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Original article

Evaluation of a non-aseptic stepwise culture technique with various carbon sources for nitrogen-fixing bacteria propagation

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Abstract

The reliance on urea fertilizers as nitrogen sources is gradually being replaced by biofertilizers containing nitrogen-fixing bacteria. However, the higher cost of biofertilizers compared to urea remains a barrier for farmers. A potential solution for farmers is to independently produce nitrogen-fixing bacteria cultures using a simple, non-aseptic culture technique. This study evaluates this technique for propagating nitrogen-fixing bacteria. It examines the effects of alternative carbon sources, such as cane sugar and white vinegar, to replace malic acid in Nfb medium. Soil samples, serving as bacterial sources, were inoculated into liquid Nfb medium, and subcultured for six stages. The most probable number (MPN) of nitrogen-fixing bacteria and medium pH were monitored at each stage. The results indicate that white vinegar supported the highest bacterial cell density, reaching 6.3×10^{10} MPN by the fifth culture stage. The increase in medium pH to an alkaline range suggests elevated ammonium concentrations, which are beneficial as a nitrogen source for plants. The culture technique demonstrated promising results and holds potential for practical application by farmers in propagating nitrogen-fixing bacteria.

Keywords: carbon source, nitrogen-fixing bacteria, non-aseptic technique

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Introduction

Nitrogen is an essential macronutrient required for plant growth. Naturally, nitrogen is available in the soil through sources such as rainfall, organic matter decomposition, and microbial inorganic redox reactions (Hartono et al. 2021). However, in intensive agricultural systems, the naturally available nitrogen in the soil is often insufficient to meet the high nitrogen demands of high-yield crops. To address this limitation, farmers have traditionally applied urea fertilizers, a synthetic nitrogen source containing approximately 46% nitrogen (Dorieh et al., 2019). Urea has long been proven as an effective nitrogen provider, supporting protein synthesis, chlorophyll production, and plant metabolic processes, thereby contributing to the high productivity of cultivated crops (Dazmiri et al., 2019).

Excessive urea application, however, can negatively impact both crops and soil health. Prolonged use may lead to salt accumulation and soil pH changes, eventually reducing soil fertility and inhibiting plant growth (Hartono et al. 2021). Overuse can also impede the uptake of other essential nutrients, such as phosphorus and potassium, making crops more susceptible to diseases and environmental stressors (Xu et al., 2020). These challenges underscore the need for sustainable nitrogen sources tailored to plant needs.

Nitrogen-fixing bacteria are soil microorganisms that capture atmospheric N_2 and convert it into ammonia (NH_3), which subsequently transforms into ammonium ions (NH_4^+) in moist environments, making it available for plant uptake (Dahal et al., 2018). These bacteria are widely found in commercial biofertilizers worldwide, including in Indonesia. Many farmers use biofertilizers to enhance soil fertility as part of organic farming systems. The application of nitrogen-fixing bacteria has been proven effective in providing a nitrogen source as an alternative to urea fertilizer (Mahmud et al., 2020). However, the cost of biofertilizers remains relatively high compared to urea fertilizer. As a result, many farmers who are unable to afford biofertilizers revert to synthetic fertilizers.

One potential solution to reduce the costs associated with purchasing commercial biofertilizers is on-site production by farmers. However, a major challenge is the lack of specialized facilities with aseptic conditions to minimize contamination by other microorganisms, which can outcompete the biofertilizer strains. Here, we propose a simple technique for propagating nitrogen-fixing bacteria that does not require aseptic conditions. The concept involves selectively enriching nitrogen-fixing bacteria naturally present in local agricultural soils.

In general, nitrogen-fixing bacteria are present in lower abundance compared to the total microbial community in the soil (Orr et al., 2011). A stepwise cultivation process using N-free bromothymol blue (Nfb) medium is expected to selectively enhance the growth of nitrogen-fixing bacterial populations. Therefore, this method can be implemented even under non-aseptic condi-

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tions. The Nfb medium contains malic acid as its sole carbon source (Narayan et al., 2018). Since malic acid is not widely available, alternative carbon sources such as cane sugar and white vinegar, which are more accessible and cost-effective, should be explored. Therefore, this study aims to evaluate the effectiveness of a non-aseptic stepwise culture technique using different carbon sources for enriching nitrogen-fixing bacterial populations.

