Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i2.2991

Research Article

Exploring The Potential of Shrimp Paste (*Belacan*) and its Indigenous Microorganisms (IMO) as a Growth Enhancer for *Capsicum annum* L. (Chilli) Plants

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ABSTRACT

In recent years, there has been growing interest in utilizing Indigenous Microorganisms (IMO) as plant growth-promoting agents, with studies demonstrating their effectiveness in enhancing crop growth. Despite local practices utilizing food-based substrates, limited reports exist on the application of IMO from fermented foods to enhance the growth of *Capsicum annum* L. (chili) plants. Therefore, this study aimed to investigate the mechanism of *Belacan* (fermented shrimp paste) as a biofertilizer in promoting the growth of *Capsicum annum* L. *Belacan* was supplemented with molasses as a carbon source, and the cultured bacteria were subsequently isolated and identified using nucleotide analysis of the 16S rRNA region. Two major bacterial isolates, *Bacillus velezensis,* and *Lysinibacillus fusiformis*, were identified as potential plant-growth-promoting bacteria, with nutrient solubilization and auxin production capability. The application of *belacan* on chili plants revealed that they significantly enhanced plant growth parameters, including shoot length, leaf length, width, thickness, and chlorophyll content, compared to the control group. These findings suggest that IMO from Malaysian fermented foods hold potential as biofertilizers to promote the growth of chili plants, contributing to the advancement of environmentally friendly and nutritionally rich agriculture.

Key words: Auxin, heritage food, plant growth promoting bacteria, phosphate solubilizing, sustainability

INTRODUCTION

Chilli (*Capsicum annum* L.) is a crop of immense economic importance on a global scale. Renowned for its high content of ascorbic acid (vitamin C), carotenoids (provitamin A), tocopherols (vitamin E), and flavonoids, chili holds significant commercial value and offers numerous medical and nutritional benefits (Azlan *et al*., 2022). Moreover, the appeal of cultivating chili has expanded to urban areas and local agriculture due to its adaptability to diverse climatic conditions. As a result, chili production has become a vital component of sustainable agriculture and food security efforts, contributing to local economies, and enhancing the availability of this nutritionally valuable crop in the global market.

However, the conventional approach to chili cultivation often relies heavily on the use of chemical fertilizers and pesticides, raising concerns about their environmental impact and long-term sustainability. In response to these challenges, there has been a growing interest in exploring environmentally friendly alternatives that align with sustainable agricultural practices. One such approach gaining popularity is the utilization of food waste through composting methods, rich in organic materials, to replace chemical fertilizer (Mohd Zaini *et al*., 2023). While this practice shows promise, its reliance on heterogeneous food waste materials presents challenges in standardizing operations and achieving consistent outcomes.

Concurrently, an interesting trend has emerged among local communities, where a traditional fermented food material, *belacan*, is being used in small amounts as a biofertilizer to promote the growth of crops, including chili. *Belacan*, a popular fermented shrimp paste in Southeast Asian cuisines, exhibits well-defined production processes and uniformity, making it an attractive alternative to heterogeneous food waste (Abedelazeez *et al*., 2023). Although primarily used as a condiment, there is an increasing practice of employing *belacan* as a biofertilizer due to its ready availability, cost-effectiveness, and perceived safety as an organic product, aligning with the demand for organic agriculture (Mohd Zaini *et al*., 2022).

The effectiveness of *belacan* as a biofertilizer may be attributed to its bacterial populations and nutrient content. Bacteria are known to confer beneficial effects by enhancing nutrient content, suppressing pathogen growth, and contributing to overall plant health (Lamont *et al*., 2017). Their presence in the plant microbiome across various plant species suggests a significant role in promoting plant growth and development (Yu *et al*., 2020).

Against this backdrop, this study aims to investigate the growth-promoting effects of *belacan* on chili plants, aligning with the

Article History
Accepted: 9 April 2025
First version online: 30 June 2025
Cite This Article:
Abedelazeez, K.J.D., Ahmad Fauzi, N.A.S., Zainuddin, N., Mohd Zaini, N.S., Yaacob, J.S. & Abd Rahim, M.H. 2025. Exploring the potential of shrimp paste (belacan) and its indigenous microorganisms (IMO) as a growth enhancer for *Capsicum annum* L. (Chilli) plants. Malaysian Applied Biology, 54(2): 104-110. https://doi.org/10.55230/mabjournal.v54i2.2991

local tradition of utilizing this substance for crop enhancement (Mohd Zaini *et al*., 2022). Our aims include evaluating the effects of *belacan*, its inherent bacteria and biofertilizing abilities, and their synergistic interaction with Nitrogen Phosphorus Potassium (NPK) fertilizer on chili plant growth. Additionally, we seek to determine the optimal concentration of these substances for maximizing its growth-enhancing effects. We hope this study contributes valuable insights into sustainable agriculture practices and may pave the way for wider adoption of this traditional fermented food material in modern farming methods.

