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The Population and Isolates of Potential ACC Deaminase-Producing Rhizobacteria from Rhizospheric Soil of Peanut under Different Moisture Level

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Abstract: ACC deaminase-producing rhizobacteria play an important role in enhancing plant growth and health, particularly under environmental stress condition. This study focused on isolating and determining the population of potential ACC deaminase-producing rhizobacteria from the rhizosphere of peanut plants (*Arachis hypogea* L.) grown under varying moisture level. Bacterial population was measured using the Standard Plate Count (SPC) method on Dworkin-Foster (DF) medium supplemented with 3 mM.L^{-1} ACC as the sole nitrogen source. The isolated bacteria were screened based on their ability to grow after 24 hours of incubation on the selective medium. Result showed that bacterial colonies on nutrient agar (NA) medium varied in color, while colonies on the selective medium were uniformly white. The total population of ACC deaminase-producing rhizobacteria generally declined as soil moisture decreased, nevertheless, the sample at 80% available water contained fewer bacteria (7.3 x 10^3 cfu.g^{-1} soil) than those (9.7 x 10^5 cfu.g^{-1}) at 50%. In an additional experiment, 9 out of 11 selected isolates were found to potentially produce ACC deaminase, with 5 of these being diazotrophic bacteria. This study contributes valuable information for designing irrigation systems in sustainable land management, particularly concerning plant-beneficial microbes that produce ACC deaminase and help plants tolerate environmental stressors.

Keywords: ACC deaminase; Peanut plant; Rhizobacterial population; Soil available water.

Introduction

Microbes, particularly rhizobacteria, are the most abundant bacteria in soil, with populations estimated at around 9 x 10^7 per gram of soil (Alexander, 1991), and ranging from 1 x 10^6 to 1 x 10^9 (Oyewole & Asiotu, 2012). The rhizosphere, enriched with nutrients secreted by plant roots, serves as the primary habitat for rhizobacteria. This zone is a centre of intense biological activity, driven by nutrient supply from root exudates. Rhizobacteria compete for water, food, and space, playing a crucial role in nutrient cycling, plant growth, and overall plant health (Oyewole & Asiotu, 2012). Their population and activity are influenced by a variety of biotic and abiotic factors. One of the key drivers of microbial growth in the rhizosphere is plant-microbe interaction. However, soil conditions such as pH, temperature, oxygen levels, moisture, and physicochemical properties, along with environmental factors like heavy metals, salinity, drought, waterlogging, plant type, and agricultural practices, also significantly affect microbial population dynamics (Rahman et al., 2021).

Water deficit, in particular, is a limiting factor for the growth and reproduction of rhizobacteria and plants. Soil moisture levels can impact the physical, chemical, and biological properties of the soil. Various studies have shown that these stressors can significantly

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influence bacterial populations in the rhizosphere. The population dynamics of rhizobacteria, in turn, directly affect soil nutrient cycling, phytohormone production, and soil health, impacting plant growth and development (Bogati & Walczak, 2022; Leizeaga et al., 2020). Abiotic stressors, such as waterlogging and drought, are often associated with the presence of ACC deaminase-producing rhizobacteria, which produce a cytoplasmic enzyme, ACC deaminase, that degrades ACC, a compound released by plants under stressful condition (Husen et al., 2021; Orozco-Mosqueda et al., 2020; Timmusk et al., 2011).

ACC deaminase-producing rhizobacteria are essential for promoting plant growth and health by producing phytohormones such as Indole-3-Acetic Acid (IAA) in addition to ACC deaminase. This enzyme breaks down ACC, the precursor of ethylene, into ammonia and α-ketobutyrate (Honma & Shimomura, 1978). As a result, ACC deaminase-producing bacteria help mitigate excess ethylene production caused by environmental stressprs, reducing its negative impact on plant growth and development. Plants associated with these bacteria often exhibit longer roots and shoots and show improved resistance to ethylene stress-induced growth inhibition (Glick, 2014).

