

Application *Azotobacter* and *Pseudomonas fluorescens* bacteria indigenous to improve plant rice production SRI method

Aplikasi bakteri *Azotobacter* dan *Pseudomonas fluorescens* indigenous untuk meningkatkan produksi padi metode SRI

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Abstract. Elita N, Susila E, Agustamar. 2022. Application *Azotobacter* and *Pseudomonas fluorescens* bacteria indigenous to improve plant rice production SRI method. *Pros Sem Nas Masy Biodiv Indon* 8: 148-152. The intensification of rice fields is dominated by nitrogen and phosphorus inorganic fertilizers. N and P have an important role in increasing rice production. The problem of N elements in wetlands is relatively short availability, easily dissolved in water, carried by percolation, surface runoff and easily evaporates with low N fertilizer absorption efficiency ranging from 30-50%. The problem of P fertilizer absorption by plants is only 10-30%. The high P residue causes the land to become critical. An effective and efficient solution is to utilize groups of rhizobacteria. The existence of indigenous rhizobacteria is very diverse in the soil, influenced by biotic and abiotic factors. This type of rhizobacteria is expected to increase the availability of local special nutrients N (*Azotobacter* indigenous), and mine local P elements (*Pseudomonas fluorescens* indigenous). These two types of indigenous rhizobacteria were applied to rice plants using the SRI method. The aim of this study was to determine the *Azotobacter* bacteria and *P. fluorescens* can be combined and determine the dose of *Azotobacter* bacteria and the appropriate dose of *P. fluorescens* bacteria can increase the production of SRI rice plants. The research was carried out in vitro in TSA medium and in a greenhouse. The results showed no inhibitory power between *Azotobacter* bacteria and *P. fluorescens*. Application in the greenhouse showed that at a dose of 20 ml/l *Azotobacter* and a dose of 30 ml/l *P. fluorescens* gave the highest vegetative growth and production in the SRI method. In conclusion, *Azotobacter* bacteria and *P. fluorescens* can be combined in one formulation. The best *Azotobacter* dose of 20 ml.l and *P. fluorescens* bacteria 30 ml/l.

Keywords: *Azotobacter*, indigenous, *Pseudomonas fluorescens*, rhizobacteria, SRI

Abstrak. Elita N, Susila E, Agustamar. 2022. Aplikasi bakteri *Azotobacter* dan *Pseudomonas fluorescens* indigenous untuk meningkatkan produksi padi metode SRI. *Pros Sem Nas Masy Biodiv Indon* 8: 148-152. Sawah intensifikasi didominasi oleh pupuk anorganik terutama nitrogen dan fosfor. N dan P memiliki peran penting dalam meningkatkan produksi padi. Permasalahan unsur N di lahan basah adalah ketersediaannya relatif sedikit, mudah larut dalam air, terbawa perkolasi, limpasan permukaan dan mudah menguap dengan efisiensi penyerapan pupuk N rendah berkisar 30-50%. Masalah pupuk P penyerapan oleh tanaman hanya 10-30%. Residu P yang tinggi menyebabkan lahan menjadi kritis. Solusi yang efektif dan efisien adalah dengan memanfaatkan kelompok rizobakteri. Keberadaan rizobakteri indigenous sangat beragam di dalam tanah, dipengaruhi oleh faktor biotik dan abiotik. Jenis rizobakteri ini diharapkan dapat meningkatkan ketersediaan unsur hara khusus N lokal (*Azotobacter indigenous*), dan menambang unsur P lokal (*Pseudomonas fluorescens indigenous*). Kedua jenis rizobakteri indigenous ini diaplikasikan pada tanaman padi dengan menggunakan metode SRI. Tujuan dari penelitian ini adalah untuk mengetahui bakteri *Azotobacter* dan *P. fluorescens* yang dapat digabungkan dan menentukan dosis bakteri *Azotobacter* dan dosis bakteri *P. fluorescens* yang tepat dapat meningkatkan produksi tanaman padi SRI. Penelitian dilakukan secara in vitro dalam media TSA dan di rumah kaca. Hasil penelitian menunjukkan tidak ada daya hambat antara bakteri *Azotobacter* dan *P. fluorescens*. Aplikasi di rumah kaca menunjukkan bahwa pada dosis 20 ml/l *Azotobacter* dan dosis 30 ml/l *P. fluorescens* memberikan pertumbuhan dan produksi vegetatif tertinggi pada metode SRI. Kesimpulannya, bakteri *Azotobacter* dan *P. fluorescens* dapat digabungkan dalam satu formulasi. Dosis *Azotobacter* terbaik 20 ml.l dan bakteri *P. fluorescens* 30 ml/l.