MATERIALS AND METHODS

Sample collection

Malaysian *belacan*, a fermented shrimp paste, was sourced from local markets. The samples were then transported to the Food Bioprocessing Research Laboratory, Universiti Putra Malaysia, for further analysis. To maintain their quality, the samples were stored in brown paper bags at a refrigerated temperature of 4°C until they were ready to be used in the experiments. Chilli seeds were acquired from Green Eagle Company at Puchong, Selangor, Malaysia. For the cultivation of the plants, soil, plastic poly bags, and chemical fertilizer NPK were obtained from D Syira Enterprise, at Seri Kembangan, Malaysia.

Isolation and identification of the bacteria

The microbial diversity of *belacan* was assessed by isolating bacteria on nutrient agar (NA) and De Man–Rogosa–Sharpe (MRS) agar. Following incubation, isolates were chosen based on morphological characteristics and preserved at -80°C in glycerol stocks for long-term storage and on Nutrient Broth (NB) slants at 4°C for short-term storage.

The genomic DNA of selected bacterial isolates was extracted using the cetyltrimethylammonium bromide (CTAB) method. Colonies were grown in NB at 28 ± 2°C. The cells were centrifuged and resuspended in Tris-EDTA (TE) buffer, followed by the addition of Sodium Dodecyl Sulfate (SDS), sodium hydroxide (NaCl), and CTAB-NaCl. After incubation at 65°C, chloroform/isoamyl alcohol was added and centrifuged, and the DNA layer was extracted with phenol/chloroform/isoamyl alcohol twice. DNA precipitation was achieved using isopropanol and ammonium acetate. Following centrifugation, the DNA was washed with ethanol, dried, resuspended in sterile distilled water, and stored at -20°C.

The 16S rDNA gene was amplified using primers BSF 8_20 and BSR 1541_20. Polymerase chain reaction (PCR) was executed with specific conditions in an Eppendorf Mastercycler Pro S Vapo. Protect. Amplified products, approximately 1500 bp, were evaluated using 0.8% agarose gel electrophoresis at 80 V for 40 min. Band sizes were estimated using a 1 kb DNA ladder. Products were sent to Apical Scientific, Selangor, Malaysia for bidirectional sequencing. Acquired sequences were aligned with BioEdit and matched against the GenBank databases at NCBI. For additional markers (ITS, EF-1α & COX1), PCR products were purified and sequenced at MyTACG, Serdang, Malaysia. Sequences were refined with BioEdit and compared to the GenBank database via NCBI BLAST for phylogenetic analyses. Gel electrophoresis further verified the DNA amplification. PCR products were subjected to electrophoresis at 90 V for 30 min, visualized under UV light, and captured using a BioRad Gel DocTM 2000 system.

Bacterial count

Belacan samples (1 mL) were diluted with 9 mL peptone water. Then, the samples were homogenized, and serial dilutions were carried out. The standard pour plate method was used for the enumeration. After the incubation, colonies were counted between 3 and 300, and the results were expressed as a logarithm of colony-forming units ($\log_{10}$cfu/ml)

*Colony Forming Units (CFU)/mL = (Number of colonies*dilution factors) / volume of the culture plate*.

Analytical procedures

The pH of the fermented *belacan* was measured at 2-day intervals during fermentation using a digital pH meter (Mettler Toledo pH). The Nitrogen/Phosphorus/Potassium (NPK) content of *belacan* at days 0 and 8 was determined at the Soil Analytical Laboratory, Faculty of Agriculture at Universiti Putra Malaysia. Phosphorus (P) content was determined using the Bray II method. Potassium (K) was analyzed via a shaking method utilizing ammonium acetate as the extracting solution. Nitrogen (N) quantification was carried out through combustion, utilizing a Carbon, Nitrogen and Sulphur analyzer.

For phosphate solubilizing activity, the bacteria were centrifuged at 10,000 rpm for 5 min. 60 µL of supernatant bacteria was pipette into the well of Pikovskaya agar. The procedure was performed in sterile conditions using a laminar flow system. The halo zone (Figure 1) was measured in a two-day interval for a total of 8 days of the incubation of plates at 28°C.