Under drought conditions, ACC deaminase-producing rhizobacteria are particularly effective in protecting plants compared to bacteria that thrive in water-abundant environments (Mayak et al., 2004). However, Tromberger et al. (2017) noted that the population of ACC deaminase-producing rhizobacteria under drought conditions varied significantly depending on the plant variety, suggesting that these populations are also influenced by the plant host. Beneficial rhizobacteria can significantly influence plant growth and health by forming biofilms, which help them survive environmental stressors and pathogen attacks (Haque et al., 2020; Karimi et al., 2022).

Microbial populations are shaped by various environmental stressors, and the types and characteristics of these isolates can vary, including whether they are diazotrophic or non-diazotrophic when grown on N-free DF medium. This study presents novel research which focuses on ACC deaminase-producing rhizobacteria and diazotroph under deficit amount of water, an approach that has not been previously explored in Lombok, West Nusa Tenggara, specifically simulating conditions akin to the semi-arid tropics of the region. The research is particularly significant in light of the increasing severity of water scarcity driven by today's changing climate. The primary aim of this study was to isolate and identify potential ACC deaminase-producing rhizobacteria from the rhizosphere of peanut plants (*Arachis hypogaea* L.) under varying soil moisture conditions.

Method

The methodology of this study followed a structured experimental procedure. It began with soil water treatment (including planting media preparation and peanut planting), followed by soil sampling. Subsequent steps included enumerating the total microbial and ACC deaminase-producing bacteria and measuring the soil respiration rate. The final step involved purifying the isolates and assessing their growth performance. A flowchart illustrating the experimental steps, from soil water treatment to the purification and evaluation of potential isolates, is provided in Figure 1.

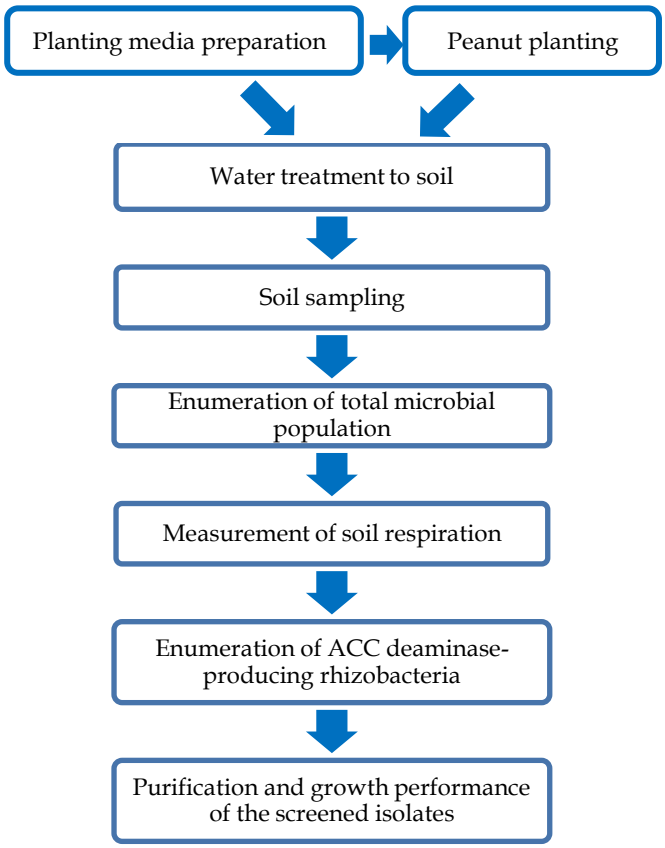

Figure 1. Experimental procedure of this study

Soil Water Treatment

Peanuts were planted in inceptisol soil originated from the experimental garden at the University of Mataram, Narmada, West Lombok, West Nusa Tenggara Province. The soil samples were sieved through a 2-mm mesh, and their initial moisture content (MC) was measured using the gravimetric method, following the Formula 1.

