., 2021a). With appropriate management, these lands hold significant potential for agricultural development (Rachman et al., 2021b). Innovations such as biofertilizers are essential to enhance crop yields by addressing nutrient availability—a key factor in successful cultivation.

Fertilization plays a pivotal role in determining cultivation success, particularly in lettuce production. Organic biofertilizers, such as Plant Growth-Promoting Rhizobacteria (PGPR), are effective in increasing productivity (Wulandari et al., 2019). PGPR contains rhizobacteria, which naturally inhabit and develop in plant roots (Kumar et al., 2021), supporting plant growth through nitrogen fixation, solubilization of insoluble phosphates, and secretion of plant hormones (Singh et al., 2023). Additionally, rhizobacteria enhance plant tolerance to abiotic stresses, such as drought and salinity (Wang et al., 2012). PGPR can be derived from various plant roots, such as bamboo, *Mimosa pudica*, elephant grass, lemongrass, and cogongrass, which are colonized by beneficial bacteria. For example, *Pseudomonas fluorescens*, found in bamboo roots, increases phosphorus solubility and controls certain pathogens (Peter & Pandey, 2014). The application of PGPR in dry-land agriculture not only enriches the soil with nutrients but also improves soil structure, which is critical for root development and nutrient absorption (Rabot et al., 2018).

The effectiveness of PGPR depends on environmental conditions, including soil type, management practices, and the crops cultivated. While numerous studies have examined the use of PGPR in lettuce cultivation (Al Turki & Abdullah, 2021; Abdel-ilah et al., 2022; Anahita et al., 2018; Naikofi & Rusae, 2017), they often focus on single-source root exudates. This study explores composite PGPR derived from multiple root exudates, such as bamboo and *Mimosa pudica*, to optimize lettuce growth and yield. The application of PGPR with appropriate composition and concentration has the potential to enhance crop performance significantly. Therefore, this research aims to evaluate the effects of PGPR and identify the optimal concentration for improving the growth and yield of lettuce (*Lactuca sativa L.*).

## MATERIALS & METHODS

### *Study area conditions*

The research was conducted in Malawili Village, Aimas District, Sorong Regency. The research site conditions are agricultural land for horticultural crops (except lettuce) with organosol soil type and low topography, located at the coordinates 0°57'21.6"S and 131°18'58.3"E. The study site employs excessive usage of inorganic fertilizers and intensive agriculture practices. The general soil fertility level in the Aimas District is low, with acidic pH (5.0-5.2), low organic matter content (1.42%-1.67%), low total nitrogen (0.13%-0.15%), and cation exchange capacity (CEC) ranging from extremely

low to moderate (14.66–21.98 cmol/kg) (Soekamto M, et al., 2023). The research began with the preparation of PGPR based on bamboo roots, mimosa roots, elephant grass roots, lemongrass roots, and cogon grass roots. The preparation of PGPR adapts methods from Ali (2016); Wahyuni et al., (2020); and Patading & Ai, (2021). The weight of each basic ingredient consists of 100 grams, which is then modified by combining the starter with the basic ingredients to produce PGPR ready for use. The characteristics of the finished PGPR are marked by a sour smell, the presence of foam, and when shaken, air bubbles appear (Ministry of Agriculture, 2021), as well as white spots on the roots or the surface of the jar, indicating that the PGPR bacteria are alive (Asfar et al., 2022). The types of bacteria identified as PGPR bacteria are *Pseudomonas*, *Azotobacter*, *Azospirillum*, *Acetobacter*, and *Bacillus* (Mokoginta, et al., 2022).

The stages in this research begin with the sowing of lettuce seeds from PT East West Seed, Indonesia. The germination process is carried out in trays with loose and fertile soil media. The seeds are planted in the tray one by one, then the tray is covered with dark-colored plastic. After 4 days, the plastic is removed and the trays are placed in a shaded area. After 15 days, the seedlings were transferred to the field. Before planting, the land to be used was cleared of weeds and the soil was processed and loosened using a hoe. The area of land used is adjusted according to the number of plots.