Methods

Soil Sampling

Wet soil samples were collected from a productive paddy field in Malang, East Java, in April 2024 during the dry season. The samples were then placed in plastic bags and stored in a cool box during transportation to the laboratory.

Abiotic Factor Analysis

Soil temperature, pH, organic matter content, total nitrogen (N), and total phosphorus (P) were measured. Soil temperature was recorded on-site using a thermometer, while pH and moisture content were determined in the laboratory using a pH meter and gravimetric methods, respectively. Organic matter, total nitrogen (N), and total phosphorus (P) were quantified using gravimetric, Kjeldahl, and spectrophotometric methods, respectively. These parameters offer valuable insights into the microbial habitat and soil nutrient condition.

Preparation of Nfb Medium

Liquid Nfb medium was prepared with the following per liter: 5 g DL-malic acid (Himedia), 0.5 g K_2HPO_4 (Merck), 0.2 g $MgSO_4 \cdot 7H_2O$ (Merck), 0.1 g NaCl (Bolab GmbH), 0.015 g $FeCl_3 \cdot 6H_2O$ (Merck), 2 mL of 2% bromothymol blue (Kanto Chemical Co., Inc.), and 5.2 g KOH (Merck). Each component was dissolved sequentially. The pH was adjusted to 6.8 using 1 M KOH or 1 M HCl (Narayan et al., 2018). Alternative carbon sources (5 g/L) tested were cane sugar (sucrose) and white vinegar (acetic acid). In the semi-solid medium used for MPN analysis, 4.5 g of bacteriological agar (Bioworld) was added per liter medium.

Non-Aseptic Stepwise Culture of Nitrogen-Fixing Bacteria

Twenty-five grams of soil were added to 700 mL Nfb medium with different carbon sources (malic acid, cane sugar, white vinegar). Control cultures excluded a carbon source but maintained equivalent final pH. Suspensions were mixed for 15 minutes, labeled as “Culture 1,” and incubated at room temperature for seven days. After incubation, the culture was homogenized and transferred 70 mL into 630 mL fresh medium for subsequent cultures. This step was repeated for six stages, resulting in a total duration of 42 days.

MPN and pH Measurement

At each stage, medium pH and bacterial cell density (MPN) of N-fixing bacteria were measured. A tenfold serial dilutions of 1 mL culture were prepared up to a 10^{-10} dilution, and 1 mL from each dilution was inoculated into five MPN tubes containing 5 mL semi-solid Nfb medium. After six days of incubation at room temperature, tubes with color changes from green to blue, and pellicle formation were scored as positive for nitrogen-fixing activity. The MPN values were calculated using the Thomas formula (Thomas, 1942).

$$MPN/g = \frac{\sum gj}{(\sum tjmj \sum (tj - gj)mj)^{(1/2)}}$$

Where:

$\sum gj$ = Number of positive tubes of the chosen dilutions

$\sum tjmj$ = Amount of diluted samples (g or mL) from all positive tubes of the chosen dilutions

$\sum (tj-gj)mj$ = Amount of diluted samples (g or mL) from negative tubes of the chosen dilutions

Data Analysis

The value of pH and MPN data were analyzed using two-way ANOVA with a 95% confidence level ($p < 0.05$). Post-hoc comparisons were conducted using the least significant difference (LSD) test when F-statistics were significant.

Results

Abiotic Soil Parameter

Soil samples were collected from the surface layer near rice plants and composited from multiple points with the following coordinates: Point 1 (-7.9226772, 112.6149481), Point 2 (-7.9225706, 112.6149950), and Point 3 (-7.9224693, 112.6150560). Figure 1 illustrates one of the sampling points, showing that the paddy plants at the time of sampling were approximately 30 days old. The abiotic characteristics, including temperature, pH, organic content, total nitrogen (N), and total phosphorus (P), were analyzed and are summarized in Table 1.