$$MC = \frac{w-d}{d} \times 100\% \quad (1)$$

MC = Moisture Content (%)
w = Wet weight (g)
d = Dry weight (g)

Further analysis involved measuring the percentage of soil water at field capacity (FC) and the permanent wilting point (PWP) to calculate the available water capacity (AWC). Three soil samples, each placed in three polybags, were watered for ten days before planting. The FC was measured by combining the three soil samples into a 100-g mixture, which was then dried for 48 hours. The sample was oven-dried, and the FC was recorded. The PWP was determined after planting by withholding water until the plants reached wilting point. A 100-g composite soil sample was analyzed to determine the PWP. Finally, the AWC was calculated by subtracting the PWP from the FC percentage. Seven days after planting, the required water volume was calculated by subtracting the percentage of available water from the initial moisture content. Plants were irrigated with volumes corresponding to 100%, 80%, and 50% of the available water, adjusting the additional water as needed.

Soil Condition and Sampling

The study employed an experimental method to assess the effects of different levels of available water on the soil of peanut plants. A 100-gram sample of rhizospheric soil was collected from each plant using a mini shovel at a depth of 10-15 cm. The sample was placed in polyethylene plastic bag and stored at -20°C until further analysis. Prior to downstream analysis, the sample was allowed to reach room temperature.

Enumeration of the Total Microbial Population

A total of 10 g of soil was transferred into 90 mL of 0.85% NaCl physiological solution and shaken at 120 rpm for 20 minutes. A 1 mL aliquot of the suspension was then transferred into 9 mL of physiological solution, and the process was repeated serially up to 10^{-7} dilution, with each dilution homogenized using a vortex. From the 10^{-6} and 10^{-7} dilutions, 0.1 mL of each suspension was inoculated using the spread plate method onto Nutrient Agar (NA) media. The cultures were incubated at room temperature for 3 x 24 hours, after which microbial growth was observed, with colony counts ranging from 30-300. The microbial population was determined using the Total Plate Count (TPC) method by manually counting the bacterial colonies and calculating the result as Colony-Forming Units (CFU) per gram of soil using the following Formula 2.

$$\text{No. of cfu/g} = \frac{\text{No. of colonies counted x dilution factor}}{\text{Volume of sample taken}} \quad (2)$$

Measurement of Soil Respiration Rate

The measurement of soil respiration was conducted according to the method described by Qur'ana (2018). A 100-gram sample of soil was placed into an airtight jar, along with two vials containing 10 mL of distilled water and 5 mL of 0.2 N KOH. The remaining soil was weighed to determine a dry weight of 50 grams. After seven days incubation, the KOH solution was transferred to an Erlenmeyer flask for titration using 0.1 N HCl. This process was repeated twice. During the first titration, two drops of phenolphthalein were added to the KOH solution, resulting in a pink color, which eventually became transparent. Subsequently, two drops of methyl orange were added until the solution turned orange, and titration continued until the color reverted to pink. The second titration was performed to measure the soil respiration rate using the following Formula 3.

$$R = \frac{(a-b)\ x\ t\ x\ 1{,}2\ x \frac{100}{adw}}{n} \quad (3)$$

R = Soil respiration (mg C-CO_2.g^{-1}.day^{-1})
a = Volume of HCl sample (mL)
b = Volume of HCL blanko (mL)
t = Normality of HCl
n = Incubation (day)
adw = Absolute dry weight (g)
1 mL HCl 0.1 N = 0.1 me CO_2
0.1 x 44 mg CO_2 = 4.4 mg CO_2
C/CO_2 = (12 x 44) x 4.4 = 1.2

