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Seed Coating with Bacteria-producing Indole-3-Acetic Acid (IAA) on Germination, Seedling Growth and Nutrient Contents of Vegetable Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

Background: Several types of bacteria capable of producing indole-3-acetic acid (IAA) are commonly used for seed coating to enhance seed germination, vigor and seedling growth. This experiment aimed to study the effects of seed coating with three IAA-producing bacterial isolates on germination, vigor, seedling growth and nutrient content in seedlings.

Methods: The experiment employed a completely randomized design (CRD) with four replications and five treatments: no coating (T1), coating with Carboxymethyl Cellulose (CMC) only (T2), coating with *Enterobacter kobei* (isolate 1) (T3), coating with *Enterobacter kobei* (isolate 2) (T4) and coating with *Burkholderia paludis* (isolate 3) (T5). Laboratory and greenhouse tests were conducted to evaluate the effects on germination and seedling growth.

Result: The results revealed that three bacterial isolates *Enterobacter kobei* (isolate 1), *Enterobacter kobei* (isolate 2) and *Burkholderia paludis* (isolate 3) produced high levels of IAA. Seed coating with these isolates significantly enhanced germination and mean germination time under laboratory and greenhouse conditions. Seeds coated with *E. kobei* (isolate 1) showed significantly greater shoot and root lengths at 10 days than non-coated seeds and higher accumulation of nitrogen, phosphorus, potassium and calcium in the seedlings. Consequently, seed coating with *E. kobei* (isolate 1) is recommended for vegetable soybean seeds to improve germination, vigor and seedling growth.

Key words: Bio-fertilizer, Organic seed treatment, Plant nutrient, Seed enhancement.

INTRODUCTION

Several plant growth regulators are classified under auxins, with the first discovered being indole-3-acetic acid (IAA), which plants endogenously produce (Glick, 2005). However, extracting IAA from plants for practical use is challenging due to its rapid degradation, instability and low concentrations, making it inconvenient for large-scale agricultural applications (Glick *et al*., 2007). Therefore, synthetic auxin-like compounds, such as NAA (1-naphthylacetic acid), IBA (4-(indol-3-yl) butyric acid), 2,4-D (2,4-dichlorophe-noxyacetic acid) and 4-CPA (4-chlorophenoxyacetic acid), have been developed and widely utilized to overcome these limitations and meet agricultural needs. In addition to synthetic alternatives, significant attention has been given to the microbial synthesis of IAA by soil-dwelling bacteria, particularly plant growth-promoting rhizobacteria (PGPR), which utilize the amino acid L-tryptophan as a precursor for IAA production (Lynch, 1985). PGPR colonize plant root systems and enhance plant growth through direct mechanisms such as improved nutrient uptake (*e.g*., nitrogen fixation, phosphorus solubilization and phytohormone production) and indirect mechanisms, including pathogen suppression *via* biocontrol activity (Forni *et al*., 2017; Santoyo *et al*., 2021). Numerous studies have demonstrated the efficacy of PGPR in enhancing crop resilience and yields under abiotic stress conditions, such as drought (Akhtar *et al*., 2021; Al-Turki *et al*., 2023; Chattaraj *et al*., 2025).

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The northern region of Thailand is a central production area for vegetable soybeans [*Glycine max* (L.) Merrill], a high-value crop with significant domestic and export demand. However, farmers in this region face recurring challenges, including low seed germination percentages, weak and non-uniform seedlings and inconsistent germination rates. These issues increase susceptibility to diseases and pests, reducing crop establishment and productivity. Consequently, farmers often resort to increasing seed rates, sowing 3-4 seeds per hole and applying more basal fertilizers to compensate for seedling losses, leading to escalated production costs.