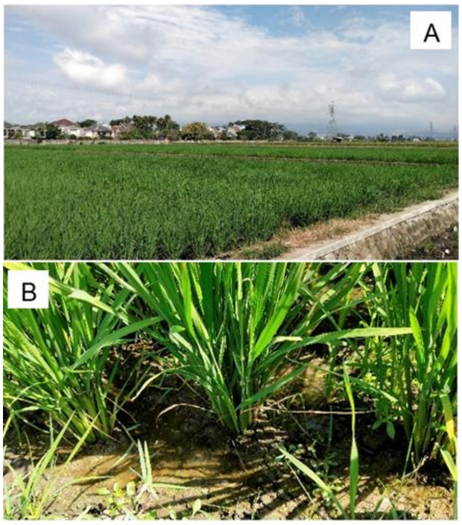

Figure 1. (A) Paddy field sampling area; (B) Specific soil sampling point.

Table 1. Abiotic parameters of paddy field soil samples

Parameter	Value	Category
Temperature (°C)	24,4 ± 0,51	Medium (Supriyadi et al., 2020)
pH	7,42 ± 0,56	Neutral (Widowati & Sukristyonubowo, 2012)
Organic Matter (%)	7,00 ± 2,19	High (Ye et al., 2024)
Total N (%)	0,19 ± 0,00	Medium (Yang et al., 2020)
Total P (%)	0,06 ± 0,00	Medium (Nishigaki et al., 2019)

Nitrogen-Fixing Bacteria in Stepwise Non-Aseptic Cultures

In this study, culture media with different carbon sources were used, and all were cultured stepwise with a seven-day period for each stage. The pH indicator (bromothymol blue) revealed notable changes in the culture media, except for the control, which remained green (neutral pH) (Figure 2). In contrast, cultures containing malic acid or white vinegar exhibited a shift to blue, indicating an increase in pH, while cane sugar-based cultures turned yellow, reflecting acidification.

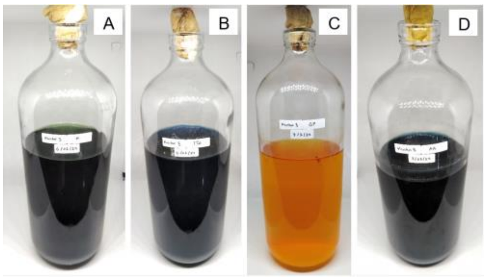

Figure 2. The pH indicator color changes in cultures with various carbon sources: (A) control (no carbon source); (B) malic acid; (C) cane sugar; (D) white vinegar.

pH Changes Across Culture Stages

The pH values for each stage are shown in Figure 3. After the first week, a significant pH increase was observed in cultures containing malic acid (pH 9.42) and white vinegar (pH 11.57). Conversely, the cane sugar culture exhibited a marked derease in pH to 3.28. Subsequent subcultures continued to show increasing pH levels for malic acid and white vinegar media, while the cane sugar culture maintained a consistently lower pH. The control culture that remained green had pH values ranging from 6.55 to 7.04.

MPN Analysis of Nitrogen-Fixing Bacteria

In addition to observing the pH values of the cultures, the cell densities of nitrogen-fixing bacteria were also determined using the MPN method after incubation (Figure 4). Initial cell densities (day 0) ranged from 6.9 × 10^4 to 7.6 × 10^5 MPN across all media. After the first week, MPN values for the control and malic acid cultures increased to 10^7, while cane sugar and white vinegar cultures remained at 10^5. By the fifth culture stage (day 35), the white vinegar-based culture achieved the highest MPN value of 1.2 × 10^{10}, surpassing malic acid. These findings indicate that white vinegar supports robust nitrogen-fixing bacterial growth, consistent with the observed pH increases.

