

ENHANCING LONGEVITY AND BACTERIAL VIABILITY OF VEGETABLE SOYBEAN SEEDS THROUGH BIO-COATING WITH *ENTEROBACTER KOBEL*

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Abstract

Vegetable soybean is valued for its high nutritional content and growing consumer demand. However, its cultivation often faces challenges related to poor seed germination, low seedling vigor, and limited storability. This study aimed to evaluate the potential of *Enterobacter kobei* as a bio-coating agent to enhance seed quality before and after storage. Seeds were coated with *E. kobei* at five different concentrations (10^3 , 10^4 , 10^5 , 10^6 , and 10^7 CFU/mL) and evaluated under two germination test conditions: sand and top of paper covered with sand. Results showed that coating seeds with *E. kobei* at 10^5 – 10^7 CFU/mL significantly improved germination percentage, speed of germination, and reduced mean germination time; MGT compared to uncoated seeds and those coated with polymer alone. The three effective treatments (10^5 , 10^6 and 10^7 CFU/mL) were subsequently subjected to storage under controlled and ambient conditions. After six months of controlled storage, seeds coated with *E. kobei* at 10^6 CFU/mL maintained high germination rates and consistent seedling shoot length, indicating stable performance. In contrast, seeds stored under ambient conditions showed a marked decline in quality after four months, particularly in germination and seedling growth. Additionally, bacterial viability was found to be influenced by inoculum density, with 10^6 CFU/mL providing the optimal balance between viable microbial population and long-term survival. These findings suggest that *E. kobei* at 10^6 CFU/mL is an effective bio-coating agent for improving seed performance and storability in vegetable soybean.

Keywords: Seed enhancement, Plant growth-promoting rhizobacteria, Seed storage, Bio-polymer

Introduction

Vegetable soybean (*Glycine max* (L.) Merrill) is a highly nutritious legume crop, rich in proteins, calcium, and compounds that significantly enhance iron absorption, making it an essential component of the human diet (Chadha & Oluoch, 2004; Nair et al., 2023). In Thailand, vegetable soybean is typically cultivated during two main seasons: the late rainy season (August–October) and the dry season (February–April). Farmers predominantly favor the late rainy season cultivation due to easier field management and lower disease pressure. Consequently, seeds harvested at the end of the dry season (April) are stored for approximately four months before sowing. However, prolonged storage often leads to a decline in seed quality (Mardmai et al., 2023), primarily resulting from lipid peroxidation of cellular membranes, membrane damage, solute leakage, reduced enzymatic activity, and a subsequent loss of seed viability and germination potential (Bailly, 2004; Bewley et al., 2013; McDonald, 1999).

Biological strategies to maintain seed quality during storage are gaining considerable interest. *Enterobacter kobei*, a plant growth-promoting bacterium; PGPB, is notable for its ability to produce indole-3-acetic acid; IAA, a key auxin that regulates fundamental physiological processes such as cell division, cell elongation, and meristematic tissue formation. Through these mechanisms, IAA promotes rapid radicle emergence, strengthens embryonic cell integrity, and enhances early seedling development. Additionally, IAA production stimulates the expression of antioxidative enzymes like superoxide dismutase; SOD and catalase; CAT, mitigating the accumulation of reactive oxygen species; ROS and thereby reducing oxidative stress-induced seed deterioration during storage (Bailly, 2004; Glick, 2012).

Seed coating technology, particularly microbial seed coating, presents a promising approach to improve seed performance. Coating seeds with *E. kobei* ensures close adherence of beneficial bacteria to the seed surface, facilitating early IAA-mediated root development, enhancing nutrient and water uptake efficiency, promoting carbohydrate accumulation, and stimulating chlorophyll biosynthesis, ultimately leading to vigorous seedling establishment (Accinelli et al., 2018; Egamberdieva et al., 2017; Halmer, 2000; Pedrini et al., 2017; Rocha et al., 2019; Taylor et al., 1998). Furthermore, *E. kobei* demonstrates additional plant growth-promoting traits, including phosphate solubilization and the production of hydrolytic enzymes

such as citrate lyase, amylase, protease, and lipase, further contributing to robust plant development etc (Kumar & Dubey, 2022).