Seed coating technology combined with PGPR offers a promising solution to improve seed efficiency and plant establishment. Seed coating involves encapsulating seeds with a thin film with beneficial substances such as PGPR, phytohormones, or micronutrients. This technology facilitates precise delivery of small amounts of inoculum directly to the seed-soil interface, ensuring optimal PGPR accessibility during germination and early seedling development (Scott, 1972). As a result, seed coating can stimulate healthy root development, improve nutrient uptake and enhance early vigor, ultimately maximizing crop production (Pérez-García *et al*., 2023). Previous research has demonstrated the potential of PGPR in improving seed quality and crop performance. For example, Kangsopa and Atnaseo (2022) reported that coating soybean seeds with a concentration of 1×10^7 CFU/mL of *Stenotrophomonas* sp., isolated from kale rhizosphere, significantly improved germination rates and speeds compared to non-coated seeds. Similarly, seed coating with 1×10^7 CFU/mL of *Bacillus* sp., isolated from kale seeds, increased fresh and dry seedling weights under laboratory conditions. These findings highlight the potential for PGPR to improve seedling establishment and overall crop performance. However, research on using IAA-producing PGPR for fresh vegetable soybean seed coatings, particularly in northern Thailand, remains limited.

Therefore, this study aims to assess the effectiveness of IAA-producing bacteria isolated from the target region on seed germination, seedling growth and nutrient content of vegetable soybeans. This research is expected to contribute to developing cost-effective and sustainable agricultural practices, ultimately improving seed quality and productivity in vegetable soybean cultivation.

MATERIALS AND METHODS

Information of bacterial isolates

The study assessed the capacity of bacteria to produce indole-3-acetic acid (IAA) within the Pong District, Phayao Province, Thailand. The chemical analysis and investigation were conducted at the Soil and Advanced Fertilizer Laboratory. Simultaneously, seed quality was evaluated at the Division of Agronomy and Modern Seed Technology Research Center, Faculty of Agricultural Production, Maejo University, from January 2024 to August 2024. Soil samples were gathered from a depth of 10-15 centimeters surrounding the roots of vegetable soybean plants and underwent serial dilution. The diluted samples were cultured on potato dextrose agar (PDA) and incubated at room temperature for 3 days. Six bacterial isolates were subsequently obtained and purified using the streak plate method on nutrient agar (NA) for further evaluation of their IAA production potential. Three of the six isolates were selected for species identification using 16S rRNA gene sequencing. Genomic DNA was extracted and the 16S rRNA gene was amplified and sequenced. The sequences were compared with those in the NCBI database using BLAST, confirming the identities as *Enterobacter kobei* (isolates 1 and 2) and *Burkholderia paludis* (isolate 3).

Microbes preparation

Once pure bacterial colonies were obtained and separated on NA, they were cultured in nutrient broth (NB) supplemented with tryptophan (Fluka, Buchs, Japan) at 0.102 g/L. The culture was then agitated at 125 revolutions per minute (rpm) for 7 days. Subsequently, the cultured bacteria were centrifuged at $5{,}000 \times g$ for 5-10 minutes to separate the bacterial cells from the liquid medium. The resulting clear solution was collected for analysis to determine the quantity of indole-3-acetic acid (IAA). For comparison with the standard IAA solution (IAA Standard), indole-3-acetic acid (MW = 175.19) was used as the standard (Phyto Technology Laboratories, Shawnee Mission, KS, United States). An IAA solution with a concentration of 10 mM was prepared initially as a stock solution (dissolved in 50% Methanol). This solution was then diluted to 1 mM IAA using 50% methanol. Standard solutions with concentrations of 0, 5, 10, 20, 50, 100 and 150 µM were prepared from this one mM IAA solution. Each standard solution was prepared in a volume of 1 mL and adjusted with 50% methanol. Then, 1 mL of the clear bacterial sample solution was added to separate test tubes. Van Urk-Salkowski reagent (2 mL) was added using Salkowski's method (Ehmann, 1977) and the mixture was thoroughly agitated. The solution was then incubated in the dark for 30 minutes and the absorbance was measured at a wavelength of 530 nm using a spectrophotometer.