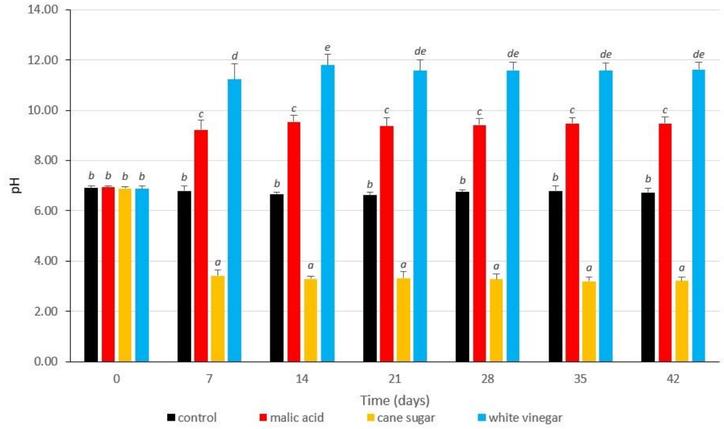

Figure 3. pH values of non-aseptic stepwise cultures with different carbon sources.

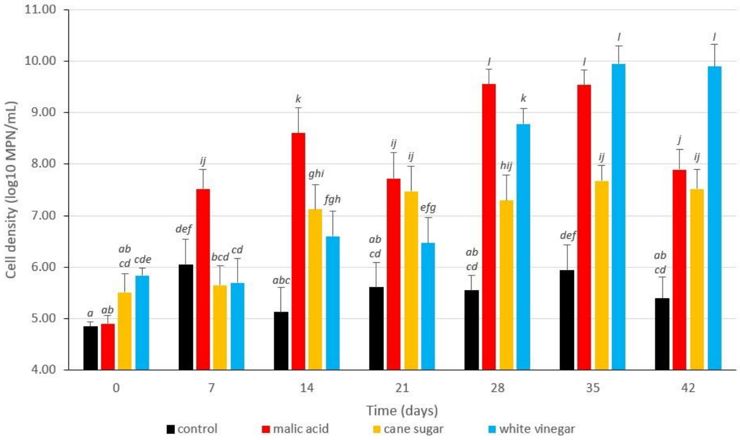

Figure 4. MPN values of nitrogen-fixing bacteria in non-aseptic stepwise cultures with different carbon sources.

Discussion

Abiotic Characteristics of Soil Samples

The recorded soil temperature and pH fall within the typical range observed in Indonesian paddy fields, which generally ranges from 24–30°C and 5.5–7.5, respectively (Supriyadi et al., 2020; Widowati & Sukristyonubowo, 2012). The measured temperature of 24.4°C aligns with the optimal range for microbial activity in soils. Soil microorganisms, including nitrogen-fixing bacteria, exhibit increased metabolic activity within a temperature range of 20°C to 30°C, facilitating enzymatic processes essential for nutrient cycling and organic matter decomposition, which in turn enhances soil fertility (Ye et al., 2024). The slightly alkaline soil pH of 7.42 is beneficial for the growth and activity of nitrogen-fixing bacteria. Many diazotrophs, such as Azotobacter species, thrive in neutral to slightly alkaline conditions, where they efficiently fix atmospheric nitrogen. A pH within this range promotes microbial stability and enhances nitrogen fixation efficiency, ultimately contributing to plant nutrient availability (Harun et al., 2020).

A 7% organic matter content provides a substantial carbon source, serving as an energy substrate for soil microbes. In addition to supporting microbial metabolism, high organic matter improves soil structure and enhances moisture retention, creating favorable conditions for microbial activity. The process of organic matter mineralization can contribute to nitrogen availability; however, the release rate is often slow and may not sufficiently meet the immediate nitrogen demand of rice crops (Tu et al., 2022). In terms of nitrogen availability, the total nitrogen (N) content of 0.19% falls within the moderate to low range for optimal rice growth. As a critical nutrient, nitrogen plays a key role in leaf development, tillering, and overall grain yield in rice plants (Ye et al., 2024). However, its effectiveness in promoting plant growth depends not only on total nitrogen content but also on its transformation into plant-available forms, such as ammonium (NH_4^+) and nitrate (NO_3^-), which are more readily absorbed by plant roots (Yang et al., 2020).