Enumeration of the Total Population of ACC deaminase-Producing Bacteria

The soil sample preparation and microbial culture procedures were conducted as follows: A total of 10 grams of soil was homogenized in 90 mL of 0.85% NaCl physiological solution using a shaker set at 120 rpm for 20 minutes. Subsequently, 1 mL of the resulting suspension was transferred into 9 mL of fresh physiological solution, and serial dilutions was performed, extending to a 10^{-7} dilution, with thorough homogenization using a vortex. From the 10^{-6} and 10^{-7} dilutions, 0.1 mL of each suspension was transferred and inoculated onto Dworkin-Foster (DF) media containing 3 mM.L^{-1} ACC compound using the spread plate method. The cultures were then incubated at room temperature for 24 hours, after which microbial growth was observed, with colony counts ranging from 30 to 300. The microbial population was estimated using the Standard Plate Count (SPC) method by manually counting the number of bacterial colonies, which were expressed as Colony-Forming Units (CFU) per gram of soil.

The preparation of DF+ACC (3 mM.L^{-1}) agar media followed the method of Penrose and Glick et al. (2007)

with some modification. One gram of rhizospheric soil was inoculated into 9 mL of sterile DF minimal salt medium containing 3 mM.L^{-1} ACC as the sole nitrogen source. This mixture was incubated at room temperature for 24 hours on a shaker at 200 rpm. After incubation, the culture was diluted to 10^{-4} dilution and then inoculated onto DF agar medium containing 500 µmol. mL^{-1} ACC, followed by incubation at 30°C for 48 hours. Pure isolates were then transferred onto slant agar medium for storage at 4°C or in 65% glycerol at -80°C, as described by Bal et al. (2013).

Purification and Growth Performance of Potential ACC Deaminase Producing Isolates

Isolates purification was performed by selecting distinct isolates from each soil sample and inoculating them onto fresh Nutrient Agar (NA) medium. The pure isolates were then recultured and screened on Dworkin-Foster (DF) and DF+ACC agar media, with growth observed after 24 hours. Subsequently, the purified isolates were assessed for Gram staining and their diazotrophic characteristics.

Result and Discussion

Bacterial colony performance on different type of agar medium

Rhizobacteria from the rhizospheric soil of peanuts were isolated on NA and Dworkin-Foster (DF+ACC) media. Figure 1 presents a representative view of microbial colonies growing on both media. The bacterial colony performances exhibited significant differences between the two types of media. Microbes growing on NA medium were denser than those growing on DF+ACC medium. The former represents the growth of total culturable microbes, while the latter reflects the growth of ACC deaminase-containing rhizobacteria. Generally, the bacteria observed on NA medium displayed variability in colony color, whereas those on DF+ACC medium formed uniform white colonies.

Soil Respiration of the Tolat Rhizobacteria

Soil respiration rates showed only small variations among the three soil samples. The highest rate was observed in sample S1, while the lowest was found in sample S3. Notably, these variations appeared to decrease as the microbial population declined. The highest respiration rate recorded was 0.009 mg C-CO_2 g^{-1} day^{-1} in S1, which had a microbial population of 1.0 × 10^8 cfu g^{-1}. In contrast, the lowest rate was 0.007 mg C-CO2 g^{-1} day^{-1} in S3, with a microbial population of 6.0 × 10^6 cfu g^{-1} (Figure 2).

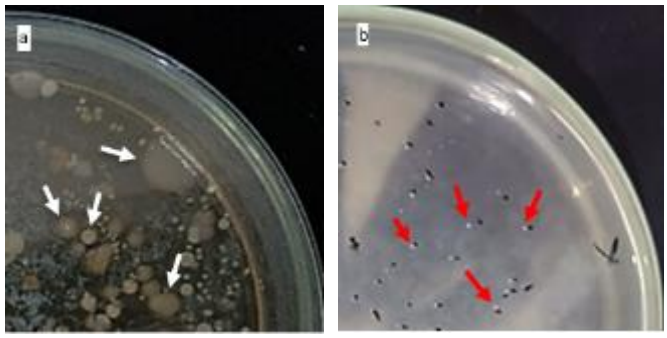

Figure 2. The representatives view of the microbial colony on NA medium (A) and DF+ACC medium (B). White arrows and red arrows point diverse microbial colonies growing on NA medium and the uniform white bacterial colony growing on DF+ACC agar medium, respectively