Coating vegetable soybean seeds

The initial germination rate of the vegetable soybean variety 67-3 was 51%, with a moisture content of 8%. The vegetable soybean seeds underwent surface sterilization using 1% sodium hypochlorite (NaOCl) for 1 minute, followed by three rinses with sterilized distilled water and drying with sterilized tissue paper. The three bacterial isolates were cultured in 500 mL Erlenmeyer flasks containing 400 mL of NB. Each isolate was inoculated using a sterile loop and incubated on a shaker at 125 rpm for 8 days. Following the incubation period, the bacterial suspension was adjusted to a concentration of 10^8 CFU/mL for seed coating. Subsequently, 50 grams of vegetable soybean seeds were coated with Carboxymethyl cellulose 0.3% w/v (CMC) using a rotary pan (model KSC-02D, CERES International Ltd., Bangkok, Thailand) at a spinning rate of 30 rpm. Five treatments were administered to the vegetable soybean seeds: no coating (T1), coating with CMC only (T2), coating with *Enterobacter kobei* (isolate 1) (T3), coating with *Enterobacter kobei* (isolate 2) (T4) and coating with *Burkholderia paludis* (isolate 3) (T5). Following the treatments, the coated seeds from each treatment group underwent moisture reduction in a forced-air oven model KKU40-2 at 33°C for 12 hours.

Seed measurement

Seed quality examination under laboratory conditions

The quality testing of 50 vegetable soybean seeds, both coated and non-coated, was conducted using the Between-Paper (BP) method with four repetitions. They were placed in a germination incubator at 25°C and 80% relative humidity with 24 hours of light exposure at 180 µE. Germination percentage was evaluated using standard procedures on days 5 (first count) and 8 (final count) (ISTA, 2019). Mean germination time was calculated by assessing normal seedlings daily for 8 days (Ellis and Roberts, 1980). Additionally, the average shoot and root lengths were measured in 10 seedlings at 10 days post-sowing following the methodology described by Jeephet *et al*. (2022).

Seed quality examination in greenhouse conditions

Germination testing of vegetable soybean seeds, both coated and non-coated, was conducted in seed trays filled with peat moss (Klasmann-Deilmann GmbH, Germany), which served as the seeding material. The first germination evaluations were assessed 5 days after planting and the final count was recorded 14 days after sowing (ISTA, 2019). The germination speed was evaluated similarly to that determined under laboratory conditions. Shoot length and fresh shoot weight were assessed 14 days after sowing. Shoots of 10 randomly selected seedlings were cut close to the planting material and then measured using a ruler (Jeephet *et al*., 2022).

Nutrient analysis in seedlings

For each treatment, vegetable soybean seedlings were prepared by germinating seeds under laboratory conditions for 10 days. The seedlings were then oven-dried at 105°C for nutrient analysis. Total nitrogen content was determined using the combustion method (Bremner, 1960), where plant tissue samples were combusted in an oxygen-rich environment and the released nitrogen gas was quantified. The phosphorus content was analyzed spectrophotometrically using the vanadomolybdate method (Legiret *et al*., 2013), in which digested plant samples reacted with vanadomolyb date reagents to form a yellow complex and the absorbance was measured. The concentrations of potassium, calcium, magnesium and iron were determined using Atomic Absorption Spectroscopy (AAS). Plant tissue samples were digested with a concentrated acid mixture and the resulting solution was analyzed based on element-specific light absorption using the AAS instrument (Paul *et al*., 2017).

Statistical analysis

The germination percentage was arcsine-transformed to normalize the data before the statistical analysis. All data were analyzed by one-way analysis of variance (ANOVA, completely randomized design) and the difference between the treatments was tested using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Quantity of Indole-3-Acetic Acid (IAA)