Phosphorus, another essential nutrient, is typically present in soil at lower concentrations, usually ranging from 0.02% to 0.1%. The measured total phosphorus (P) content of 0.06% falls within this range and is generally sufficient to meet the phosphorus requirements of rice plants. Sufficient phosphorus availability not only supports plant growth but also plays a crucial role in enhancing the nitrogen-fixing efficiency of diazotrophic bacteria. As a key component of ATP and nucleic acids, phosphorus is essential for the energy-intensive process of biological nitrogen fixation (Nishigaki et al., 2019).

Based on the soil characteristics observed in the samples, it can be inferred that nitrogen-fixing bacteria are likely present. This assumption is supported by the fact that the soil conditions align well with those required for the optimal growth of nitrogen-fixing bacteria. Therefore, the sample is suitable for use in the subsequent stage as a bacterial source in non-aseptic propagation.

Culture Performance with Different Carbon Sources

In the malic acid-based culture, the pH value increased significantly to 9.23 after seven days of incubation. This elevated pH level remained consistent from the second culture stage (day 14) through the sixth stage (day 42). In a nitrogen-free medium, as used in this experiment, the observed pH increase may result from nitrogen fixation activity, which releases alkaline compounds. Previous studies have reported that the pH values in media containing Azotobacter cultures, with malic acid as the sole carbon source, typically range between 8 and 9 (Arsita et al., 2020; Aasfar et al., 2021). The increase in pH occurs as extracellular ammonia, released from the cell, reacts with water molecules to form ammonium ions (NH_4^+) and hydroxide ions (OH^-). This process begins with the fixation of a single nitrogen gas (N_2) molecule by the nitrogenase enzyme, reducing it to two ammonia (NH_3) molecules. While some ammonia is assimilated into biomass for cellular growth, the excess is excreted into the surrounding environment, where it undergoes hydrolysis, leading to pH elevation (Baldani et al., 2014; Ni et al., 2023).

When malic acid was replaced with white vinegar, the pH of the culture increased significantly to 11–12 within the first seven days, which was considerably higher than the pH observed in the malic acid-based culture. Despite this alkalization, the MPN values indicated that the density of nitrogen-fixing bacteria in the white vinegar culture was initially lower than in the malic acid culture during the early stages of growth. This discrepancy suggests that the extreme alkalinity may have initially inhibited bacterial proliferation, which is not entirely consistent with the expected correlation between pH and bacterial density (Navarro et al., 2016). However, by the fifth culture stage, bacterial density in the white vinegar culture had increased and reached levels comparable to those in the malic acid culture, with a maximum MPN value of approximately 10^9. This suggests that, over time, the bacteria adapted to the alkaline conditions and were able to achieve similar population densities as those cultured with malic acid.

Malic acid provides optimal support for the growth of nitrogen-fixing bacteria, as demonstrated by the highest MPN values observed among all tested carbon sources at the first incubation stage (day 7). At this stage, the MPN value increased from 10^5 to 10^7, indicating rapid bacterial proliferation. This accelerated growth suggests that malic acid is readily utilized by the bacteria, providing an efficient energy source that promotes biomass production. From a metabolic perspective, malic acid (C4) molecules enter the tricarboxylic acid (TCA) cycle directly, facilitating rapid oxidation and generating a total of 12 ATP molecules through the respiratory pathway (Xu et al., 2024). In contrast, white vinegar, in the form of acetic acid (C2), must first be converted into acetyl-CoA by acetyl-CoA synthetase, which consumes the equivalent of 1 ATP before entering the TCA cycle. As a result, the total energy yield per molecule of acetic acid is 9 ATP (He et al., 2022; Sun et al., 2020). This indicates that the oxidation of malic acid generates more ATP than acetic acid, thereby supporting better cell growth. Although not as efficient as malic acid, white vinegar has also been shown to support cell growth, achieving comparable cell densities after several culture stages.