To determine the production of auxin (indole-3-acetic acid) (IAA), the isolates were assessed using the Salkowski reagent method (Das *et al*., 2019). In this process, the isolates' broth cultures were spun down, and the clear liquid on top was mixed with Salkowski reagent at a 1:2 ratio. The Salkowski reagent was prepared by combining 150 mL of concentrated sulfuric acid (H_2SO_4), 250 mL of distilled water, and 7.5 mL of a solution containing 0.5 M $FeCl_3 \cdot 6H_2O$, following a standard procedure (Patten & Glick, 2002). This mixture was left in the dark at room temperature for 30 min, resulting in color development. Isolates that turned pink or red were identified as - IAA producers and used in subsequent experiments. To quantify the IAA produced, a spectrophotometric method was employed at 535 nm, and the concentration was determined using a standard IAA curve. The bacterial concentration was also measured using a standard curve within the range of 0.5-10 µg IAA.

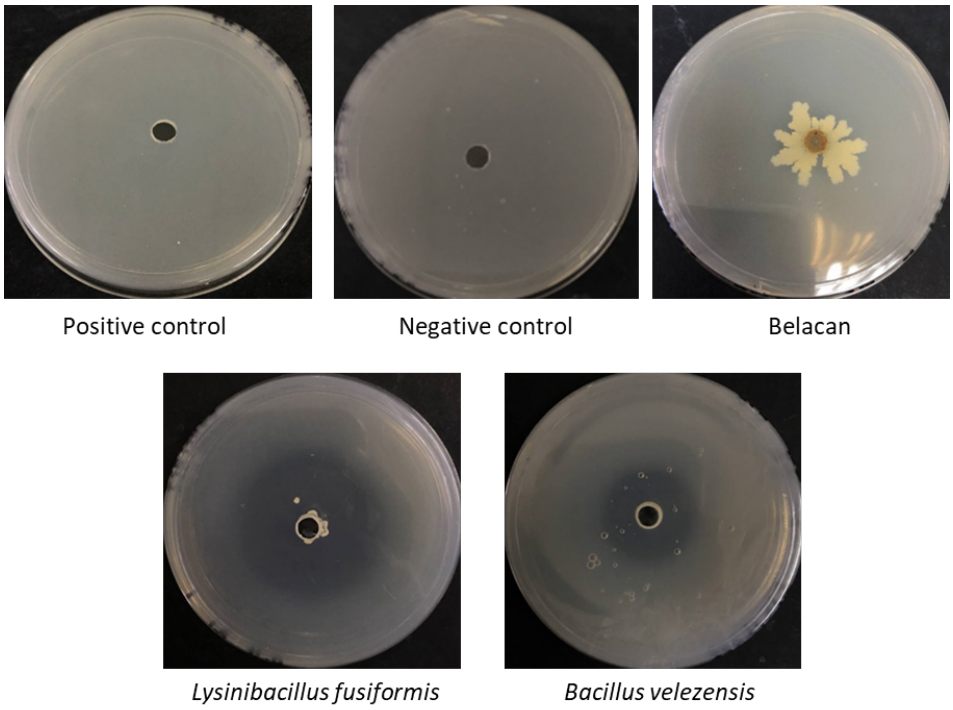

Fig. 1. The example of the halo zone produced during the phosphate solubilization test. NPK was used as the positive control, while water served as the negative control.

Treatments, experimental design, and greenhouse size

The experimental site chosen for this study was a greenhouse located at the Faculty of Food Science and Technology, at Universiti Putra Malaysia. The planting commences on August 12, 2022, and concludes on April 15, 2023. This study employed ten treatments, as follows:

1. A positive control of NPK (chemical fertilizer),
2. Negative control (no fertilizer),
3. Stock (undiluted) of *belacan* + IMO + NPK,
4. First dilution of *belacan* + IMO + NPK,
5. Stock (undiluted) of *belacan* - IMO - NPK,
6. First dilution of *belacan* + IMO - NPK,
7. Stock (undiluted) of *belacan* + IMO - NPK,
8. First dilution of *belacan* - IMO + NPK,
9. Stock (undiluted) of *belacan* - IMO + NPK,
10. First dilution of *belacan* - IMO - NPK.