From soil samples collected at a depth of 10-15 cm in a vegetable soybean plot in Pong District, Phayao Province, Thailand, six bacterial isolates were obtained (Fig 1). Among these, three isolates exhibited the ability to produce indole-3-acetic acid (IAA), a plant growth regulator in the auxin group, when cultured in nutrient broth. The IAA production levels ranged from 10 to 87 µg/mL (Fig 1). Isolate 1 produced the highest IAA concentration at 87 µg/mL, followed closely by isolate 2 with 86 µg/mL, while isolates 3, 4, 5 and 6 produced IAA at 18, 15, 12 and 10 µg/mL, respectively. The top three IAA-producing isolates were

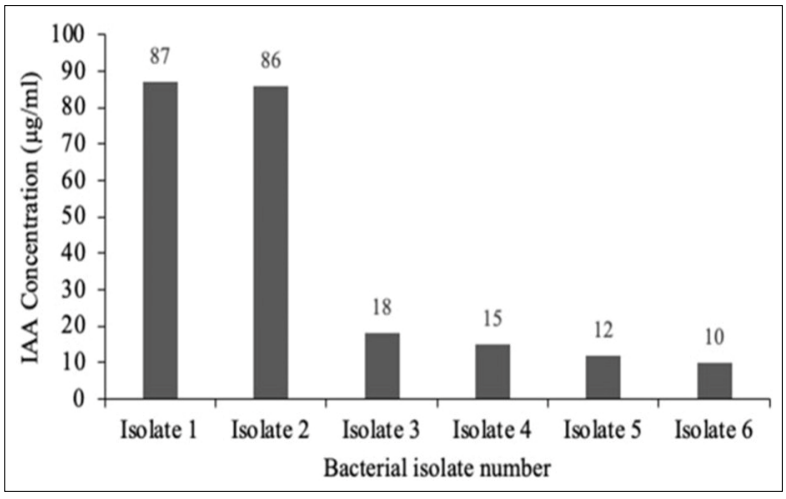


Fig 1: Screening of indoleacetic acid (IAA)-producing strains isolated from the vegetable soybean rhizosphere soil collected from Pong District, Phayao Province, Thailand.

further identified through 16S rRNA gene sequencing, which classified them as *Enterobacter kobei* (isolate 1), *Enterobacter kobei* (isolate 2) and *Burkholderia paludis* (isolate 3) (Table 1). While isolates 1 and 2 were identified as *E. kobei*, their similarity percentages were 99.72% and 99.59%, respectively. Jeephet *et al*. (2024) confirmed IAA production by *Enterobacter* sp. at 9.28 µg/mL using Salkowski's method. Similarly, Zhang *et al*. (2021) reported 3378–3477 µg/mL of IAA from *Enterobacter* sp. isolated from soil and corn stalks. Mohite (2013) found that *B. megaterium*, *L. casei*, *B. subtilis*, *B. cereus* and *L. acidophilus* produced 15-65 µg/mL of IAA, while Datta *et al*. (2011) reported 0-44 µg/mL from 15 *Bacillus* isolates. Kangsopa and Atnaseo (2022) observed IAA levels of 10.78 and 4.18 µg/mL from *Stenotrophomonas* sp. and *Bacillus* sp., respectively. *E. kobei* and *B. paludis* synthesize IAA *via* tryptophan-dependent pathways. In *E. kobei*, tryptophan is converted to IAA through the indole-3-pyruvic acid (IPyA) pathway involving aminotransferases and decarboxylases (Jha *et al*., 2011). *B. paludis* likely utilizes multiple pathways, including the IAM pathway *via* indole-3-acetamide hydrolase. These mechanisms enable all three isolates to efficiently produce IAA, supporting their role in plant growth promotion (Glick *et al*., 2007; Guo *et al*., 2011; El-Beltagi *et al*., 2024).

Seed quality

Under laboratory conditions, applying indole-3-acetic acid (IAA)-producing bacteria from the three isolates to vegetable soybean seeds significantly enhanced root germination during the initial 4-day period compared to non-coated seeds, with statistically significant differences. Seed coating with isolate 1 resulted in a 73% increase in germination, followed by *E. kobei* (isolate 2), which showed a 61% increase. Seeds coated with *E. kobei* (isolate 1) and *E. kobei* (isolate 2) also exhibited significantly faster germination rates than other treatments. Under greenhouse conditions, seed coating with CMC alone and coating with *E. kobei* (isolate 1) and *E. kobei* (isolate 2) accelerated germination rates. *E. kobei* (isolate 1) consistently showed significantly higher germination percentages than other methods, while non-coated seeds and seeds coated with CMC alone had longer mean germination times (Table 2).