On the other hand, in the cane sugar culture, the number of nitrogen-fixing bacteria increased significantly from 10^5 (day 0) to 10^7 (day 14) and remained at this level until the end of the observation period. This bacterial count exceeded the cell density observed in the vinegar culture up to the third culture stage (day 21). Despite the relatively high bacterial density, the pH of the sucrose culture dropped drastically from 6.8 in the first culture stage (day 7) to 3.4, after which it remained relatively stable until the final culture stage. This significant pH reduction is likely attributed to fermentation activity, which leads to the accumulation of organic acids. Sucrose, a disaccharide derived from sugarcane plants, consists of glucose and fructose (Kizling et al., 2020). Once hydrolyzed within the cell, these monosaccharides are further metabolized via glycolysis. Under anaerobic conditions, the resulting pyruvate undergoes fermentation, producing organic acids that are subsequently released into the environment, leading to a decline in medium pH (Aasfar et al., 2021). The development of this highly acidic environment likely inhibits nitrogen fixation activity, as many nitrogen-fixing bacteria are sensitive to extreme acidity. Consequently, bacterial proliferation is suppressed, preventing further increases in cell density. As the culture progresses, the microbial community shifts, becoming dominated by fermentative microbes, which are more tolerant to low pH conditions.

Although sucrose is an easily accessible carbon source, its breakdown into glucose and fructose can lead to changes in osmotic pressure. This enzymatic reaction increases the number of dissolved particles in the culture medium, creating a hypertonic environment, where the solute concentration is higher than that of the bacterial cytoplasm, causing water to flow out of the cells. This can result in cellular dehydration, reduced turgor pressure, and disrupted cellular functions. Under such conditions, bacterial cells must expend additional energy to regulate their internal osmotic balance, diverting resources away from essential metabolic activities, including nitrogen fixation. As a result, nitrogen-fixing efficiency declines, ultimately affecting bacterial proliferation and function. Nitrogen-fixing bacteria such as Rhizobium and Azotobacter are particularly sensitive to osmotic stress, which can inhibit their growth and nitrogen-fixing capability (Singh et al., 2020).

In the control culture, there were minimal changes detected in pH or the cell density of nitrogen-fixing bacteria. A slight increase was observed after seven days of incubation, rising from 10^4 to 10^6, but in subsequent culture stages, the cell density declined and stabilized at around 10^5. This increase was likely due to the utilization of simple carbon compounds naturally present in the soil. The preparation of the soil suspension in the medium dissolved these compounds, making them accessible to nitrogen-fixing bacteria. However, this condition did not last long, as soil generally contains significantly more complex organic compounds than simple ones, which may have been depleted during the initial incubation stage (Ni et al., 2020).

This study demonstrated that the proposed non-aseptic culture technique successfully increased the population of nitrogen-fixing bacteria by four orders of magnitude (10^5 to 10^9) as the culture stages progressed. Additionally, white vinegar, a cost-effective and widely available carbon source, was proven to be a viable alternative to malic acid for propagating nitrogen-fixing bacteria. By the fourth or fifth subculture, white vinegar-based media achieved high bacterial cell densities, making them suitable for application as a biofertilizer. However, the elevated pH levels observed in these cultures could influence soil pH upon application. In slightly acidic soils, this increase in pH may be beneficial for enhancing microbial activity and supporting plant growth. Nevertheless, proper dilution and precise dosage calibration are essential for field applications to prevent excessive bacterial introduction, which could potentially disrupt the soil microbial community. Further field trials are necessary to evaluate the effectiveness of this technique under varying environmental conditions and crop types. Additionally, certain minor chemical components used in the Nfb medium in this study could likely be omitted, as their natural presence in the soil may be sufficient to support bacterial growth.

