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# ABUNDANCE OF N2-FIXING RHIZOBACTERIA OF DIFFERENT DRYLAND AGRICULTURE ECOSYSTEMS IN DRY CLIMATE ZONE OF LOMBOK-INDONESIA

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## ABSTRACT

Soil microbial communities are crucial in ecosystem diversity and are directly related to soil fertility. Lombok is an island in central Indonesia that has low soil fertility and a limited amount of available water. Beneficial microorganisms can be used as a low-cost and environmental-friendly tool to increase productivity in dryland agriculture systems. Screening to obtain superior rhizosphere bacteria is one of the options to support the nutrient supply in arid soils. Composites soil samples were taken from five ecosystems in Lombok, West Nusa Tenggara, an arid region in the eastern part of Indonesia to obtain the isolates of nitrogen-fixer rhizobacteria (NFR). Nine Azotobacter and Azopirillium spp were isolated from, rainfed, maize, mixed crop, natural forests, and savanna ecosystems. Abundance of total bacteria and N2-fixers in all ecosystems was relatively high (more than 108 cfu g-1), and the highest total population was recorded in the natural forest. The abundance of N2-fixer rhizobacteria recorded the highest Azotobacter population at 2.64 x 108 cfu g-1 in the maize ecosystem and the highest Azospirillum population at 2.32 x 108 cfu g-1 in the natural forest ecosystem. Additionally, the highest contain of organic C and total nitrogen were obtained in natural forest and savanna ecosystem. Eighteen isolates were obtained and characterized microscopic and macroscopically, consisted of nine Azotobacter sp and nine Azospirillium isolates which are potentially to be used as biogent for improving the growth of upland rice on dry climate zone.

**Keywords:** Beneficial Microbes; Isolates; Dryland; N-fixing Rhizobacteria, Upland Rice.

## 1. INTRODUCTION

Lombok Island, located in West Nusa Tenggara Province, is one of the seven unique ecosystems in Indonesia based on its biogeographic attributes. There are various types of ecosystems in Lombok with the dominant ecosystem being natural forests, mangrove forests, mixed fields, rice fields, and mining. Unique flora and fauna communities with high biodiversity, including soil microbes, can be found in those ecosystems [1]. Ecosystem function depends largely on the functional diversity and activity of the underground microbial system. Soil microbial functional diversity is extremely sensitive to changes in the soil microenvironment and so can reflect changes in soil quality [2]. Natural forests are generally high in species diversity, both flora, and fauna. Half of the countries with tropical climates have dry season climates which are home to dry forests and savannas [3].

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The analysis of microbial networks is now a privileged tool to assess microbial interactions in ecosystems [4]. The soil microbial community is largely responsible for ecosystem diversity which directly implicates soil fertility [5]. Lombok Island has low soil fertility and a limited amount of available water [6]. It is because one of the characteristics of the climate in Lombok is the low rainfall and the short number of rainy days, making Lombok vulnerable to drought. The average rainfall in West Nusa Tenggara is in the range of <2000 mm/y over the last 160 days [7]. Other problems are also often faced are N nutrient and P content is low and is not available for plants [8]. Management of dryland agriculture is not easy to do due to limiting factors in the cultivation process. The majority experienced degradation due to the low content of organic matter, high erosion, and inappropriate land management [9].

Beneficial microorganisms can be applied as a low-cost and environmental-friendly strategy to increase productivity is relevant in dryland agriculture. They interact with each other to form a balance and contribute to nutrient cycles, such as carbon and nitrogen, detoxification, and reclamation of the soil, as well as microclimate control which is important for environmental health [10]. The beneficial interactions of these microbes with the plants include the nutrients supply to crops, plant growth stimulation, producing phytohormones, biocontrol of phytopathogens, improving soil structure, bioaccumulation of inorganic compounds, and bioremediation of metal-contaminated soils [11]. Microorganisms also have high metabolic potential and can adapt quickly to fluctuating environmental conditions. Thus, selecting and enriching beneficial microorganisms around plants will help increase plant productivity, and have been proposed as agriculturally beneficial microorganisms.