The results indicate that seed coating significantly improved germination percentages, particularly with *E. kobei* (isolate 1) and *E. kobei* (isolate 2), which were identified as *E. kobei* with percentage similarities of 99.72% and 99.59%, respectively, based on 16S rDNA sequencing. *E. kobei* was found to produce IAA at concentrations of 87 µg/mL and 86 µg/mL, which significantly enhanced seed quality. IAA stimulates amino acid synthesis in the embryo and activates enzymes involved in starch degradation within the endosperm (Chakraborti and Mukherji, 2003; Mendes *et al*., 2007), providing energy for seed germination and accelerating the process. Previous studies have reported that *E. kobei* is a high IAA-producing bacterium capable of enhancing seed germination and vigor (Zhang *et al*., 2021).

This study's increased radicle emergence percentage and faster germination rates support these findings. Nakamura *et al*. (1978) demonstrated that appropriate IAA concentrations significantly promote germination and the

Table 1: Identification of IAA-producing bacteria by 16S rDNA sequencing.

Isolate number	Identified bacteria	% Similarity	Accession number
Isolate 1	*Enterobacter kobei*	99.72	CP017181.1
Isolate 2	*Enterobacter kobei*	99.59	CP017181.1
Isolate 3	*Burkholderia paludis*	99.32	KT159931.1

Table 2: Radicle emergence (RE), emergence percentage (EME), germination percentage (GE) and mean germination time (MGT) of vegetable soybean seed after coating with bacteria-producing Indole-3-Acetic Acid (IAA), tested under laboratory and greenhouse conditions.

Treatment[1]	Laboratory condition			Greenhouse condition		
	RE (%)	GE (%)	MGT (day)	EME (%)	GE (%)	MGT (day)
T1	94 b [2,3]	51 c	9.02 a	36 c	30 d	6.48 a
T2	99 a	65 bc	9.42 a	69 a	70 b	6.35 a
T3	99 a	88 a	7.88 c	74 a	81 a	5.10 b
T4	99 a	82 ab	7.37 c	73 a	79 ab	5.12
T5	99 a	76 bc	8.29 b	54 b	72 c	5.24 b
F-test	*	**	*	**	**	**
CV.%	4.44	7.24	1.20	7.48	6.02	1.83

*,** : Significantly different at P≤0.05 and P≤0.01, respectively.

[1]T1 = non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4 = Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Data are transformed by the arcsine before statistical analysis and the back-transformed data are presented.

[3]Means within a column followed by the same letter are not significantly at P≤0.05 by DMRT.

concentrations produced by *E. kobei* (isolate 1) and *E. kobei* (isolate 2), 87 and 86 µg/mL, respectively, fall within the effective range for improving the quality of vegetable soybean seeds. Furthermore, Ogawa *et al*. (2003) reported that IAA concentrations of 20 mg/L^{-1} used for seed treatment increased gibberellic acid (GA) levels, which are critical in promoting seed germination. Additionally, *Enterobacter* sp. has been shown to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that degrades ACC into α-ketobutyric acid and ammonia, effectively lowering ethylene levels and promoting seed germination (Glick *et al*., 2007).

Seedling growth of vegetable soybean

Under laboratory conditions, evaluations over the first 3 days revealed that seed coating with all three bacterial isolates enhanced shoot length compared to non-coated seeds and those coated with CMC alone. During the 10-day assessment period, *E. kobei* (isolate 1) significantly promoted shoot growth compared to all other treatments. By day 4, seeds coated with *B. paludis* (isolate 3) exhibited slower shoot development, while *E. kobei* (isolate 2) achieved comparable shoot lengths to *B. paludis* (isolate 3), both of which were significantly greater than non-coated seeds (Table 3).