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References

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. *Frontiers in Microbiology*, *12*(2021). https://doi.org/10.3389/fmicb.2021.628379

Arsita, R., Karim, H., Hala, Y., Iriany, N., & Jumadi, O. (2020). Isolation and identification of nitrogen-fixing bacteria in the corn rhizosphere (Zea mays l.) originating from Jeneponto Regency, South Sulawesi. *IOP Conference Series: Earth and Environmental Science*, *484*(1). https://doi.org/10.1088/1755-1315/484/1/012051

Baldani, J. I., Reis, V. M., Videira, S. S., Boddey, L. H., & Baldani, V. L. D. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. *Plant and Soil*, *384*(1–2), 413–431. https://doi.org/10.1007/s11104-014-2186-6

Dahal, S., Franklin, D. H., Cabrera, M. L., Hancock, D. W., Stewart, L., Ney, L. C., Subedi, A., & Mahmud, K. (2018). Spatial Distribution of Inorganic Nitrogen in Pastures as Affected by Management, Landscape, and Cattle Locus. *Journal of Environmental Quality*, *47*(6), 1468–1477. https://doi.org/10.2134/jeq2018.06.0241

Dorieh, A., Mahmoodi, N. O., Mamaghani, M., Pizzi, A., & Mohammadi Zeydi, M. (2019). Effect of different acids during the synthesis of urea-formaldehyde adhesives and the mechanical properties of medium-density fiberboards bonded with them. *Journal of Applied Polymer Science*, *136*(14). https://doi.org/10.1002/app.47256

Hartono, A., Nugroho, B., Nadalia, D., & Ramadhani, A. (2021). Dinamika Pelepasan Nitrogen Empat Jenis Pupuk Urea Pada Kondisi Tanah Tergenang. *Jurnal Ilmu Tanah Dan Lingkungan*, *23*(2), 66–71. https://doi.org/10.29244/jitl.23.2.66-71

Harun, N. S., Hanafiah, M. M., Nizam, N. U. M., & Rasool, A. (2020). Water and soil physicochemical characteristics of different rice cultivation areas. *Applied Ecology and Environmental Research*, *18*(5), 6775–6791. https://doi.org/10.15666/aeer/1805_67756791

He, Y., Xie, Z., Zhang, H., Liebl, W., Toyama, H., & Chen, F. (2022). Oxidative Fermentation of Acetic Acid Bacteria and Its Products. *Frontiers in Microbiology*, *13*(2022). https://doi.org/10.3389/fmicb.2022.879246

Dazmiri, MK., Kiamahalleh, MV., Dorieh, A., & Pizzi, A. (2019). Effect of the initial F/U molar ratio in urea-formaldehyde resins synthesis and its influence on the performance of medium density fiberboard bonded with them. *International Journal of Adhesion and Adhesives*, *95*. https://doi.org/10.1016/j.ijadhadh.2019.102440

Kizling, M., Dzwonek, M., Nowak, A., Tymecki, Ł., Stolarczyk, K., Więckowska, A., & Bilewicz, R. (2020). Multi-substrate biofuel cell utilizing glucose, fructose and sucrose as the anode fuels. *Nanomaterials*, *10*(8), 1–16. https://doi.org/10.3390/nano10081534

Mahmud, K., Makaju, S., Ibrahim, R., & Missaoui, A. (2020). Current progress in nitrogen fixing plants and microbiome research. Plants, *9*(1), 97. https://doi.org/10.3390/plants9010097

Narayan, R., Gupta, N. C., & Shahi, D. K. (2018). Isolation, Morphological and Cultural Characterisation of Azospirillum Isolated from Rhizospheric Soils of Various Non-Leguminous Crops of Ranchi Having Acidic pH. *International Journal of Current Microbiology and Applied Sciences*, *7*(08), 329–338. https://doi.org/10.20546/ijcmas.2018.708.037

Navarro-Noya, Y. E., Luna-Guido, M., & Dendooven, L. (2016). Cultivable Nitrogen Fixing Bacteria from Extremely Alkaline-Saline Soils. *Advances in Microbiology*, *06*(06), 412–423. https://doi.org/10.4236/aim.2016.66041

Ni, G., Leung, P. M., Daebeler, A., Guo, J., Hu, S., Cook, P., Nicol, G. W., Daims, H., & Greening, C. (2023). Nitrification in acidic and alkaline environments. In *Essays in Biochemistry* (Vol. 67, Issue 4, pp. 753–768). Portland Press Ltd. https://doi.org/10.1042/EBC20220194