Table 1. The relationship between bacterial population and soil respiration

Soil samples	Population of the total rhizobacteria	Soil respiration (mg C-CO2.g^{-1}.day^{-1})
S1	1.0 x 10^8	0.009
S2	1.4 x 10^7	0.008
S3	6.0 x 10^6	0.007

S1: 100% available water, S2: 80% available water, S3: 50% available water

Based on table 1, which shows the number of bacterial populations on NA media, soil sample S1 (1.0 × 10^8 cfu.g^{-1}) has the highest bacterial population, followed by S2 (1.4 × 10^7 cfu.g^{-1}) and S3 (6.0 × 10^6 cfu.g^{-1}). This shows that different water availability affects the size of bacterial populations. The available water content in S1 is higher compared to that of S2 and S3. In conditions with 60-100% available water, optimal environmental conditions for bacterial growth are created due to the sufficient availability of water (Kusumastuti, 2013). According to Panggabean et al. (2016), higher water availability is associated with increased bacterial population growth to some extent.

The basic characteristics of the microbial population in the rhizosphere are directly or indirectly influenced by the exudates released by plant roots and the availability of soil nutrients. The population in the rhizosphere consists of soil microflora and fauna. The rhizosphere is an area of intense microbial activity, making it essential to qualitatively and quantitatively analyze the microbial population, its interactions with the root surface, and its contributions to plant health. One type of microbe that is directly involved in plant growth and health is rhizobacteria—bacteria that inhabit the soil zone around the roots, that can form symbiotic relationships on the root surface and within the root tissue.

This study found that the bacterial population reaches approximately 10^8 cfu per gram of soil in a

nutrient-rich medium. This result corresponds with several studies that estimate rhizobacterial populations in the rhizosphere at approximately 9 x 10^7 (Alexander, 1991); 1 x 10^6 to 1 x 10^9 (Oyewole & Asiotu, 2012) and 2 x 10^6 to 1 x 10^9 per gram of soil (Brahmaprakash et al., 2017). The highest population of rhizobacteria are typically found in soil at depths of up to 30 cm, with the population gradually decreasing in deeper layers. The rhizobacteria are the most abundant microbial group among other microbes and soil fauna. The presence of root exudates, which act as nutrients—such as amino acids, organic acids, carbohydrates, nucleic acid derivatives, growth factors, vitamins, and mucilage—is the main cause of the abundance of these rhizobacteria in the rhizosphere. In addition, the dynamics of soil moisture and other type of abiotic stressor greatly influence the population, community, and activity of microbes within it (Borowik & Wyszkowska, 2016; Oyewole & Asiotu, 2012; Rahman et al., 2021).

Soil water availability is a limiting factor that influences the growth and development of rhizobacteria and plants, thereby affecting overall plant growth and development. The microbial community in the soil is closely associated with plant health, productivity, and environmental adaptation. Research has shown that host-microbe interactions can enhance plant tolerance to environmental stressors, such as drought. This finding encourages researchers to manipulate and enhance microbial communities to stimulate plant growth during periods of stress (Armada et al., 2018). Changes in soil moisture can impact microbes associated plants, such as plant growth-promoting rhizobacteria (PGPR) (Xu et al., 2018). This observation may be valid, as our study indicates that the population of microbes tends to decrease as moisture levels decline (Figure 1).

Water availability also influences water activity (aw) for bacteria. Water activity, also referred to as free water, is the water available for bacterial survival. Water activity (aw) is an index of the amount of water used by bacteria for their growth. The higher the water activity, the greater the water availability; thus, the potential for bacterial growth is also higher. Bacteria have higher aw requirements compared to other microbes (Asiah & Djaeni, 2021). In general, bacteria can grow at aw levels of 0.90-0.999 (Suryani & Taupiqurrahman, 2021). Almost all types of bacteria cannot grow below aw 0.90 (Achadiyah, 2017). A water activity of 0.92 is equivalent to a water content availability of 33.5% wet weight (Hayati et al., 2005). In the S3 sample, which had the lowest water availability, a substantial bacterial population was still found. This indicates that the S3 sample treatment still supports the optimal growth and development of bacteria.