Moreover, recent advancements in bio-coating technology, particularly the encapsulation of microbial strains using biochar and sodium alginate, have been shown to significantly enhance bacterial viability during storage by providing a protective microenvironment against desiccation, oxidative stress, and ultraviolet radiation (Accinelli et al., 2018; Syaziana et al., 2024). Maintaining the viability of *E. kobei* on coated seeds is critical to ensuring consistent IAA production and sustained plant growth-promoting activities after sowing. Therefore, bio-coating vegetable soybean seeds with *E. kobei* offers a dual benefit of extending seed longevity and preserving bacterial viability, which collectively contribute to improved seedling vigor and overall crop performance.

Given these advantages, this study aims to evaluate the effects of bio-coating vegetable soybean seeds with *E. kobei* on seed longevity and bacterial viability, using sand and top-of-paper covered with sand tests under controlled and ambient conditions. Moreover, microbial bio-coating represents an eco-friendly alternative to chemical treatments, enhancing seed storability and seedling establishment, while supporting the competitiveness of Thailand's vegetable soybean industry and contributing to global climate-smart agriculture.

Materials and methods

1. Location of experiment, duration, and seed quality

This study was carried out between October 2024 and May 2025 at the Seed Technology Laboratory and the Modern Seed Technology Research Center under the Agronomy Program, as well as the Soil and Advanced Fertilizer Laboratory, all within the Faculty of Agricultural Production at Maejo University. The vegetable soybean cultivar 66-1 used in the experiment exhibited an initial germination rate of 87%.

2. Characteristics of *Enterobacter kobei*

To obtain bacterial isolates for this study, soil sampling was conducted in the rhizosphere of vegetable soybean plants in Chiang Muan District, Phayao Province, Thailand. Soil samples were collected from a depth of 10–15 cm and subjected to a serial dilution procedure. The diluted suspensions were plated onto potato dextrose agar; PDA and incubated at ambient room temperature for three days. Following incubation, eleven distinct bacterial colonies were

isolated and purified through repeated streaking on nutrient agar; NA to ensure culture purity (Thomma & Sirithorn, 2012). Selected bacterial isolates were subjected to species identification using 16S rRNA gene sequencing. Genomic DNA was extracted, and the 16S rRNA gene was amplified and sequenced. The resulting sequences were compared with those in the NCBI GenBank database using the BLAST algorithm, which confirmed the identity of the isolate as *Enterobacter kobei* (GenBank accession no. CP017181).

3. Microbes preparation

After obtaining pure bacterial colonies on NA, the isolates were cultivated in nutrient broth (NB) containing 0.102 g/L of tryptophan (Fluka, Buchs, Japan) and incubated with continuous shaking at 125 rpm for seven days. Upon completion of the incubation period, the cultures were centrifuged at $5,000\times g$ for 5–10 minutes to separate the bacterial cells from the supernatant. The bacterial pellets were then resuspended in sterile distilled water, and the concentration was adjusted to 10^5 to 10^7 CFU/mL in preparation for mixing with the seed coating formulation (Thomma & Sirithorn, 2012).

4. Coating vegetable soybean seeds

Prior to coating, vegetable soybean seeds were surface-disinfected using 1% sodium hypochlorite; NaOCl for 1 minute, followed by triple rinsing with sterile distilled water. The seeds were then air-dried to reach a moisture content of approximately 9%. For the coating process, a solution was prepared by dissolving 0.1% (w/v) carboxymethyl cellulose; CMC in 99 mL of distilled water, which served as the coating polymer. Subsequently, 1 mL of bacterial suspension was added to the polymer solution, and the mixture was thoroughly homogenized before application. A total of 50 g of seeds were subjected to seven different treatments as follows: T1 = uncoated seeds (Control); T2 = seeds coated with polymer only; T3 = seeds coated with *E. kobei* at 10^3 CFU/mL; T4 = *E. kobei* at 10^4 CFU/mL; T5 = *E. kobei* at 10^5 CFU/mL; T6 = *E. kobei* at 10^6 CFU/mL; and T7 = *E. kobei* at 10^7 CFU/mL. After coating, all seeds were dried again to approximately 9% moisture content prior to further evaluation of seed quality parameters.