Kata kunci: *Azotobacter*, indigenous, *Pseudomonas fluorescens*, rhizobacteria, SRI

INTRODUCTION

The intensive rice paddies have been managed with high organic fertilizer, especially N and P elements. The N element problem in wetlands is relatively short availability, easily dissolved in water, carried by percolation, surface

flow and volatile. The efficiency of N (Urea) fertilizer uptake in the tropics by lowland rice crops is relatively low at 30-50% (Prasad and De Data 1979). This low efficiency of urea fertilizer increases the cost of production borne by farmers. The problem is that the availability of P elements is low, 10-30% of P elements can be absorbed by plants, so

the soil structure becomes denser, and the organic matter content of the soil decreases (La Habi 2018). The high P residue causes the land to become critical. Intensive use of chemical fertilizers on agricultural land in the long term will lead to a decrease in soil organic content, damaged soil structure and environmental pollution (Ghimire 2017).

Effective and efficient solutions are needed, namely the biological approach by utilizing the rhizobacteria group to be expected to answer the problems faced. The existence of indigenous rhizobacteria is very diverse in the soil. This is influenced by biotic and abiotic factors in the soil.

Azotobacter bacteria have the advantage that is different from other bacteria and are known to produce varieties of vitamins, amino acids, plant growth hormones, antifungal agents, hydrogen cyanide, and siderophores. *Azotobacter* has growth-promoting substances such as indoleacetic acid, gibberellic acid, arginine, etc., which directly affect shoots and root length and seed germination of some agricultural crops. *Azotobacter* can grow and survive in extreme environmental conditions, namely, tolerant to higher salt concentrations, pH values, and even in dry soils with maximum temperatures. Different factors affect the *Azotobacter* population in the soil, such as pH, phosphorus content, soil aeration and moisture content, etc. (Gurikar et al. 2016).

The results of the research Elita et al. (2012, 2014) have found indigenous *Pseudomonas fluorescens*. and indigenous *Azotobacter* on SRI Method rice cultivation. The characteristics and nature of *P. fluorescens* and indigenous *Azotobacter* are more effective, adaptive and efficient in their development and growth because they are empowered in their natural ecosystems.

The results of the study (Sharma et al. 2017) that *Bacillus megaterium* bacteria, *P. fluorescens* and *Azotobacter chroococcum* were formulated on bioinoculants given to wheat plants able to increase wheat yields 1.5-1.7 times compared to controls. Furthermore, the results of the research (Rai et al. 2017) of biological fertilizers containing *A. chroococcum*, *Azospirillum brasilense*, and *Pseudomonas putida* increased the growth of *Rauwolfia serpentina* parameters such as root length, shoot length, number of leaves, number of roots and dry weight and fresh from roots and shoots. According to (Rai et al. 2018) the application of *Azotobacter* bacteria and *P. fluorescens* can increase the germination of wheat seeds.

The results of the study (Kumar et al. 2018), showed that a combination of three rhizobacteria, including *Pseudomonas* sp. bacteria could significantly increase germination, roots and shoot length and fresh and dry weight of wheat plants compared to single inoculation of non-inoculated rhizobacteria and controls. These three rhizobacteria can be used for bioinoculant production, which is effective in replacing chemical fertilizers and harmful pesticides for environmentally friendly and sustainable wheat production and increasing yields and regaining the natural agro-ecosystem environment.