The soil respiration test results in Table 1 show that S1 (0.009 mg C-CO2 $\cdot g^{-1} \cdot day^{-1}$) has the highest respiration rate compared to S2 (0.008 mg C-CO2 $\cdot g^{-1} \cdot day^{-1}$) and S3 (0.007 mg C-CO2 $\cdot g^{-1} \cdot day^{-1}$). Soil respiration is the process of releasing CO_2 from the soil as a result of microbial respiration process (Qur'ana, 2018). According to Menti et al. (2020), soil respiration can be used as an indicator of microbial activity in the soil. A high level of soil respiration is generally accompanied by a high microbial population in the soil (Sopiah & Arifudin, 2016). Bacteria are among the microbes with the largest populations found in soil, apart from fungi (Ristiati et al., 2018). This indicates the high level of respiration in each sample is due to the elevated bacterial population.

Population densities of ACC deaminase-producing rhizobacteria in the peanut rhizosphere

The population densities of rhizobacteria on NA and DF+ACC medium were enumerated from rhizosphere of peanut with different moisture level. The result showed that the population of rhizobacteria in three samples on NA medium had relatively larger population than that on DF+ACC medium ranging from 6.0 x 10^6 to 1.0 x 10^8 and 7.33 x 10^3 to 9.7 x 10^5, respectively (Table 1).

Table 2. Bacterial growth on selective medium

Soil sample	Moisture level (%)	Population of the bacteria (CFU. g^{-1})	
		DF	DF+ACC 3 $mM.L^{-1}$
S1	100	4.8 x 10^6	9.0 x 10^5
S2	80	1.6 x 10^6	< 30 x 10^3 (7.3 x 10^3)
S3	50	1.6 x 10^6	9.7 x 10^5

S1: 100% available water, S2: 80% available water, S3: 50% available water

In this experiment, DF medium was used as a control to determine the presence of bacterial isolates capable to grow without any nitrogen source. The DF medium (without the addition of ACC) serves as a control for identifying bacterial isolates that can grow on this medium, which are thus indicated as diazotrophic bacteria. Ratnaningsih et al. (2023) and Syah (2021) also stated that DF minimal salt medium, without any nitrogen source, is used to assess diazotroph. Based on the experiments detailed in Table 2, some bacteria were able to grow on DF medium from the three soil samples. Diazotrophic bacteria that grow on DF medium can fix atmospheric N_2. Additionally, several types of diazotrophic bacteria are known to produce the ACC deaminase enzyme (Astuti et al., 2021). Mir et al. (2022) reported that 6 out of 15 diazotrophic bacterial isolates were able to produce the ACC deaminase enzyme. Triyani & Hafsan (2021) also noted that *Azospirillum* is an example of diazotrophic bacteria capable of fixing atmospheric nitrogen and producing the ACC deaminase enzyme. It is suggested that several

associative diazotrophic bacteria and endophytes can produce the ACC deaminase enzyme, but the exact mechanism requires further study (Carvalho et al., 2014).

The bacterial growth on DF+ACC medium, as shown in Table 2, indicates that they are able to utilize ACC as the sole nitrogen source in the medium (Ariyani et al., 2021). This aligns with the study by Nugroho et al. (2023), who stated that bacterial isolates capable of growing on DF+ACC medium are indicators of bacteria producing the ACC deaminase enzyme. Based on Table 2, the most optimal growth of ACC deaminase-producing bacteria was found in samples S1 (9.0×10^5 cfu.g^{-1}) and S3 (9.7×10^5 cfu.g^{-1}) with 100% and 50% water availability, respectively. Meanwhile, bacterial growth in S2 (< 30×10^3 (7.3×10^3) cfu.g^{-1}) with 80% water availability was less than optimal. Nevertheless, the results of this experiment suggest that water availability at certain levels does not significantly affect the bacterial population producing ACC deaminase, indicating that these bacteria may play a key role in plant host tolerance. However, further study is required to confirm these findings.