Rhizospheric bacteria that benefit plants through several indirect and direct mechanisms are broadly called plant growth-promoting rhizobacteria (PGPR) [12]. In particular, plant growth promoting rhizobacteria (PGPR) are microorganisms, which form symbiotic interactions with plant roots, promoting plant health and productivity through different mechanisms such as the production of plant hormones (auxins, cytokinin, and gibberellins); inhibition of plant senescence; $N_2$-fixation; phosphate solubilization and mineralization of other nutrients; and siderophores production [13]. Rhizosphere microbial exploration can be developed to support environmentally friendly agriculture [14]. Therefore it is necessary to research to obtain the N2-fixer isolates that could be used as bioagents or biofertilizers to improve the fertilizer efficiency and crop productivity of the dryland ecosystem.

## 2.MATERIALS AND METHODS

### 2.1Materials

This study was conducted in Lombok Island, West Nusa Tenggara, Indonesia which consisted of four selected ecosystems: Rainfed Ecosystem (Pujut, Central Lombok), Maize ecosystem (Pringgabaya, East Lombok), Mixed Crop Ecosystem (Sambelia, East Lombok), Natural Forest Ecosystem (Mount Rinjani National Park, Tetebatu, East Lombok), and Savanna Ecosystem (Sembalun, East Lombok) (Figure 1 and Table 1).

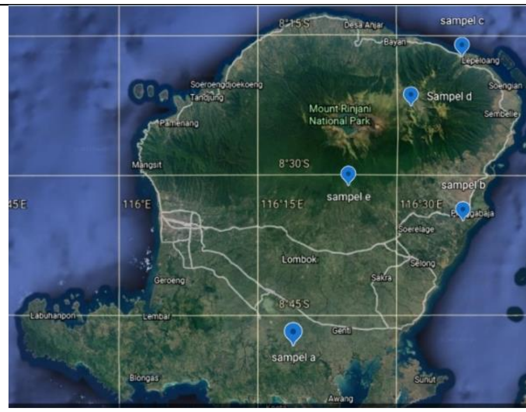



**Figure 1:** Sampling Location (Blue Pinpoint) in 5 different ecosystems in Lombok, West Nusa Tenggara

**Table 1: Coordinate Point of NTB Dryland Ecosystem Sampling Location**

| Ecosystem | Date | Location: Area | Location: Coordinate Point | Location: Elevation (meter above the sea level) | Vegetation |
|---|---|---|---|---|---|
| Rainfed | 22/06/2021 | Pujut, Central Lombok | S 80 48’ 29,82” ; E 1160 18’ 52,81” | 100 | Upland Rice |
| Maize | 23/06/2021 | Pringgabaya, East Lombok | S 80 35’ 6,91” ; E 1160 37’ 14,45” | 34 | Maize |
| Mixed crop | 15/05/2021 | Sambelia, East Lombok | S 80 17’ 31,98” ; E 1160 37’ 7,02” | 37,85 | Cashew |
| Natural Forest | 16/05/2021 | TNGR, Tetebatu, East Lombok | S 80 22’ 46,4” ; E 1160 31’ 28,4” | 1461 | Trees |
| Savanna | 23/06/2021 | Sembalun, East Lombok | S 80 31’ 20,83” ; E 1160 24’ 46,8” | 775 | Grass |

### 2.1.1 Sampling Location Description

Location 1 was a rainfed ecosystem in Pujut Village, Central Lombok. Geologically it includes the Malit land plain formed from Old Volcanic rocks with almost no irrigation in the form of rivers. Agricultural activities were very limited, only once a year, during rainy season, with short-lived seasonal crops with mixed cropping systems or intercropping of several commodities such as upland rice, maize, cassava, sweet potato, peanuts, and green beans. Location 2 was a maize ecosystem in Pringgabaya Village, East Lombok. The type of soil in this location was Inceptisol with high potassium and phosphorus content. Meanwhile the N content, was very low. Maize was the main commodity of this region, with a rice-maize-maize cropping pattern. Some farmers grow chilies and vegetables in between rice and maize cultivation.

Location 3 was a mixed crop ecosystem in Sambelia, East Lombok. This location was known as a cashew production area and has relatively similar characteristics as other districts such as lowland area, marginal soils with dry climate, low rainfall, and low number of rainy days. Cashew can grow very well here because of its extensive root system that can reach deep groundwater supplies [15].