The results of the study (Kumar et al. 2018) that sources of organic nutrients such as vermicompost increase micronutrients (Fe, Mn, and Zn) in the soil which results in improved quality of rice seeds. The soil N content is higher than control.

The purpose of this study was to determine the *Azotobacter* bacteria and *P. fluorescens* can be combined in one formulation and to know the exact dose of *Azotobacter* bacteria with the appropriate dose of *P. fluorescens* bacteria can increase the production of SRI rice plants.

MATERIALS AND METHODS

Time and place

The study was conducted from Mei to November 2021. Place of Microbiology Laboratory and Greenhouse Payakumbuh State Agricultural Polytechnic

Materials and tools

The material used was indigenous *Azotobacter* bacteria as a result of the 1st year study and *Pseudomonas* (previous research results). The tools used are petri dish, test tubes, spectrophotometers, correcting tubes, along with the micropipette tip, Beaker glass, Erlenmeyer, loopful, test tube rack, stirring rod, plastic baskets, seed germination beds, pots.

Competition test for *Azotobacter* bacteria isolates and indigenous RPPT and indigenous *Pseudomonas fluorescens* in vitro

Work procedures

Evaluation of competition power between *Azotobacter* bacterial isolates and indigenous (selected) RPPT and indigenous *P. fluorescens* in vitro was carried out in TSA medium to predict the possibility of using indigenous N-fixing bacteria isolates and phosphate solvents in combination. The first isolates of *Azotobacter* and indigenous RPPT bacteria were etched evenly on the TSA medium. Filter pieces of paper with a diameter of 1 cm were immersed in the second bacterial suspension and placed on the medium inoculated with bacterial isolates I. The culture was incubated in a room with a temperature of 27°C for four days. The inhibitory power of the second phosphate to bacteria I is shown by the presence of a transparent area (halo) around the piece of paper overgrown with a second paper (Schaad et al. 2001).

Test of *Azotobacter* and *Pseudomonas fluorescens* indigenous bacteria with SRI method

Work procedures

The experiment was conducted using a completely randomized factorial pattern design with factor I dose of *Azotobacter* bacteria (0, 10, 20, 30 ml/l) and factor II dosage of *P. fluorescens* (0 and 20 ml/l) treatment. Each treatment combination was repeated three times, so there were 24 experimental units.

Planting media in the form of soil from the intensified rice fields planted with SRI method were dried aerated and sieved with a 10 mesh sieve and sterilized with the aim of turning off all organisms contained in soil samples, so that only the inoculated microbes developed and the response was correct true due to the isolates given. Sterilization is carried out inside.

Rice seeds soaked in disinfectant in the form of 2% sodium hypochlorite for 5 minutes, then rinsed with sterile water three times and dried in laminar water flow for 1 hour. 1 gram of seed was soaked for 24 hours in *Azotobacter* indigenous suspension at 26°C. After treatment, the seeds are dried again in laminar airflow. The nursery is carried out in the nursery of rice over the seed bed. Seedlings aged 12 days are transferred to the pot. Observations made were: (1) Plant Height (cm), (2) Number of panicles per clump (panicle), (3) Number of grains per panicle (grain), (4) Weight of 1000 seeds (g), (5) Production of grain dry per pot (grams). To examine the effect of treatment on responses observed, analysis of variance was performed using the Statistical Analysis System (SAS) program. Furthermore, the Duncan Multiple Test (DNMRT) multiple region test was performed to see differences in treatment at the 5% level.

RESULTS AND DISCUSSION

Competition test for *Azotobacter* bacteria isolates and indigenous RPPT and indigenous *Pseudomonas fluorescens* in vitro

The results of the competition power test between the isolates of *Azotobacter* bacteria and indigenous RPPT and indigenous *P. fluorescens* by invitro in TSA medium were obtained by indigenous N fixing bacteria isolates and phosphate solvents can be combined. There is no inhibitory power of indigenous *P. fluorescens* bacteria against *Azotobacter* bacteria. Both cover each other as shown in Figure 1.