For root length, seeds coated with *E. kobei* (isolate 1) and *E. kobei* (isolate 2) demonstrated significantly greater root growth than non-coated seeds. By day 6, seedlings coated with *B. paludis* (isolate 3) showed root lengths approaching those of *E. kobei* (isolate 1) and *E. kobei* (isolate 2). Over the 10 days, seed coatings with all three bacterial isolates consistently resulted in longer root lengths than non-coated seeds (Table 4). Fig 2 shows that seedlings from seeds coated with *E. kobei* (isolate 1) exhibited enhanced shoot and root development compared to non-coated seeds. Additionally, seeds coated with *E. kobei* (isolate 2) and *B. paludis* (isolate 3) showed a consistent trend of improved seedling growth relative to non-coated seeds. The evaluation of shoot and root lengths revealed significant differences in vegetable soybean seedlings across treatments over the 10-day period. Seed coating with *E. kobei* (isolate 2) significantly promoted shoot and root growth compared to non-coated seeds. IAA production levels for *E. kobei* (isolate 1) and *E. kobei* (isolate 2) were 87 and 86 µg/mL, respectively, suggesting that bacterial-derived IAA plays a key role in promoting cell elongation in shoots and roots. IAA stimulates cell

Table 3: Effect of bacteria producing indole-3-acetic acid (IAA) on shoot length in 10 days.

Treatment[1]	Shoot length (cm)								
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
T1	0.42b	1.25b	2.35ab[2]	3.25bc	5.19b	5.49b	8.38b	8.95b	9.35b
T2	0.46b	1.37ab	2.45ab	3.56ab	4.85b	5.15b	6.20c	6.90c	8.25c
T3	0.52a	1.44a	2.62a	3.54ab	7.57a	7.87a	9.27a	10.03a	10.75a
T4	0.53a	1.42a	2.48ab	3.69a	6.90a	7.70a	8.39b	9.89a	9.51b
T5	0.50a	1.43a	2.16b	3.12c	3.39c	3.69c	5.39d	6.62c	8.43c
F-test	**	**	**	**	**	**	**	**	**
CV.%	8.54	11.25	9.74	6.70	10.46	9.96	2.58	1.22	2.76

** : Significantly different at P≤0.01.

[1]T1 = Non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4 = Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

Table 4: Effect of bacteria producing indole-3-acetic acid (IAA) on root length in 10 days.

Treatment[1]	Root length (cm)								
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
T1	0.60c[2]	6.02b	5.12b	9.01c	10.20b	10.50b	10.79c	11.46b	12.55b
T2	1.11b	6.08b	5.08b	9.11b	10.26ab	10.48b	10.70c	11.62b	12.12b
T3	1.18a	6.83a	7.67a	10.44a	11.00a	11.30a	12.90a	13.50a	14.05a
T4	1.19a	6.96a	7.52a	9.22b	10.52ab	11.18a	12.30a	13.40a	13.86ab
T5	1.02b	5.39c	7.21a	9.32b	10.64ab	11.20a	11.28b	13.31a	13.72ab
F-test	**	**	**	**	**	**	**	**	**
CV.%	5.24	6.25	11.42	2.20	1.59	1.54	0.82	0.79	0.60

** : Significantly different at P≤0.01.

[1]T1 = Non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4 = Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

elongation, division and differentiation (Shahab *et al*., 2009; Saleem *et al*., 2021; Hosni *et al*., 2023). Previous studies have reported that *Enterobacter* sp. produces indole-3-acetic acid (IAA), which enhances root growth by promoting cell division and elongation at the root tip. This increases the root surface area, improving nutrient absorption efficiency (Yang *et al*., 2009; Guo *et al*., 2011). In contrast, seed coating with *B. paludis* (isolate 3) was observed to hinder root growth. This may be due to its IAA production, which could activate ACC synthase, leading to the synthesis of ACC, a precursor of ethylene. Ethylene inhibits root growth by reducing primary root length (Glick, 2005).