Location 4 was a natural forest ecosystem in Tetebatu Village, East Lombok, precisely in the Mount Rinjani National Park (Taman Nasional Gunung Rinjani, abbreviated as TNGR) area. This area was a part of tropical rain forest that consists of various types of ecosystems ranging from lowland tropical forests, and mountainous tropical forests to sub-alpines. The TNGR area is very rich in flora and fauna biodiversity as a source of germplasm [16].

Location 5 was a savanna ecosystem in Sembalun Village, East Lombok. This location included in the sub-alpine zone which was dominated by the type of grass *Themeda* sp. which forms a mountainous savanna and is occasionally overgrown with shrubs such as *Vaccinium* sp. (mountain tea) and *Anaphalis Viscida* (Candar Nyawa) which are a type of Edelweiss known as the perennial flower of the Asteraceae family [17]. In the sub-alpine zone, the substrate was rocky and a little soil that was poor in nutrients, therefore generally in this zone trees rarely grow and become several pioneer species that are scattered or only found in sheltered places, due to low-temperature conditions and often poor soils, the growth process is very slow [18].

### 2.2 Methodology

Soil sampling was carried out from May to Juni 2021. Soils were sampled from May to June 2021. Random sampling points were chosen and rhizosphere soil with a depth of 0-20 cm were taken with root intact. Samples were placed in plastic zip bag and stored inside a cooler box prior to laboratory analysis. Soil microbial analyses were conducted at Soil Biotechnology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. Identification of microbial community is carried out microscopically and macroscopically.

#### 2.2.1 Isolation of Azotobacter and Azospirillum

Azotobacter and Azospirillum were isolated from soil samples in 250 ml liquid Ashby's and Malat medium. The medium was incubated in 120 rpm rotary shaker for 72 hours under ambient temperature. After 72 hours, isolates from Malat media were transferred into liquid Okon media (selective media for Azospirillium isolation) aseptically and reincubated under the same condition. This process was repeated five times in order to obtain pure culture. Meanwhile, after three days the Azotobacter colonies in the form of pellicle on the surface of Ashby's media were transferred aseptically into solid Ashby's media by streak method. Streaked solid medium were incubated in 28°C for 72 hours [19].

#### 2.2.2 Identification of Bacterial Morphology Macroscopically and Microscopically

Observation of colony morphology of non-symbiotic $N_2$-fixing bacteria by Ashby's Agar media for Azotobacter sp and Okon media for Azospirillum bacteria was seen from colony color, colony edge, colony shape, colony surface, colony elevation, gram test and cell shape.

The morphological observations of isolates weremicroscopically, includingd the shape of the bacteria under a microscope with 1000 times magnification and the types of bacteria using the Gram staining method. According to the gram staining method, bacteria are divided into 2 groups, namely Gram-Positive Bacteria and Gram-Negative Bacteria. The staining index is that gram-positive bacteria will be violet and gram-negative bacteria will be red [20].

## 3.RESULT AND DISCUSSION

### 3.1 Soil Chemical Properties

The results of the analysis of the chemical properties of the soil and the total population of bacteria from 5 ecosystems are shown in Table 2.

**Table 2: The chemical soil properties of studied area**

| Ecosystem | Sample Code | Parameter | | | | |
|---|---|---|---|---|---|---|
| | | C-Organic (%) | N-Total (%) | C/N | pH | CEC cmol $kg^{-1}$ |
| Rainfed | A | 0,63 | 0,07 | 9 | 7,85 | 37,96 |
| Maize | B | 0,88 | 0,22 | 4 | 7,54 | 17,35 |
| Mixed Crop | C | 0,68 | 0,07 | 9 | 6,93 | 19,54 |
| Natural Forests | D | 2,18 | 0,37 | 6 | 6,18 | 23,33 |
| Savanna | E | 2,18 | 0,37 | 6 | 6,16 | 35,8 |

The soil pH at the sampling location showed a tendency for the pH to be slightly acidic to neutral and ranged between 6.16-7. 85. The growth and development of bacteria are strongly influenced by soil pH. *Azotobacter* sp. can grow optimally at a suitable soil pH of 4.5 – 8.5 [21]. *Azospirillum* sp. can live optimally in an environment with a pH of 6.8 – 7.9 [22].

The results of the organic carbon analysis showed varying values. Ecosystem location natural forests and savanna have the highest organic carbon value (2.18%) were in the medium category [23]. These two locations were different from other locations that have a low organic carbon value, which is <1%.