In Figure 1 it can be seen that there is no separation from the proliferation of the two bacteria, both of which are joined together. This shows that *Azotobacter* bacteria and *P. fluorescens* can be combined in the same formula. The results of the study (Rai et al. 2017) that the *A. chroococcum*, *A. brasilense* and *P. putida* bacteria with cow dung media as biological fertilizers application in *R. serpentina* plants can increase root length, shoot length, number of leaves,

the roots and weight are dry and fresh from the roots and shoots. This biological fertilizer increases the availability of nitrates, nitrites and phosphates in the roots and leaves of the *R. serpentina* plant. This formulation also increases the alkaloid content in the roots of this plant. Soil microbial populations also increased, showing increased soil fertility with this application of biological fertilizers. The results show that replacing chemical fertilizers with microbial biofertilizers is possible with even higher doses.

Test of *Azotobacter* and *Pseudomonas fluorescens* indigenous bacteria with SRI Method

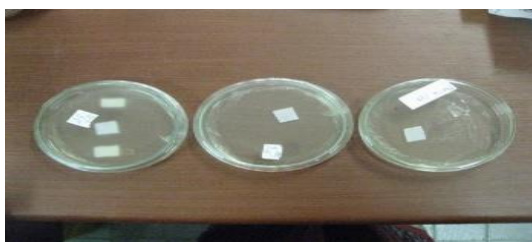
Vegetative observations from the treatment of *Azotobacter* and *P. fluorescens* doses of Junjuang variety rice plants using SRI method after statistically analyzed there was no interaction between *Azotobacter* and *P. fluorescens* doses. The results of observations of vegetative growth are presented in Table 1.

The numbers in the columns followed by the same lower case are not significant at the 5% real level according to the DNMRT test.

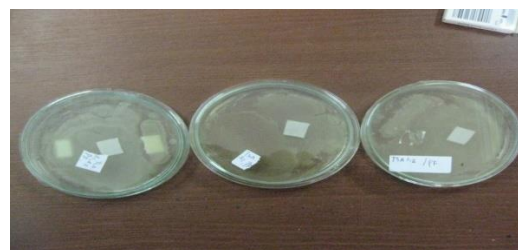
In Table 1 *Azotobacter* at a dose of 20 ml/l gives a higher plant height and number of tillers. The population of *Azotobacter* bacteria at a dose of 20 ml/l gave a response to providing N elements so as to increase the vegetative growth of the SRI rice plant method. Research on *A. chroococcum* spp. for crop production manifests significantly to plant nutrition and soil fertility. *Azotobacteria* synthesize auxins, cytokines, and GA-like substances, and these growth ingredients are the main substances that control growth. These hormonal substances, which originate from the rhizosphere or root surface, affect plant growth (Wani et al. 2013).

Table 1. Vegetative growth observation from of *Azotobacter* and *P. flourescens* bacteria on Junjuang variety plant of SRI method

<i>Azotobacter</i> dose	Plant height (cm)	Number of tillers (tillers)
A0 (0 ml/l)	127,5 d	21,5 d
A1 (10 ml/l)	134,1 c	25,0 c
A2 (20 ml/l)	144,3 a	31,7 a
A3 (30 ml)	137,3 b	28,0 b
<i>P. fluorescens</i> dose		
P0 (0 ml/l)	133,67 B	25,75 B
P1 (30 ml/l)	137,92 A	27,33 A



Initial conditions of competition power test



After 2 days observed

Table 2. Generative growth observation of *Azotobacter* and *P. fluorescens* bacteria to Junjuang variety plant of SRI method

<i>Azotobacter</i> dose	Number of panicles	Number of grains/panicles	The number of pithy / panicle grains	Weight of 1000 seeds	Production per hectare
A0 (0 ml/l)	17,0 d	183,4 d	157,1 d	19,8 d	5,9 d
A1 (10 ml/l)	19,3 c	206,4 c	169,9 c	20,6 c	7,7 c
A2 (20 ml/l)	23,7 a	225,7 a	200,8 a	22,6 a	12,3 a
A3 (30 ml)	20,7 b	210,6 b	179,5 b	21,1 b	8,8 b
<i>P. fluorescens</i> dose					
P0 (0 ml/l)	19,67 B	194,18 B	171,47 B	20,5 B	8,2 B
P1 (30 ml/l)	20,67 A	218,86 A	182,19 A	21,6 A	9,2 A