Under greenhouse conditions, leaf development typically begins around day 6 after sowing. Evaluations revealed that seed coating with Isolate 1 significantly increased the leaf count compared to all other treatments and this effect persisted throughout the 10-day period. Seed coating with *E. kobei* (isolate 2) also resulted in a higher leaf count compared to non-coated seeds. Shoot height measurements revealed that all three bacterial isolates significantly promoted shoot growth compared to non-coated seeds, with *E. kobei* (isolate 1) and *E. kobei* (isolate 2) exhibiting the most substantial increases in shoot height (Table 5). The results show that coating seeds with IAA-producing bacteria significantly improves vegetable soybean seedling growth compared to non-coated seeds (Table 6). After germination, seedlings rapidly develop cotyledons, elongated hypocotyls and true leaves by day 6, likely due to bacterial IAA stimulating root and shoot elongation (Glick, 2005; Shahzadi *et al*., 2022). Faster



T1 = Non-coated seed; T2 = Coated seed + CMC only; T3 = Coated seed + *Enterobacter kobei* (isolate 1); T4 = Coated seed + *Enterobacter kobei* (isolate 2); and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

Fig 2: The seedling growth of vegetable soybean was examined under laboratory conditions 10 days after planting.

Table 5: Effects of bacteria producing indole-3-acetic acid (IAA) on number of vegetable soybean leaves (leaves/plant).

Treatment[1]	Number of vegetable soybean leaves (leaves/plant)				
	6th day	7th day	8th day	9th day	10th day
T1	1b[2]	2b	3b	3b	4b
T2	1b	2b	3b	3b	4b
T3	2a	3a	4a	4a	6a
T4	1b	2b	3b	3b	5ab
T5	1b	2b	3b	3b	4b
F-test	**	**	ns	ns	*
CV.%	11.21	15.05	13.97	13.97	12.28

ns, *,** : not significantly different, significantly different at P≤0.05 and P≤0.01, respectively.

[1]T1 = non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4 = Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

establishment enhances nutrient uptake through leaves, supporting vigorous growth (Yang *et al*., 2009). Similarly, Bhandari *et al*. (2009) reported that IAA affects root length and plant height in *Verbascum thapsus*, with 50 ppm increasing leaf and node numbers and 200 ppm enhancing leaf area, flowering and yield.

Amount of plant nutrients

Seed coating with indole-3-acetic acid (IAA)-producing bacteria significantly enhanced the accumulation of macronutrients and micronutrients in 8-day-old vegetable soybean seedlings, except for magnesium, which showed no significant differences among treatments. Seeds coated with *E. kobei* (isolate 1) had the highest nitrogen and calcium content. All seed coating treatments (T2–T5) increased phosphorus levels compared to non-coated seeds. Coating with CMC alone (T2) or in combination with *E. kobei* (isolate 1) resulted in higher potassium content than *B. paludis* (isolate 3) and non-coated seeds. Additionally, *E. kobei* (isolate 1) exhibited a significantly higher iron content compared to *B. paludis* (isolate 3), although no significant difference was observed relative to other treatments (Table 7).

Coating with *E. kobei* (isolate 1) improved nutrient uptake, particularly nitrogen, calcium and iron, due to IAA production. As a plant growth-promoting hormone, IAA stimulates root cell elongation and division, leading to extensive lateral root branching and dense root hair formation (Glick *et al*., 2007; El-Beltagi *et al*., 2024). This enhances root surface area, facilitating efficient nutrient absorption, especially nitrogen, which is transported through nitrate transporters activated by IAA (Hu *et al*., 2021; Luvizotto *et al*., 2010). Moreover, IAA production promotes the release of siderophores, which chelate insoluble iron in the soil, converting it into a bioavailable form for plant uptake-a crucial process in alkaline or iron-limited soils (Cen *et al*., 2024). Enhanced calcium uptake, closely linked to water movement, was observed as a result of expanded root surface area (Esitken *et al*., 2010). In contrast, CMC-coated seeds (T2) showed the highest potassium content, likely due to the polymer's moisture-retention ability, which supports nutrient absorption during early seedling growth. Magnesium levels did not differ significantly among treatments, possibly due to lower demand and limited involvement during early growth (Kisvarga *et al*., 2023). Overall, these findings support the potential of *E. kobei*

Table 6: Effect of indole-3-acetic acid (IAA)-producing bacteria on shoot height at 10 days.