Growth Performance of ACC deaminase bacterial isolates

Eleven isolates were selected based on their distinct colony morphology and their representation of each sample. Nine out of them showed potential to produce ACC deaminase based on their growth performance on DF+ACC agar media. Five isolates were considered diazotrophic due to their ability to grow on DF medium. All isolates exhibited characteristics indicating whether they are Gram-negative or Gram-positive bacteria (Table 3).

Table 3. The growth performance of bacterial isolates on different types of medium

Isolates	Growth performance			
	NA	DF	DF+ACC	Gram
S1U1	+	+	+	Negative
S1U2	+	+	+	Positive
S1U3	+	+	+	Positive
S1U4	+	-	+	Positive
S2U1	+	+	+	Negative
S2U2	+	-	+	Negative
S2U3	+	-	-	Negative
S2U4	+	-	-	Negative
S3U1	+	-	+	Positive
S3U2	+	+	+	Positive
S3U3	+	-	+	Positive

(+) = grow; (-) = not grow

The bacterial population on selective (DF+ACC) medium was generally lower than that on nutrient-rich medium due to the minimal salt composition of DF. The densities of ACC deaminase-containing rhizobacteria ranged from 10^3 to 10^5 cfu per gram soil. This result aligns with the study by Nadeem et al. (2012), which showed that ACC-degrading rhizobacteria from avocado roots under salinity stress ranged from 1.5×10^4 to 8.5×10^6 cfu·g^{-1}, which is lower than those on NA medium. The trend of the bacterial population on selective medium differs from that on NA medium. The bacterial population in sample S3 is similar to that in sample S1, indicating that ACC deaminase isolates have a specific osmotic mechanism for maintaining their population under water-deficit conditions. Although the bacterial population was the lowest at 80% moisture level, it was higher at 50% available water. This result suggests that 100% moisture, or non-stressed soil, provides the most optimal conditions for bacterial growth. **Ali et al. (2013)** also found that ACC deaminase-producing *Pseudomonas* exhibits better ACC deaminase activity in non-stress conditions compared to drought stress. Similarly, Husen et al. (2021) found that ACC deaminase-producing bacteria are sensitive to certain osmotic pressures, suggesting that the bacterial population is dynamic under different stress conditions.

Several bacterial isolates that grew on DF+ACC medium in previous experiment was purified multiple times to obtain pure bacterial isolates. Fitriasari et al. (2020) stated that purification may need to be conducted several times until pure bacterial isolates are achieved. In this experiment, purification was performed using streak method on NA medium (Triyani & Hafsan, 2021). Of the 12 isolates purified in this experiment, 11 were selected for re-culturing on DF and DF+ACC medium using the point method with a toothpick (Arifin et al., 2019). The 11 bacterial isolates were derived from the three treatments used in this experiment: S1 (100% available water) with 4 isolates (S1U1, S1U2, S1U3, and S1U4); S2 (80% available water) with 4 isolates (S2U1, S2U2, S2U3, and S2U4); and S3 (50% available water) with 3 isolates (S3U1, S3U2, and S3U3). In sample S3 (50% available water), only 3 pure bacterial isolates were selected because one of the 4 isolates was identified as an actinomycete. The criteria for selecting the 11 pure isolates in this experiment were based on size, shape, surface, edges, color, and density (Permana et al., 2021).