The numbers in the columns followed by the same lower case are not significant at the 5% real level according to the DNMR test

The application of *P. fluorescens* at a dose of 30 ml/l gave the highest yield for plant height and number of tillers. In line with the results of research Elita et al. (2012) that the application of phosphate-solubilizing microorganisms to rice plants using the SRI method was able to significantly increase vegetative growth compared to controls. Miftahurrohmat and Sutarman (2020), *P. fluorescens*. Bacteria serves to maintain plant health and promote vegetative plant growth by producing enzymes that work in the mineralization process of P-organic to P-organic available to plants.

Generative growth observation of the treatment of *Azotobacter* and *P. fluorescens* doses of Junjuang variety rice plants using SRI method after statistically analyzed, there was no interaction between *Azotobacter* and *P. fluorescens* doses. The results of generative growth observations are presented in Table 2.

In Table 2, the dose of *Azotobacter* bacteria 20 ml/l gave high yields on panicle number, number of grains/panicle, number of rice grains/panicle weight of 1000 seeds and production per hectare reached 12.3 tons/ha higher than other treatments.

Azotobacter can be used as the main inoculum used in biofertilizers to restore nitrogen levels in cultivated land. Nitrogen mineralization in the soil is low due to the immobilization of nitrogen from the soil, negatively affecting the amount of N available to plants (Tang et al. 2020). El-Komy et al. (2020) *Azotobacter* sp. plays a role in the growth and development of host plants by releasing phytohormones and increasing the availability and absorption of nutrients through nitrogen fixation and phosphorus dissolution.

Azotobacter can be used as the main inoculum used in biological fertilizers to restore nitrogen levels in cultivated land. *Azotobacter* is well characterized due to the production of exopolysaccharide (EPS) which has a huge effect on plant growth (Gauri et al. 2012).

The growth rate of *Azotobacter* bacteria has to do with phosphate dissolution activities in various environmental conditions. The close relationship between the ability of phosphate dissolution and the growth rate of *Azotobacter* bacteria is an indicator of active metabolism. *Azotobacter* has the potential for biofertilizers which can result in

chemical nitrogen reduction and phosphate fertilizer which increases crop production. (Nosrati et al. 2014).

Rhizosphere microorganisms such as *Piriformospora indica* and *A. chroococcum* have beneficial interactions because they can increase plant growth and increase the protein content of *P. indica* (Kumar et al. 2015).

The application of *P. fluorescens* can also improve generative growth parameters, namely panicle number, number of grains/panicle, number of grained / panicle grains, 1000 seeds weight and production per hectare only reach 9.2 tons/ha. The balance of N and P elements in the soil can increase the N and P content in grain and straw (Yang et al. 2020).

The application of *P. fluorescens* bacteria can increase the P content of the soil, thereby increasing the activity of the nitrogenase enzyme, which leads to higher N₂ fixation and leads to better root nodule development (Hao et al. 2019). The activity of *P. flourescens* bacteria will increase the available P content of the soil, soil CO₂ production, dehydrogenase enzymes, and decreased Al-dd content in the land (Marlina and Gusmiatun 2020).

The ability of endophytic bacteria (*P. fluorescens*) to produce acid gluconic acid (GA), which can dissolve insoluble phosphate, and stimulate plant growth *Pisum sativum* L. The results showed that many endophytic strains produced GA. and had a moderate to high phosphate solubilization capacity, when inoculated to medium to high-level GA crops that show the stimulating effects of plant growth so as to trigger faster growth (Oteino et al. 2015).

In conclusion, *Azotobacter* bacteria and *Pseudomonas fluorescens* can be combined in the same formula. Vegetative growth and generation of rice plants cultivated by the SRI method increased by *Azotobacter* bacteria and *P. fluorescens* at doses of 20 ml/l and 30 ml/l.

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