Treatment[1]	Shoot height (cm)						
	4th day	5th day	6th day	7th day	8th day	9th day	10th day
T1	1.42b	2.34c	4.86c	7.86c	11.93b	13.30c	14.23b
T2	1.75ab	2.43bc	4.78c	7.78c	14.64a	13.90bc	16.94a
T3	1.96a	2.80a	6.27a	9.27a	15.62a	15.35a	17.40a
T4	1.94a	2.65b	5.59b	8.59b	15.10a	15.07a	17.10a
T5	1.84ab	1.93d	5.23bc	8.23bc	14.80a	14.40ab	16.92a
F-test	**	**	**	**	**	**	**
CV.%	5.27	4.73	6.04	3.87	4.91	4.44	4.23

**: Significantly different at P≤0.01.

[1]T1 = non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4= Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

Table 7: Effect of indole-3-acetic acid (IAA)-producing bacteria on the nutritional content of vegetable soybean seedlings at 10 days.

Treatment[1]	Nitrogen (%)	Phosphorus (%)	Potassium(%)	Calcium (%)	Magnesium (%)	Iron (%)
T1	7.13c[2]	0.58b	2.07b	0.72bc	0.20	0.11ab
T2	7.40b	0.62a	2.36a	0.76bc	0.21	0.12ab
T3	8.83a	0.62a	2.35a	0.84a	0.20	0.15a
T4	7.34b	0.62a	2.10b	0.75bc	0.20	0.12ab
T5	7.65b	0.61a	2.27ab	0.68c	0.20	0.09b
F-test	**	**	**	**	ns	**
CV.%	4.52	6.24	5.42	4.85	4.29	5.60

ns, ** : not significantly different and significantly different at P≤0.01, respectively.

[1]T1 = non-coated seed, T2 = Coated seed + CMC only, T3 = Coated seed + *Enterobacter kobei* (isolate 1), T4 = Coated seed + *Enterobacter kobei* (isolate 2) and T5 = Coated seed + *Burkholderia paludism* (isolate 3).

[2]Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

isolate 1 as a bioinoculant that enhances nitrogen, calcium and iron uptake through IAA production, promoting root development, increasing surface area and improving iron availability *via* siderophore release (Glick *et al*., 2007).

CONCLUSION

The responsiveness of IAA-producing bacteria revealed that six isolates could produce IAA, with three highly efficient isolates selected for a study on seed coating to evaluate their impact on germination, growth and nutrient content of vegetable soybean seedlings. The results demonstrated that seeds coated with *E. kobei* (isolate 1) and *E. kobei* (isolate 2) showed significant improvements in radicle emergence, emergence percentage, germination percentage, mean germination time, shoot length, root length and shoot height compared to other methods. Furthermore, coating seeds with *E. kobei* (isolate 1) resulted in higher nitrogen, phosphorus, potassium and calcium content in seedlings compared to non-coated seeds. Notably, *E. kobei* (isolate 1), which produced IAA at a concentration of 87 µg/mL, exhibited higher efficiency than other isolates. Both *E. kobei* (isolate 1) and *E. kobei* (isolate 2) demonstrated the ability to produce IAA, significantly stimulating plant seed germination and cell elongation. Seed coating with these isolates enhances seed quality. Therefore, *E. kobei* (isolate 1) is recommended for seed coating to improve the quality of vegetable soybean seeds.

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Conflict of interest

All authors declare that they have no conflict of interest.

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