*IMO – Indigenous microorganisms; NPK – Nitrogen Phosphorus Potassium; "-" without; "+" with

The treatment combinations were arranged in a randomized complete block design (RCBD) with four replications. The greenhouse size used for the experiment was 11 × 4 meters, which provided a total area of 44 square meters. Each block consisted of four plants, which were individually planted in plastic bags sized 16 × 16 cm, spaced 1 meter apart from each other.

Germination and planting of the chili

To initiate the germination process, the chili seeds were soaked in water at room temperature for 12 to 24 hr to soften the seed coat. Once adequately soaked, a seed-starting mix with peat moss soil was transferred to the tray, leaving a small space at the top. The soil was maintained in a moist condition, ensuring that waterlogging was avoided. Small holes were created in the soil (approximately 1/4 inch deep), and one seed was placed in each hole. The seeds were then covered with soil and gently pressed down to ensure proper seed-to-soil contact (Figure 2).

A warm and humid environment (70% to 90%) is maintained for optimal germination by covering the seed tray with plastic wrap or a clear lid (Figure 2A & 2B), creating a greenhouse effect that retains moisture and heat. The tray is kept in a warm place, with temperatures between 25 to 30°C, with a minimum of 20°C and a maximum of 35°C. Regular watering, using tap water, is performed by checking the soil daily and watering when the top layer feels dry to the touch while avoiding overwatering. The germination period typically takes around 7 to 14 days for chili seeds to sprout. Once germination occurs and seedlings emerge, the plastic wrap or lid is removed to allow for proper air circulation. The seed tray is then transferred to a sunny location to provide sufficient light (>50%) for seedling growth (Figure 2C). When the seedlings reach a height of 2 to 3 inches, they are transplanted into individual pots or a larger container filled with well-draining soil. The seedlings are progressively exposed to outdoor conditions for acclimation to temperature, sunlight, and wind fluctuations.

The soil mixture of Coconut Coir Dusit (CCD) and Bio Soil (a blend of organic materials, including compost, vermiculite, and perlite) was prepared in a ratio of 1 CCD to 2 Bio Soil (Figure 2). The prepared soil mixture was then used to fill 16x16 plastic poly bags, leaving approximately an inch of space from the top (Figure 2D). The soil mixture was lightly pressed to remove air pockets and ensure a level surface. Before transplanting the seedlings, the soil mixture in the poly bags was thoroughly watered using a tap water drip system, running for a duration of 15 to 20 min daily with an average of 2000 mL over 60 min to achieve even moisture throughout. This step helps prevent the roots from drying out during the transplanting process. After one week of transplanting, the seedlings were fertilized with a balanced liquid fertilizer and the treatments (Figure 2D).

Fig. 2. (A) and (B) The seed tray was covered with plastic wrap (C) Chilli seedlings during the germination stage (D) 16×16 plastic poly bag with the soil mixture of Coconut Coir Dusit (CCD) and Bio Soil, and chili seedlings after a week of transfer

Fertilizer preparation and application

The autoclaved molasses at a concentration of 20% was mixed with an equal ratio of *belacan*, creating a homogenous blend. This mixture was fermented for 7 days at room temperature to develop beneficial microbial activity and nutrient transformation. For the first dilution of the treatments, 1 mL of the stock solution was diluted with 9 mL of tap water.

In treatments involving IMO, the fermentation process was halted by storing the samples in small batches at a temperature range of -2 to -5°C to preserve the microbial composition. For treatments without IMO, the samples underwent pasteurization to eliminate potential IMO presence. Microbial growth tests were conducted to validate the effectiveness of the pasteurization process. In certain study groups, a combination of NPK fertilizer and organic treatments was mixed in equal proportions to harness the combined benefits of both types of fertilizers.

After a week of transplanting the seedlings, the treatments and NPK fertilizer were administered once a week, in the morning from 7 to 9 am. A standardized volume of 10 ml of the treatments or NPK fertilizer was applied to each plant to ensure consistency in the treatment process.

Measurements and data collection

Measurements of plant height, leaf length, leaf width, stem thickness, and chlorophyll content were recorded every two weeks from the 5th to the 14th week of the experiment. Multiple measurements were taken for each parameter at different locations within the experimental plot or greenhouse to account for natural variability among the plants.

For plant height, a measuring tape was used to measure the distance from the base of the plant to the tip. Leaf length and width were measured by selecting a mature and healthy leaf from the middle of the plant. The leaf was placed flat against a ruler or tape measure, and the length was measured from the base to the tip along the center vein. The width was measured by placing the ruler horizontally across the widest part of the leaf, perpendicular to the central vein. Multiple leaves from different parts of the plant were measured to obtain an average. To measure stem thickness, a stem approximately 5 cm above the soil level was selected. A caliper or ruler was used to measure the diameter of the stem.