#### *Research design*

This study employed a randomized block design (RBD) consisting of four treatments, each repeated three times, resulting in a total of 12 plots with dimensions of 1 m × 1 m per plot. The treatments were based on varying concentrations of PGPR, including P0 (7.5 mL/L), P1 (10.5 mL/L), P2 (13.5 mL/L), and P3 (16.5 mL/L). Lettuce seedlings were transplanted into planting holes with a depth of 3 cm and a spacing of 20 cm × 20 cm. Each plot and plant sample were labeled accordingly to ensure proper identification. PGPR was applied three times during the first, second, and third weeks by evenly watering the plants with the assigned concentrations. The total volume of PGPR applied across all treatments was 4320 mL, equivalent to 1440 mL per week. The application volumes were adjusted for each treatment as follows: P0 (225 mL), P1 (315 mL), P2 (405 mL), and P3 (495 mL).

One week after planting, replanting was conducted to replace any non-viable seedlings, followed by routine weed monitoring and weeding, as followed previous study (Edi & Bobihoe 2010; Husen et al., 2021). Irrigation was performed regularly in the morning and evening throughout the cultivation period. The lettuce plants were harvested 20 days after planting. Harvesting was carried out for each treatment plot, followed by the weighing of produce. Observations were conducted on 8 plants per plot, with 3 replications for each treatment, resulting in a total of 96 observation samples.

#### *Measurement of observation variables*

The observation variables measured in this study included plant height, which was recorded in centimeters from the base of the stem to the tip of the tallest leaf, and the number of fully expanded leaves observed at 2, 3, and 4 weeks after planting (WAP) (Alatas et al., 2021). The weight of fresh biomass was determined by harvesting and weighing eight sample plants from each treatment unit. Additionally, the weight of biomass per plot was calculated by weighing the total plants in each treatment plot, excluding the sample plants. Fresh weight for consumption was measured by weighing all harvested samples after removing roots and any non-consumable leaves, such as yellow or damaged ones. Root length was measured in centimeters from the base of the root to the tip of the longest root (Restiani et al., 2015). The harvest results for each treatment concentration were obtained through careful weighing of the plants.

#### *Data analysis*

The results of the subsequent research were analyzed using Analysis of Variance (ANOVA). If the treatment being tested has a significant effect, it will be followed by an Honest Significant Difference (HSD) test at a 95% confidence level with a 5% significance level. The data were processed using Excel 2016 (Microsoft, USA).

## RESULTS &amp; DISCUSSION

The produced PGPR has signs such as foam and white spots on the roots or the surface of the jar. The presence of these signs indicates that the bacteria in the root culture are alive. (Asfar et al., 2022). The use of PGPR on lettuce plants has a very significant effect on all observed parameters. In general, the treatment concentrations P0: 7.5 mL/L; P1: 10.5 mL/L; and P2: 13.5 mL/L increased plant height, number of leaves, weight of fresh biomass, weight of biomass per plot, fresh weight for consumption, and as well as root length. The application of PGPR can have both direct and indirect effects. Directly, there is an increase in nutrient absorption in the plant organs, while indirectly the plants become more resistant to pest attacks and attacks from plant-destroying pathogens. In this case, the bacteria from PGPR compete for space in the pathogen's roots, as they produce specific chelators (*called siderophores*) (Grobelak et al., 2015). The use of PGPR concentrations describes the fulfillment of plant growth at treatment concentrations P0: 7.5 mL<sup>-1</sup>; P1: 10.5 mL/L; P2: 13.5 mL/L, which showed an increase in all parameters used both at 2 - 4 Week After Planting (WAP), but at P3: 16.5 mL/L there was a decrease despite the high concentration given (see Table 1).