The total nitrogen content in 5 locations has a variation of values that are not much different. The location of rainfed and mixed crop ecosystems has the lowest total nitrogen value (0.07%), while the location of natural forest and savanna ecosystems has the highest total nitrogen value (0.37%).

The organic carbon and total nitrogen analysis displayed that the C/N ratio of the soil at the sampling location was in the low-medium category with a C/N ratio ranging from 4-9. The location of the rainfed ecosystem has the lowest soil C/N ratio with a value of 4. Meanwhile, the location of the rainfed and mixed crop ecosystems has the highest soil C/N ratio with a value of 9. These results indicated that the level of organic matter decomposition in several research locations varied and have an unbalanced mineralization process. The C/N ratio of 15–30 indicates a balanced mineralization process with immobilization [24]. All soil samples showed moderate to high value of cation exchange capacity (Table 2).

The highest CEC value was obtained from the rainfed ecosystem location with a value of 37.96 cmol $kg^{-1}$ and the lowest was from the maize ecosystem location with a value of 17.35 cmol $kg^{-1}$. This result may relate to the pH value of all soil samples, ranged from slightly acidic to neutral contained alkaline cations leachate such as calcium, magnesium, potassium, and sodium. The moderate to high value of CEC can also be attributed to several factors besides pH, such as clay content, base saturation, organic matter content, and mineral type [25].

### 3.2 Population of Total Bacteria and Nitrogen Fixers

The results of the analysis of the population of nitrogen-fixing bacteria (Azotobacter and Azospirillum) from 5 ecosystems are shown in Table 3**.**

**Table 3: The abundance of total bacteria and Nitrogen-fixing rhizobacteria (Azotobacter and Azospirillum) of different dryland ecosystem**

| Ecosystem | Sample Code | Bacteria Population ($10^8$ cfu $g^{-1}$) | NFB Population ($10^8$ cfu $g^{-1}$) | |
|---|---|---|---|---|
| | | | Azotobacter | Azospirillum |
| Rainfed | A | 1,91 ± 0,01 a | 1,92 ± 0,01 a | 1,75 ± 0,01 c |
| Maize | B | 2,62 ± 0,02 d | 2,63 ± 0,01 c | 1,94 ± 0,01 d |
| Mixed Crop | C | 2,33 ± 0,01 c | 2,61 ± 0,02 c | 1,09 ± 0,02 a |
| Natural Forests | D | 1,94 ± 0,02 b | 1,94 ± 0,01 a | 2,32 ± 0,01 e |
| Savanna | E | 2,32 ± 0,01 c | 2,33 ± 0,01 b | 1,46 ± 0,01 b |

The results of the analysis of the total bacterial population varied at each sampling location. The range of bacterial populations ranged between 1.92 x $10^8$ to 2.64 x $10^8$ cfu $g^{-1}$. The highest bacterial population in the field ecosystem and the lowest in the rice field ecosystem (Table 3).

This study focused on the isolation and characterization of two genus of nitrogen fixers, *Azotobacter* spp. And Azospirillum spp. The range of $N_2$-fixers rhizobacteria (NFR) found was 1.10 x $10^8$ to 2.64 x $10^8$ cfu $g^{-1}$ of soil. This difference in population number may has been caused by the different vegetation that grows on it and the suitability of the initial bacterial ecosystem with the medium.

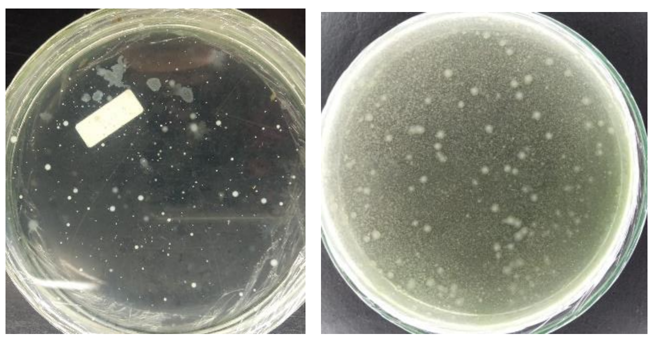
### 3.3 Characteristics of Isolated N-Fixers

Identification and characterization of nitrogen-fixing bacteria isolates (Azotobacter and Azospirillum) are shown in Table 4.