Based on the results of this study presented in Table 3, five bacterial isolates—S1U1, S1U2, S1U3, S2U1, and S3U2—were able to grow on DF media after 24 hours, suggesting they are diazotrophic. Syah (2021) reported that bacterial isolates capable of growing on DF media within 24 hours are likely diazotroph that can fix free nitrogen. In addition to their growth on DF media, these five isolates also thrived on DF+ACC media, indicating their ability to produce the ACC deaminase enzyme which degrade ACC sources present in the DF+ACC media. Kruasuwan & Thamchaipenet (2018) noted that the ACC deaminase activity in the diazotrophic bacteria

Entrobacter sp. was assessed by culturing them in both DF and DF+ACC media to measure the amount of α-ketobutyrate produced from ACC cleavage. Thiebaut et al. (2022) stated that several diazotrophic bacteria, such as *Herbaspirillum seropiddecae*, can produce the ACC deaminase enzyme to degrade ACC compounds as a nitrogen source. Ong et al. (2018) reported that 41 out of 147 diazotrophic bacterial isolates from saline soil were capable of producing ACC deaminase. This aligns with Karthikeyan et al. (2012), who found that one out of four diazotrophic isolates from the rhizosphere of periwinkle plants affected by salinity stress produced the ACC deaminase enzyme, specifically identified as *Achromobacter xylosoxidans*. Additionally, Imran et al. (2021) stated that diazotrophic bacteria tolerant to drought stressor can produce ACC (1-aminocyclopropane-1-carboxylate) deaminase, which catabolizes ACC—an ethylene precursor produced by plants—into α-ketobutyrate and NH_3.

Several bacterial isolates were indicated to be capable of producing ACC deaminase in the experiments, though they are not diazotrophic because they only grew on DF+ACC medium within 24 hours. Some of these isolates include S1U4, S2U2, S3U1, and S3U3. According to Jaya et al. (2019), bacterial isolates that can grow within 24 hours on DF+ACC medium are believed to optimally code for the ACC deaminase enzyme. Ratnaningsih et al. (2023) reported that, in their research on 16 bacterial isolates from the rhizosphere of pineapple plants affected by abiotic and biotic stress on DF+ACC medium, one bacterial isolate (CHTB 2C, *Brevundimonas* sp.) had the highest ACC deaminase activity, producing α-ketobutyrate at 13,370 nm α-ketobutyrate mg^{-1} h^{-1} within 24 hours. Gupta et al. (2022) also reported that two bacterial isolates (*Pseudomonas aeruginosa* and *Bacillus subtilis*) from the rhizosphere of peas affected by salinity stress were isolated on DF+ACC medium within 24 hours and were able to degrade the ACC compound into α-ketobutyrate, producing 489 nm α-ketobutyrate mg^{-1} h^{-1} and 257 nm α-ketobutyrate mg^{-1} h^{-1}, respectively. Both bacterial isolates were also able to produce NH_3. Based on the experiments conducted, two bacterial isolates (S2U3 and S2U4) were unable to grow on DF+ACC or DF media within 24 hours, indicating that they were unable to produce ACC deaminase in that time frame and were not diazotrophic.

Conclusion

This study concludes that ACC deaminase-producing rhizobacteria play a critical role in supporting plant growth under environmental stressor, particularly varying moisture level. The population of these beneficial bacteria generally decreased with lower soil moisture, except at 50% available water, where it was higher than that at 80% moisture. This suggests that certain moisture condition may favor the growth of ACC deaminase-producing bacteria, which can contribute to plant stress tolerance. Nevertheless, future researches are required to strongly confirm this result statistically. Furthermore, 9 out of the 11 isolates tested showed potential for ACC deaminase production, with 5 being diazotrophic, highlighting their dual role in nitrogen fixation and stress mitigation. These findings provide valuable insights for optimizing irrigation practices and leveraging plant-beneficial microbes in sustainable agriculture.

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Author Contributions

Conceptualization, D.K.J and L.E.S.; methodology, D.K.J; software, A.I.; investigation, A.I and E.M.; writing—original draft preparation, D.K.J; writing—review and editing, L.E.S.; visualization, A.I.; supervision, L.E.S.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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