**Tabel 1.** Results of observations and measurements of various parameters on lettuce plants after the application of PGPR

Concentration (PGPR/L water)	Parameters										
	TT (cm)			Number of Leaves			WFB (g)		WBPP Plot (g)	FWC (g)	RL (cm)
	Week After Planting (WAP)										
	2	3	4	2	3	4	4	4	4	4	
P0 = 7.5 mL	7.60a	9.00a	11.4a	4.0a	5.7a	10.3a	333.3a	400.0a	200.0a	6.3a	
P1 = 10.5 mL	8.00ab	10.2ab	13.3ab	5.7ab	7.7ab	12.0ab	466.7ab	500.0ab	230.0ab	6.9ab	
P2 = 13.5 mL	9.00c	11.0b	14.6b	6.7b	8.3b	14.0b	633.3b	683.3c	300.0b	9.6c	
P3 = 16.5 mL	8.40bc	10.6ab	13.4ab	5.0ab	8.0ab	12.3ab	500.0ab	533.3bc	250.0ab	8.0bc	
<b>BNJ (5%)</b>	0.6	1.9	2.8	1.2	2.5	2.0	192.0	123.2	82.7	2.0	

*Note:* Numbers followed by the same alphabet in the same column indicate no significant difference based on the BNJ 5% test.

PH: Plant Height, NL: Number of Leaves, WFB: Weight of Fresh Biomass, WBPP: Weight of Biomass Per Plot, FWC: Fresh Weight for Consumption, RL: Root Length

The effect of PGPR application on lettuce height showed the highest average results at 2, 3, and 4 MST in the P2 treatment with a concentration of 13.5 mL/L, while the lowest average was obtained in the (P0) treatment with a concentration of 7.5 mL/L. From (Table 1), it is shown that the best treatment among the given concentrations is PGPR P2: 13.5 mL/L with a plant height of 14.6 cm in the fourth week after planting. The macro and micro nutrients needed by the plant will be fulfilled if the concentration of PGPR given is appropriate, which can mobilize nutrient absorption through the roots, thereby supporting the vegetative growth of the plant, including plant height. This is in accordance with the research by Sholihah & Meidianie (2016), which found that the application of PGPR at 100 grams per liter resulted in a higher plant height compared to PGPR at 150 grams per liter in lettuce plants. The presence of Rhizobium found in the roots of the sensitive plant can fix atmospheric nitrogen into ammonia (NH<sub>3</sub>), which is then converted into amino acids and subsequently transformed into nitrogen compounds for plant growth and development. Thus, PGPR functions to enhance the absorption of nitrogen nutrients in plants, which can increase plant height and branching (Wulandari et al., 2019; Jumin, 2017).

The variable number of leaves differed significantly at the concentration of P2: 13.5 mL/L (age 2 WAP and 4 WAP). The highest number of leaves at all observation ages was shown by the PGPR concentration P2: 13.5 mL/L (Table 1). The PGPR concentration P2: 13.5 mL/L is considered capable of meeting the sufficient macro and micro nutrient needs for lettuce plant growth. The availability of nutrients in sufficient quantities promotes the leaf metabolism process for optimal growth (Omaranda et al., 2016). PGPR contains several bacteria such as *Bacillus sp.*, *Pseudomonas sp.*, *Azospirillum sp.*, and *Azotobacter sp.*, which actively colonize the roots. Plants with well-developed roots will efficiently absorb nutrients,

making them less susceptible to pathogens by synthesizing and regulating the concentration of various growth regulators (*phytohormones*) such as *IAA*, *gibberellin*, *cytokinin*, and *ethylene* in the root environment (Anggari, 2017). All these hormones function well in lettuce plants and lead to increased yields. Cytokinin in the leaves can enhance chlorophyll synthesis because cytokinin can increase the formation of one or more proteins that will bind chlorophyll, thereby inhibiting senescence and abscission in the leaves, making them more stable (Lidar & Mutryarni, 2018).