5. Seed storages

Seeds from all treatments were placed in aluminum foil pouches (10×15 cm, W×L) and stored under two conditions: ambient storage ($27\pm2^\circ\text{C}$ with $80\pm5\%$ relative humidity) and controlled storage (4°C with 50% relative humidity). Quality assessments were

conducted every two months for up to six months, using randomly sampled seeds from each treatment.

6. Seed measurement

6.1 Sand testing

Sand with a uniform particle size of less than 0.05 mm was used as the germination medium. Prior to use, the sand was sterilized by dry heat at 200°C for 2 hours to eliminate microbial contamination. The sterilized sand was then adjusted to a moisture content of approximately 60% and placed in plastic germination containers measuring 180 mm in length, 140 mm in width, and 90 mm in height. A 3 cm layer of moist sand was poured into the base of each container, upon which the seeds were evenly positioned. An additional 2 cm layer of the same moist sand was used to cover the seeds completely. Germination tests were conducted in a controlled growth chamber maintained at 25°C with 80% relative humidity, under continuous light conditions at an intensity of 180 μ E. Each treatment consisted of four replications with 50 seeds per replicate. Seed germination was evaluated on days 5 and 8 following the International Seed Testing Association (ISTA, 2023). Speed of germination was assessed by counting the number of seeds that had developed into normal seedlings each day from day 5 to day 8, following the protocol outlined by AOSA (1983). The mean germination time (MGT) was determined using the formula $MGT = \sum(n \times d) / N$, where n is the number of seeds that germinated on day d , and N is the total number of germinated seeds during the observation period (Ellis & Roberts, 1981). Seedling growth was evaluated on the 8th day after sowing. Ten seedlings were randomly selected per treatment to measure shoot length and shoot fresh weight. Shoots were excised at the base, close to the surface of the sand medium, and subsequently measured for length and weighed to determine fresh weight (Kangsopa et al., 2023).

6.2 Top of paper covered with sand testing; TPS

The TPS method followed the same preparation protocol for sand and container setup as described in the standard sand germination test. In this method, a sheet of germination paper was laid flat at the bottom of each plastic box to provide a uniform surface. Fifty seeds were evenly distributed on the paper and subsequently covered with a 2-centimeter layer of sterilized sand (ISTA, 2023). The germination boxes were sealed to maintain humidity and incubated under the same environmental conditions as the sand

germination test. Seed quality parameters, including germination percentage and seedling vigor, were assessed using the same procedures.

7. Statistical analysis

The germination percentage was arcsine-transformed to normalize the data before statistical analysis using the SAS 9.10 program. 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

1. Seed germination and vigor

Under sand conditions, seed coating with *Enterobacter kobei* at concentrations of 10^5 , 10^6 , and 10^7 CFU/mL, as well as uncoated seeds, resulted in significantly higher germination percentages and speed of germination compared to other treatments. Moreover, coating seeds with *E. kobei* at these concentrations notably reduced the mean germination time, indicating a statistically faster germination compared to other methods.

Under top of paper covered with sand conditions, seed coating with *E. kobei* at 10^5 , 10^6 , and 10^7 CFU/mL still led to higher germination percentages and speed of germination than most other treatments; however, these values were not significantly different from those of seeds coated with the coating agent alone. Notably, coating with *E. kobei* at 10^7 CFU/mL significantly reduced the mean germination time compared to other treatments as shown in Table 1.

Table 1 Germination percentage; GE, speed of germination; SGE and mean germination time; MGT of vegetable soybean seeds after coating with *Enterobacter kobei* were tested under sand and top of paper covered with sand conditions

Treatment	Sand			Top of paper covered with sand		
	GE (%)	SGE (plant/day)	MGT (day)	GE (%)	SGE (plant/day)	MGT (day)
T1	87 ^{a1, 2}	14.78 ^a	6.10 ^a	61 ^{bc}	9.55 ^b	6.41 ^a
T2	65 ^c	11.84 ^b	6.41 ^a	72 ^{ab}	12.08 ^{ab}	6.27 ^a
T3	78 ^b	12.70 ^b	6.29 ^a	67 ^{bc}	10.79 ^b	6.27 ^a
T4	77 ^b	12.76 ^b	6.15 ^a	56 ^c	8.68 ^b	6.48 ^a