The SPAD-502 meter was used for chlorophyll measurement. Healthy and fully expanded leaves were selected for measurement, and their surfaces were cleaned to remove any interference. The meter probe was placed gently on the leaf surface, covering a representative area, and the measurement button was pressed to obtain the SPAD value, representing the relative chlorophyll content.

Statistical analysis

The variability within and between groups was evaluated using the two-way analysis of variance (ANOVA) approach. Additionally, a mean comparison of pairwise differences between the treatment groups was performed using the least significant differences (LSD) method. All the analyses were carried out using Minitab software version 2.1.

RESULTS AND DISCUSSION

The isolation and identification of potential plant-growth-promoting bacteria in the *belacan*

In general, all *belacan* and molasses ratios yielded substantial bacterial growth, and subsequent isolation revealed the presence of two prominent types of bacteria. To maintain experimental simplicity and ensure consistency, we selected the 1:1 ratio of molasses to fermented foods as the foundational medium for bacterial cultivation in this study., which produced 13.4 × 10^7 $\log_{10}$ cfu/mL.

Read 1 (R1) and Read 2 (R2) colors were isolated from *belacan* (Figure 3). The identity of the two selected bacterial isolates was confirmed by molecular identification. PCR analysis of the nucleic acid extracted from these isolates produced an amplicon at 1500 bp on a 1% agarose gel. Read 1 (R1) was *Lysinibacillus fusiformis* strain and Read 2 (R2) was *Bacillus velezensis* strain. The partial 16S rDNA sequences of four isolates were determined and aligned to other known sequences deposited in GenBank. The sequence similarity between all identified species showed identities of 99 -100% with reference isolates in the GenBank (Table 1).

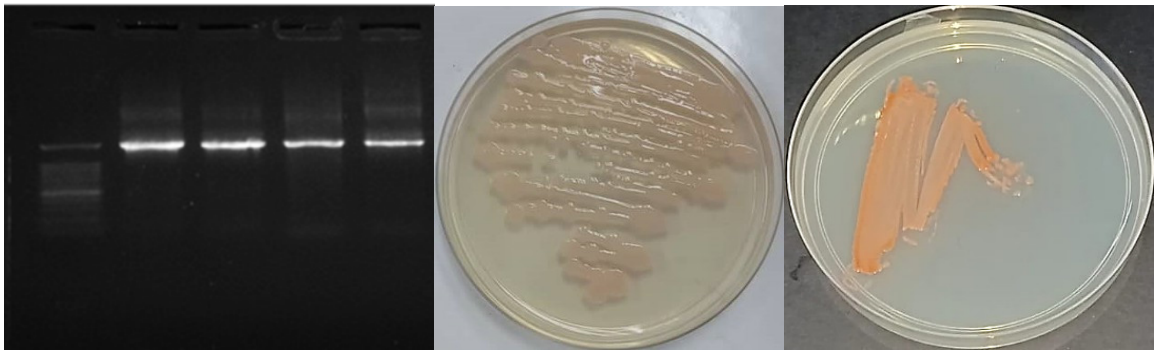

Fig. 3. Selected bacterial colonies from *belacan* and their 16S rDNA amplification, showing fragments of 1500 base pairs on a 1% agarose gel.

Table 1. Identification of bacterial isolates using 16S rDNA gene sequencing

Isolates	Species	Max similarity (%)	Accession Number
R1	*Lysinibacillus fusiformis*	99.70-100	MW035604.1
R2	*Bacillus velezensis*	99.82-100	MT649755.1

Based on the literature search, bacterial isolates R1 and R2 showed the potential to be plant-growth-promoting microorganisms (Tiwari *et al*., 2019). From a morphological viewpoint, the color and size of these two colonies vary from red to reddish, with small to medium in size. Colony margins also varied, entire and filiform, with flat to raised elevations. The existence of morphological differences or variations between bacterial strains can occur due to differential gene expression (Muzaifa *et al*., 2023). This discovery implies the presence of a diverse array of microbial strains within the fermentation matrix, potentially contributing to the intricate flavor profile and functional attributes of *belacan*. This is further substantiated by the substantial number of colonies observed on the initial MRS agar, with each colony likely representing a distinct bacterial species. As previously reported in our earlier publication, the microflora of *belacan* is known to encompass various microbial communities, including fungi, yeast, and different types of bacteria (Mohd Zaini *et al*., 2022).