The increase in plant height growth is accompanied by an increase in the number of leaves because the growth rate increases with the age of the plant (Nugroho, 2015). This occurs due to the presence of chlorophyll pigments that facilitate light absorption through the process of photosynthesis. When the process of photosynthesis occurs, it affects the vegetative and generative development processes of the plant and influences the fresh weight of the lettuce shoots. The application of PGPR concentrations significantly affects the fresh weight of lettuce shoots and the fresh weight of consumption, and it has a notable impact on the shoot weight per plot and the root length of lettuce plants after harvest (Table 1). The presence of plant growth-promoting hormones (*cytokinin*) in PGPR affects the fresh weight of lettuce shoots, which plays a role in cell expansion and differentiation, influencing the harvest yield. (Husnihuda et al., 2017). The lettuce cultivation technique that has been carried out describes that the application of PGPR into the soil media has a very significant impact and supports high yields of lettuce plants effectively. This occurs due to the stimulation of PGPR, which triggers an increase in microorganisms. Microorganisms can increase biomass production and plant tolerance to heavy metals and other soil conditions (Baharlouei et al., 2011). Concentration P0: 7.5 mL/L and P1: 10.5 mL/L have less than optimal results, whereas P2: 13.5 mL/L and P3: 16.5 mL/L show high results, especially at concentration P2: 13.5 mL/L (Figure 1).

Usually, the bacteria contained in PGPR are microorganisms that live in colonies around plant roots, thereby stimulating plant growth. This occurs because PGPR acts as a biofertilizer that can improve the physical, chemical, and biological properties of the soil by enhancing the availability of macro and micronutrients within the soil. Especially the quality of the physical properties of the soil, which is considered a key element in soil management for the benefit of land production (Nakajima et al., 2016), and good physical soil quality will also result in high plant productivity (Riskawati et al., 2021). Because the physical properties of soil play an important role in plant growth, i.e., as a medium/container for the presence of nutrients, water, and air or gases needed by plants; as a regulator of water availability for plants, and; as a regulator of the supply of gases needed by plants (Rachman, 2019).



**Figure 1.** The harvest of lettuce plants with concentration of P0: 7.5 mL/L; P1: 10.5 mL/L; P2: 13.5 mL/L; P3: 16.5 mL/L.

The addition of PGPR to the planting medium provides moisture and aeration to the planting environment, thereby affecting the rate of nutrient absorption, which can influence the physiological processes of the plant, such as in the roots.

Plants release compounds to alter the rhizosphere and affect the physical, chemical, or biological properties of the soil, enhancing nutrient absorption through the root system, which is a key factor regulating the microecological functions of the rhizosphere in the soil (Wu et al., 2014). Thus, the efficiency of fertilization with continuous application of organic fertilizers is important to optimize the physical properties of the soil (Rauber et al., 2018), especially to support good moisture and aeration for maximum root growth. Good roots and a favorable soil nutrient status determine the availability of microbes in the rhizosphere. Each plant releases root exudates with different compositions, which also act as microbial selectors; enhancing the development of certain microbes and inhibiting the growth of others. This occurs because root exudates are important carriers of material cycles, energy exchange, and information transfer between the underground parts of plants and the soil (Ma et al., 2022), consisting of water, air, minerals, organic matter, and living organisms. Therefore, the root exudates of plants found on the surface between the roots and the soil will have an initial effect on the physical and chemical properties of the soil through the microorganisms and plants present in the soil (Brevik et al., 2015; Giri et al., 2018). Plant roots are the main source of nutrients for microorganisms living in the rhizosphere. This was stated by the German microbiologist Lorents Hiltner, who noted that the abundance of microbes is much higher in the rhizosphere compared to the soil (Yin et al., 2018). Thus, the use of root applications containing high levels of exudates and microbes, when applied through PGPR, will be beneficial for plants. An adequate and balanced concentration of PGPR positively affects the growth, development, and yield of lettuce plants.

## CONCLUSIONS

The concentration of plant growth promoting rhizobacteria (PGPR) affects all observed variables to plant height, number of leaves, weight of fresh biomass, weight of biomass per plot, fresh weight for consumption, and root length of lettuce. The best concentration for supporting the growth and yield of lettuce is PGPR 13.5 mL/L. The application of PGPR to soil planting media at the correct concentration can increase the yield of lettuce plants. PGPR promotes sustainable agriculture by lowering pollution from synthetic fertilizers and enhancing soil microbial health to increase long-term productivity. Furthermore, PGPR aids plant adaptation to severe climates, promotes the development of novel biofertilizers, and improves global food security through efficient yield improvements.

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