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

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# 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 1e 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 element 20 seudomonas 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 degramment and P. fluorescens 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 greenhous 13 he results showed no inhibitory 13 er between Azotobacter bacteria and P. fluorescens. Application in the SRI method. In conclusion, Azotobacter bacteria and 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, Suz 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 memanfatkan kelompok rizobakteri. Keberadaan rizobakteri indigenus sangat beragam di dalam tanah, dipengaruhi oleh faktor biotik dan abiotik. Jenis rhizobakteri indinarapkan dapat meningkatkan ketersediaan unsur hara khusus N lokal (Azotobacter indigenous), dan menambang unsur P lokal (Pseudomond 30 urescens indigenous). Kedua jenis rizobakteri indigenus 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 antasa 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 ment 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 183 P residue causes the land to become critical. Intensive use of chemical fellizers 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 face (14) he 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, gibb 25 lic acid, arginine, etc., which directly affect shoots and roo 2 ength 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, p1 sphorus 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 em 7 wered in their natural ecosystems.

The results of the study (Sharma et al. 2017) that Bacillus megaterium bacteria, P. fluorescens and Azotobacter chroococcum formulated were bioinoculants given to wheat plants able to increase wheat yields 1.5-1.7 times compared to controls. Furthermore, the results of the research (Rai4t al. 2017) of biological fertilizers containing A. chroococcum, Azospirillum brasilense, and Pseudomonas putida increased the growth of Rauwolfia serpentina parameters such as dot length, shoot length, number of leaves, number of roots and dry 32 ght and fresh from roots and shoots. According to (Rai et al. 2018) the application of Azotobac 7 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. 8 cteria 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 35 noculant 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 nu 15 nts 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 that 21 ontrol.

The purpose of this study was to determine the *Azotobacter* bacteria and *P. fluorecens* 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.

### 10 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

Wor31 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. fluoresce 28 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. mservations made were: (1) Plant Height (cm), (2) 11 mber 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 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,



Initial conditions of competition power test

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. serpentind* alant. 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 Pseodomonas fluorescens indigenous bacteria with SRI Method

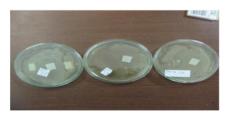
Vegetative observations from the treatment of *Azotobacter* and *P. fluorescens* doses of Junjuang variety rice plants using SRI method after statistically analyzed there was no interaction between *Azotobacter* and *P. fluorescens* doses. The results of observations of vegetative growth are presented 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 6 plant nutrition and soil fertility. Azotobacteria synthesize auxins, cytokines, and GA-like substances, and these growth ingredients are the main bstances 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

	1	
Azotobacter 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
P. fluorescens dose		
P0 (0 ml/l)	133,67 B	25,75 B
P1 (30 ml/l)	137,92 A	27,33 A



After 2 days observed

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

Azotobacter 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
P. fluorescens 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 DNMRT 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 appl 3 tion 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* bact 2 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. 2021). 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 producten 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 interaction 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 10 erative growth parameters, namely panicle number, number of grains/panicle, number of grained / panicle grains, 1000 seeds weight and 16 duction per hectare only reach 9.2 tons/ha. The balance of N and P elements in the soil callincrease the N and P content in grain and straw (Yang et al. 2020).

The application of *P. fluorescens* bacteri 29 an increase the P content of the soil, thereby increase 19 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 5nd (Marlina and Gusmiatun 2020).

The ability of endophytic bacteria (*P. fluorescens*) to produce acid gluconic acid (GA), which can dissolve insoluble ph phate, and stimulate plant growth *Pisum sativum* L. The results showed that many endophytic strains produced GA. and had a moderate to high phosphate 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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