The *in vitro* fertilizing potential of *belacan* and its bacteria

Based on Table 2, *Lysinibacillus fusiformis* (R1) and *Bacillus velezensis* (R2) can solubilize phosphate, as indicated by the presence of an inhibition zone on the Pikovskaya agar. The inhibition zone of both bacteria showed an increasing trend after 8 days. Both of them were not significantly different; however, NPK (as positive control) and negative control (water) did not have the inhibition zone, indicating that they could not solubilize the phosphate. Previous literature indicated that these bacteria can convert insoluble forms of phosphorus into soluble forms, consequently enhancing the subsequent accessibility of phosphorus to plants (Elhaissoufi *et al*., 2022). This could also be correlated with a study by Alori *et al*., (2017) showing that the phosphate-solubilising bacteria can potentially replace the conventional bio-fertilizer in commercial agriculture.

Indole-3-acetic acid (IAA) serves as the primary plant auxin, overseeing essential growth and developmental functions like cell division, elongation, tissue specialization, apical dominance, and reactions to stimuli such as light, gravity, and pathogens (Ali *et al*., 2022). Based on Table 2, both R1 and R2 contained a significant amount of IAA, as indicated by the statistical significance relative to the controls; however, *Bacillus velezensis* (R2) has a significantly higher level of IAA compared to *Lysinibacillus fusiformis* (R1) with a value of 23.67 ± 0.47^b. The positive control had a low level of IAA, while the negative control had no detectable IAA. *Belacan* had an exceptionally high IAA level of 162.39 ± 4.16^a, which is significantly higher than all other treatments and controls. *Belacan*, or its fermentation process, may contain substances or compounds that naturally have a high

IAA content or promote IAA production.

Table 2. The *in vitro* biofertilising potential of *belacan* and its bacteria. The superscript letters indicate significance within the same column

Treatment	Phosphate solubilization zone	Auxin level
Positive Control (NPK)	0.00 ± 0.000^{b}	2.39 ± 0.236^{d}
Negative Control (Water)	0.00 ± 0.000^{b}	0.00 ± 0.000^{d}
Lysinibacillus fusiformis (R1)	1.77 ± 0.252^{a}	12.56 ± 0.472^{c}
Bacillus velezensis (R2)	1.53 ± 0.252^{a}	23.67 ± 0.472^{b}
Belacan	0.00 ± 0.000^{b}	162.39 ± 4.16^{a}

Plant growth under *belacan* treatments

The investigation of the plant growth parameters - plant's height, leaf length, leaf width, stem thickness, and chlorophyll content involved three main areas:

1. The impact of fermented food concentration.
2. The impact of IMO without NPK.
3. The impact of IMO with NPK.

The fermentation process of *belacan* appears to enrich the substrate with beneficial microbes that actively promote plant growth. To the best of our knowledge, this is the first study to elucidate the effect of indigenous microorganism (IMO) as a biofertilizer within *belacan*. In the conducted growth experiments, distinct patterns emerged regarding nutrient supplementation (Table 3). Firstly, the combination of *belacan* (diluted or undiluted) with IMO and NPK (nitrogen, phosphorus, and potassium) consistently resulted in the strongest overall growth increments, as evidenced by Treatments 4 and 8. Secondly, in situations where NPK was absent, but IMO was present, growth increments remained at an average level (2 & 6), indicating the significance of IMO support. However, when both NPK and beneficial IMO were lacking (5 & 9), plant growth was notably poor, emphasizing the pivotal role played by these nutrients and microorganisms in fostering healthy and vigorous plant development under experimental conditions.

Table 3. The effect of *belacan* biofertilizer treatments on the plants' height, leaf length and width, stem thickness, and chlorophyll content of chili plants for each group of treatments