**Table 4: Morphological Characteristics of Nitrogen-fixing Bacterial Isolates (Azotobacter sp and Azospirillum sp)**

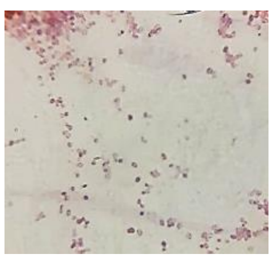
| Isolate Code | Macroscopic Characteristics | | | | Microscopic Characteristics | |
|---|---|---|---|---|---|---|
| | Colonies Shape | Colony color | Margin | Elevation | Gram test | cell form |
| **Ashby's Media** | | | | | | |
| Azt-a1 | Circular | Milky-white | Entire | Convex | - | coccus |
| Azt-a3 | Circular | Milky-white | Entire | Convex | - | coccus |
| Azt-b1 | Circular | Clear-white | Undulate | Umbonate | - | coccus |
| Azt-b2 | Circular | Clear-white | Undulate | Convex | - | coccus |
| Azt-b3 | Circular | Milky-white | Undulate | Convex | - | coccus |
| Azt-c1 | Circular | Milky-white | Entire | Umbonate | - | coccus |
| Azt-c2 | Circular | Clear-white | Entire | Convex | - | coccus |
| Azt-c3 | Circular | Milky-white | Undulate | Umbonate | - | coccus |
| Azt-d1 | Circular | Milky-white | Entire | Convex | - | coccus |
| **Okon Media** | | | | | | |
| Azs-a2 | Circular | White | Entire | Flat | - | basil |
| Azs-b2 | Circular | White | Entire | Flat | - | basil |
| Azs-b3 | Circular | White | Entire | Flat | - | basil |
| Azs-c1 | Circular | White | Undulate | Convex | - | basil |

| | | | | | | |
|---|---|---|---|---|---|---|
| Azs-d1 | Circular | White | Entire | Convex | - | spiral |
| Azs-d2 | Circular | White | Entire | Flat | - | spiral |
| Azs-e1 | Circular | White | Entire | Flat | - | basil |
| Azs-e2 | Circular | White | Undulate | Flat | - | basil |
| Azs-e3 | Circular | White | Undulate | Flat | - | spiral |

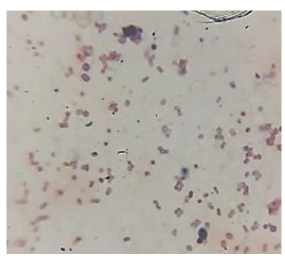


**(1)** **(2)**

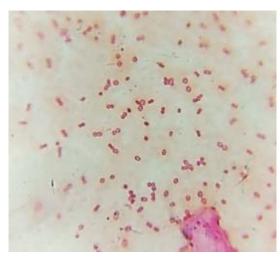
**Figure 2:** Macroscopic observation of bacterial characteristics **(1)** Azospirillum **(2)** Azotobacter