Ni, X., Liao, S., Tan, S., Wang, D., Peng, Y., Yue, K., Wu, F., & Yang, Y. (2020). A quantitative assessment of amino sugars in soil profiles. *Soil Biology and Biochemistry*, *143*, 107762. https://doi.org/https://doi.org/10.1016/j.soilbio.2020.107762

Nishigaki, T., Tsujimoto, Y., Rinasoa, S., Rakotoson, T., Andriamananjara, A., & Razafimbelo, T. (2019). Phosphorus uptake of rice plants is affected by phosphorus forms and physicochemical properties of tropical weathered soils. *Plant and Soil*, *435*(1–2), 27–38. https://doi.org/10.1007/s11104-018-3869-1

Orr, C.H., James, A., Leifert, C., Cooper, J.M., & Cummings, S.P. (2011). Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. *Applied and Environmental Microbiology*, *77*, 3. https://doi.org/10.1128/AEM.01250-10

Singh, R. K., Singh, P., Li, H. B., Song, Q. Q., Guo, D. J., Solanki, M. K., Verma, K. K., Malviya, M. K., Song, X. P., Lakshmanan, P., Yang, L. T., & Li, Y. R. (2020). Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: A comprehensive study of plant-microbe interactions for growth enhancement in Saccharum spp. *BMC Plant Biology*, *20*(1). https://doi.org/10.1186/s12870-020-02400-9

Sun, S., Ding, Y., Liu, M., Xian, M., & Zhao, G. (2020). Comparison of Glucose, Acetate and Ethanol as Carbon Resource for Production of Poly(3-Hydroxybutyrate) and Other Acetyl-CoA Derivatives. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.00833

Supriyadi, Pratiwi, M. K., Minardi, S., & Prastiyaningsih, N. L. (2020). Carbon Organic Content under Organic and Conventional Paddy Field and its Effect on Biological Activities (A Case Study in Pati Regency, Indonesia). *Caraka Tani: Journal of Sustainable Agriculture*, *35*(1), 108–116. https://doi.org/10.20961/carakatani.v35i1.34630

Thomas, H. A. (1942). Bacterial Densities From Fermentation Tube Tests. *Journal AWWA*, *34*(4), 572-576. https://doi.org/10.1002/j.1551-8833.1942.tb19721.x

Tu, D., Jiang, Y., Salah, A., Cai, M., Peng, W., Zhang, L., Li, C., & Cao, C. (2022). Response of Source-Sink Characteristics and Rice Quality to High Natural Field Temperature During Reproductive Stage in Irrigated Rice System. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.911181

Widowati, L. R., & Sukristyonubowo, . (2012). Dynamics of pH, Ferrum and Mangan, and Phosphorus on Newly Opened Paddy Soil having High Soil Organic Matter on Rice Growth. *Jurnal TANAH TROPIKA (Journal of Tropical Soils)*, *17*(1), 1–8. https://doi.org/10.5400/jts.2012.17.1.1

Xu, B., Zhang, W., Zhao, E., Hong, J., Chen, X., Wei, Z., & Li, X. (2024). Unveiling malic acid biorefinery: Comprehensive insights into feedstocks, microbial strains, and metabolic pathways. *Bioresource Technology*, *394*, 130265. https://doi.org/https://doi.org/10.1016/j.biortech.2023.130265

Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., Zhu, Z., Ge, S., & Jiang, Y. (2020). Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00904

Yang, J., Liang, X., Li, H., Chen, Y., & Tian, G. (2020). Effects of no-till and rice varieties on nitrogen and phosphorus balance in rice fields. *Environmental Sciences Europe*, *32*(1). https://doi.org/10.1186/s12302-020-00302-z

Ye, C., Zheng, G., Tao, Y., Xu, Y., Chu, G., Xu, C., Chen, S., Liu, Y., Zhang, X., & Wang, D. (2024). Effect of Soil Texture on Soil Nutrient Status and Rice Nutrient Absorption in Paddy Soils. *Agronomy*, *14*(6). https://doi.org/10.3390/agronomy14061339