Treatment	Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Stem Thickness (mm)	Chlorophyll (μg/cm²)
Negative control (water)	34.250 ± 2.625^{i}	17.542 ± 1.1414^{g}	5.6042 ± 0.5894^{f}	5.1217 ± 0.5755^{d}	23.092 ± 5.895^{f}
Stock + IMO – NPK	55.958 ± 15.730^{d}	21.792 ± 3.021$^{c\,d}$	6.9792 ± 0.9610^{d}	6.5138 ± 1.3020^{c}	51.950 ± 11.018^{b}
Stock – IMO + NPK	43.000 ± 12.904^{f}	19.583 ± 2.376^{e}	6.000 ± 0.6255^{e}	5.0954 ± 0.7958^{d}	38.125 ± 3.441^{c}
Stock + IMO + NPK	61.833 ± 18.199^{b}	22.583 ± 3.998^{c}	7.4375 ± 1.2186$^{b\,c}$	7.0196 ± 1.6682^{b}	55.313 ± 14.465^{a}
Stock – IMO - NPK	39.583 ± 9.108^{g}	18.208 ± 1.532$^{f\,g}$	5.5000 ± 0.4663^{f}	5.4363 ± 0.9763^{d}	31.996 ± 1.067^{d}
Diluted Stock + IMO – NPK	58.708 ± 18.679^{c}	21.167 ± 3.703^{d}	7.2292 ± 1.0527$^{c\,d}$	6.3658 ± 1.2358^{c}	53.096 ± 14.374^{b}
Diluted Stock – IMO + NPK	49.500 ± 13.609^{e}	19.417 ± 2.781^{e}	5.6042 ± 0.5513^{f}	5.2083 ± 0.7777^{d}	38.429 ± 3.072^{c}
Diluted Stock + IMO + NPK	66.292 ± 21.798^{a}	24.458 ± 4.950^{a}	7.6875 ± 1.5798$^{a\,b}$	7.4958 ± 2.3597^{a}	54.450 ± 12.297^{a}
Diluted Stock – IMO - NPK	35.750 ± 5.900^{h}	18.958 ± 2.010$^{e\,f}$	5.5000 ± 0.4170^{f}	5.1125 ± 0.6423^{d}	26.213 ± 3.504^{e}
Positive control (NPK)	63.083 ± 18.909^{b}	23.479 ± 4.694^{b}	7.8542 ± 1.4998^{a}	7.2021 ± 1.9464$^{a\,b}$	55.542 ± 14.206^{a}

Each value from the table represents the mean ± standard deviation (n=4). Means with different letters represent the significant differences (Tukey's test, p<0.05) between negative control (water), positive control NPK (chemical fertilizer), and *belacan* treatments.
IMO – Indigenous microorganisms; NPK – nitrogen, phosphate, potassium

The significance of plant-growth-promoting IMO has been well-established in previous literature, and in our experiments, we successfully isolated and identified several potential bacteria associated with IMO. This aligns with our prior publication (Mohd Zaini *et al*., 2022), which highlighted *Bacillus* spp. is well recognized for its ability to boost plant growth and act as a biofertilizer and biocontrol agent to combat plant diseases, as described in Ahemad and Kibret (2014) and Tiwari *et al*. (2019). An excessive population of IMO can have detrimental effects on plant health, thus, an appropriate dosage is essential during application. In our experiment, we have shown that undiluted *belacan* application can lead to lower growth, as compared to the first dilution. When IMO numbers are too high, they may compete with each other for limited resources, potentially disrupting the microbial balance in the soil (Li, 2022). This imbalance can lead to unfavorable interactions between plants and microbes, increase the risk of plant diseases, alter nutrient availability, and induce stress responses in plants (Kumar & Verma, 2018). Therefore, maintaining a balanced microbial community in the rhizosphere is crucial to ensure that IMO provides the intended benefits without causing

unintended harm to plant growth and health.

CONCLUSION

Overall, this study sheds light on the intricate relationship between fermented food substrates, particularly *belacan*, and plant growth. The identification of bacteria further emphasizes the potential of these substrates as valuable tools for enhancing agricultural practices and maximizing crop yields. The findings presented herein not only expand our understanding of the microbial dynamics in fermented foods but also pave the way for future research and practical applications in the field of plant growth promotion.

ACKNOWLEDGEMENTS

The authors thank the Universiti Putra Malaysia Inisiatif Putra Siswazah Grant, with a reference to UPM.RMC.800-2/1/2022/GP-IPS/9740400 and the Ministry of Higher Education, Malaysia (FRGS grant no. 01-01-20-2323FR, with reference code: FRGS/1/2020/STG01/UPM/02/2) for the financial support.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abedelazeez, K.J.D., Nurul Solehah, M.Z., Jaafar, A.H., Meor Hussin, A.S., Wan-Mohtar, W.A.A.Q.I. & Abd Rahim, M.H. 2023. Production, organoleptic, and biological activities of *belacan* (shrimp paste) and *pekasam* (fermented freshwater fish), the ethnic food from the Malay Archipelago. Sains Malaysiana, 52(4): 1217–1230. https://doi.org/10.17576/jsm-2023-5204-14