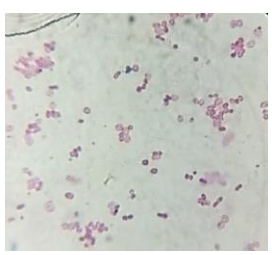
Azt-a1



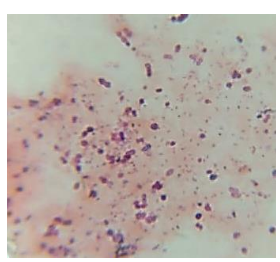
Azt-a3



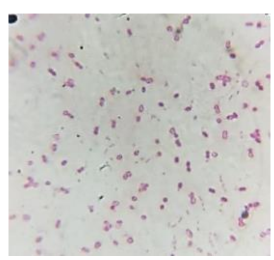
Azt-b1



Azt-b2



Azt-b3



Azt-c1

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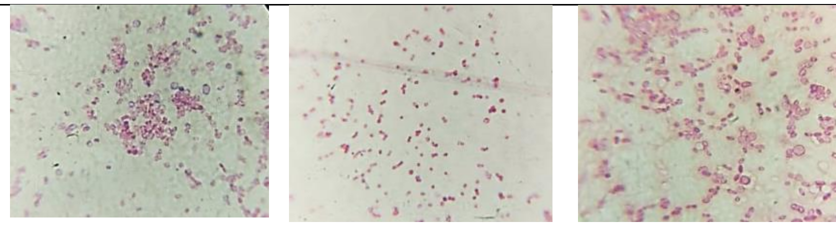
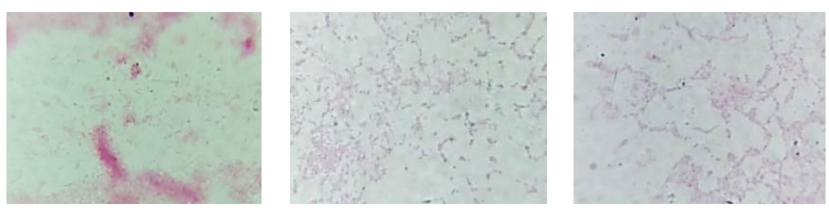


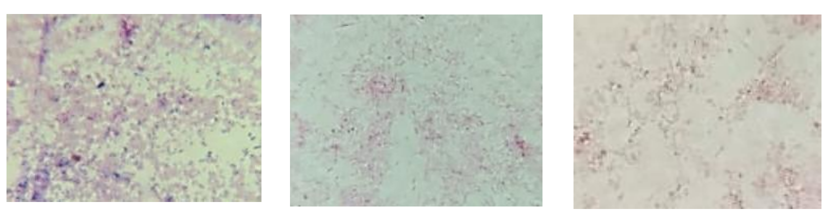
Azt-c2 Azt-c3 Azt-d1

**Figure 3:** Macroscopic observation of Azotobacter characteristics through gram staining showed coccus-form cells and gram-negative test

Azs-a2 Azs-b2 Azs-b3



Azs-c1 Azs-d1 Azs-d2



Azs-e1 Azs-e2 Azs-e3

**Figure 4:** Macroscopic observation of Azospirillum characteristics through gram staining showed bacilli and spiral form cells and gram-negative test

Figure 2,3,4 showed microscopic and macroscopic characters of Azotobacter as shown in Bergey's Manual of Determinative Bacteriology [26]. Genus Azotobacter colonies have entire to undulate margin, convex, has coccus or diplococcus shape and formation, and gram-negative in nature. Despite being well known as coccus bacteria, some Azotobacter strain are pleomorphic or varied in cell shapes [27]. The microscopic character of Azospirillum isolate was in the form of bacil and spiral cells. All isolates were Gram-negatives which are characteristics of the genus Azospirillum [28].

Nine Azotobacter isolates coded Azt-a1, Azt-a3, Azt-b1, Azt-b2, Azt-b3, Azt-c1, Azt-c2, Azt-c3, and Azt-d1 were obtained. Nine isolates were also obtained from Okon medium isolation, which were coded as Azs-a2, Azs-b2, Azs-b3, Azs-c1, Azs-d1, Azs-d2, Azs-e1, Azs-e2, and Azs-e3. Macroscopic characteristics of Azospirillium are in the form of circular colonies, white colony color, convex and flat elevations, entire and undulate margin.

## 4.CONCLUSION

The abundance of total bacteria and $N_2$-fixers in all ecosystems was relatively high (more than $10^8$ cfu $g^{-1}$), and the highest total population was recorded in the natural forest. The abundance of $N_2$-fixer rhizobacteria recorded the highest Azotobacter population at 2.64 x $10^8$ cfu $g^{-1}$ in the maize ecosystem and the highest Azospirillum population at 2.32 x $10^8$ cfu $g^{-1}$ in the natural forest ecosystem. While the highest organic carbon and total nitrogen were obtained in natural forest and savanna ecosystems. The population of total bacteria and NFR were relatively high (>$10^8$ cfu $g^-$1). Eighteen isolates of NFR were characterized microscopic and macroscopically, consisting of nine Azotobacter isolates (Azt-a1, Azt-a3, Azt-b1, Azt-b2, Azt-b3, Azt-c1, Azt-c2, Azt-c3, and Azt-d1), and Azospirillium isolates (Azs-a2, Azs-b2, Azs-b3, Azs-c1, Azs-d1, Azs-d2, Azs-e1, Azs-e2, and Azs-e3) as a potential bioagent to be used for improving the fertilizers efficiency and agronomic traits of upland rice.

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