Ahemad, M. & Kibret, M. 2014. Mechanisms and applications of plant growth promoting *Rhizobacteria*: Current perspective. Journal of King Saud University - Science, 26(1): 1–20. https://doi.org/10.1016/j.jksus.2013.05.001

Ali, H., Sallahuddin, N., Ahmed Shamsudin, N.H., Mohd Zain, N.A., Ibrahim, M.H. & Yaacob, J.S. 2022. Abiotic stress induces morphological, physiological, and genetic changes in *Orthosiphon Stamineus Benth*. *in vitro* cultures. Horticulturae, 8(2): 153. https://doi.org/10.3390/horticulturae8020153

Alori, E.T., Glick, B.R. & Babalola, O.O. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology, 8: 971. https://doi.org/10.3389/fmicb.2017.00971

Azlan, A., Sultana, S., Huei, C.S. & Razman, M.R. 2022. Antioxidant, anti-obesity, nutritional and other beneficial effects of different chili pepper: A Review. Molecules, 27(3): 898. https://doi.org/10.3390/molecules27030898

Das, S., Nurunnabi, T.R., Parveen, R., Nur Mou, A., Islam, M.E., Islam, K.M.D. & Rahman, S.M.M. 2019. Isolation and Characterization of Indole Acetic Acid Producing Bacteria from Rhizosphere Soil and their Effect on Seed Germination. International Journal of Current Microbiology and Applied Sciences. 8(*3*): 2319-7706. https://doi.org/10.20546/ijcmas.2019.803.146

Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y. & Bargaz, A. 2022. Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. Journal of Advanced Research, 38: 13–28. https://doi.org/10.1016/j.jare.2021.08.014

Kumar, A. & Verma, J.P. 2018. Does plant—microbe interaction confer stress tolerance in plants: a review. Microbiological Research, 207: 41–52. https://doi.org/10.1016/j.micres.2017.11.004

Lamont, J. R., Wilkins, O., Bywater-Ekegärd, M. & Smith, D. L. 2017. From yogurt to yield: Potential applications of lactic acid bacteria in plant production. Soil Biology and Biochemistry, 111: 1-9. https://doi.org/10.1016/j.soilbio.2017.03.015

Li, W. 2022. Cooperation vs. competition: microbiome diversity and interactions. Harvard University. https://sitn.hms.harvard.edu/flash/2022/cooperation-vs-competition-microbiome-diversity-and-interactions/ (accessed 7.23.24)

Mohd Zaini, N.S., Idris, H., Yaacob, J.S., Wan-Mohtar, W.A., Putra Samsudin, N.I., Abdul Sukor, A.S., Lim, E.J. & Abd Rahim, M.H. 2022. The potential of fermented food from Southeast Asia as biofertiliser. Horticulturae, 8(2): 102. https://doi.org/10.3390/horticulturae8020102

Mohd Zaini, N.S., Khudair, J.D.A., Mohsin, Z.A., Jitming Lim, E., Minato, W., Idris, H., Yaacob, J.S. & Abd Rahim, M.H. 2023. Biotransformation of food waste into biofertilisers through composting and anaerobic digestion: a review. Plant, Soil and Environment, 69(9), 409–420. https://doi.org/10.17221/101/2023-PSE

Muzaifa, M., Murlida, E., Nilda, C., Rozali, Z.F. & Rahmi, F. 2023. Isolation and identification of protease-producing bacteria from *belacan depik*, a traditional fermented fish of the Gayo tribe. IOP Conference Series: Earth and Environmental Science, 1177(1): 12038. https://doi.org/10.1088/1755-1315/1177/1/012038

Patten C.L., Glick B.R. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Applied Environmental Microbiology, 68(8): 3795-3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002

Tiwari, S., Prasad, V. & Lata, C. 2019. Chapter 3 - *Bacillus*: plant growth promoting bacteria for sustainable agriculture and environment. In: New and Future Developments in Microbial Biotechnology and Bioengineering. J.S. Singh and D.P. Singh (Eds.). Elsevier. Amsterdam. pp. 43-55. https://doi.org/10.1016/B978-0-444-64191-5.00003-1

Yu, A.O., Leveau, J.H.J. & Marco, M.L. 2020. Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria. Environmental Microbiology Reports, 12(1): 16–29. https://doi.org/10.1111/1758-2229.12794