Table 1 (Continue)

Treatment	Sand			Top of paper covered with sand		
	GE (%)	SGE (plant/day)	MGT (day)	GE (%)	SGE (plant/day)	MGT (day)
T5	86 ^a	14.49 ^a	5.76 ^b	78 ^a	13.25 ^a	5.94 ^a
T6	89 ^a	15.40 ^a	5.82 ^b	76 ^a	13.32 ^a	6.20 ^a
T7	87 ^a	14.48 ^a	5.78 ^b	75 ^a	13.50 ^a	5.39 ^b
F-test	**	**	**	**	**	**
CV.(%)	6.26	7.74	2.86	12.00	19.47	5.49

** : significantly different $P \leq 0.01$

¹Data are transformed by the arcsine before statistical analysis and back-transformed data are presented

²Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

2. Seedling growth

Under sand conditions, seed coating with *E. kobei* at 10^5 CFU/mL significantly enhanced shoot length and shoot fresh weight compared to other treatments, although no significant differences were observed when compared with coatings at 10^6 and 10^7 CFU/mL. Under top of paper covered with sand conditions, seed coating with *E. kobei* at 10^5 CFU/mL significantly increased shoot length and shoot fresh weight compared to uncoated seeds, indicating a statistically positive effect on early seedling growth as shown in Table 2.

Table 2 Shoot length and shoot fresh weight of vegetable soybean seeds after coating with *Enterobacter kobei* were tested under sand and top of paper covered with sand conditions

Treatment	Sand		Top of paper covered with sand	
	Shoot length (cm)	Shoot fresh weight (g)	Shoot length (cm)	Shoot fresh weight (g)
T1	16.97 ^{bc1}	19.91 ^{bcd}	14.32 ^c	20.51 ^b
T2	16.20 ^c	18.49 ^d	15.47 ^{bc}	22.10 ^{ab}
T3	17.52 ^{bc}	19.64 ^{cd}	15.46 ^{bc}	21.88 ^{ab}
T4	17.67 ^{bc}	20.20 ^{bc}	15.38 ^{bc}	22.65 ^{ab}
T5	19.61 ^a	21.88 ^a	16.91 ^a	23.19 ^a

Table 2 (Continue)

Treatment	Sand		Top of paper covered with sand	
	Shoot length (cm)	Shoot fresh weight (g)	Shoot length (cm)	Shoot fresh weight (g)
T6	18.48 ^{ab}	21.29 ^{ab}	16.07 ^{ab}	22.32 ^{ab}
T7	18.36 ^{ab}	21.17 ^{ab}	16.15 ^{ab}	22.29 ^{ab}
F-test	**	**	**	**
CV.(%)	5.94	5.20	12.14	7.96

** : significantly different $P \leq 0.01$

¹Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

3. Seed quality after storage

Following seed quality evaluations of vegetable soybean coated with *Enterobacter kobei* at five different concentrations, the results indicated that coatings with *E. kobei* at 10^5 , 10^6 , and 10^7 CFU/mL were the most effective in enhancing germination, seedling vigor, and early growth performance. These selected treatments were subsequently subjected to storage under different conditions to evaluate post-storage seed quality. The experimental results are presented as follows:

3.1 Seed germination and vigor

Under controlled conditions, seeds coated with *E. kobei* at concentrations of 10^5 – 10^7 CFU/mL exhibited significantly higher germination percentages than those subjected to other treatments, particularly during the 0–6 month storage period, as evaluated under both sand and top of paper covered with sand conditions. Notably, coating with *E. kobei* at 10^6 CFU/mL consistently resulted in significantly greater germination percentages compared to uncoated seeds across both testing methods as shown in Table 3. The speed of germination was also significantly enhanced by coating with *E. kobei* at 10^5 – 10^7 CFU/mL throughout the 0–6 month storage period under both testing conditions. Coating *E. kobei* at 10^6 CFU/mL consistently produced the highest speed of germination. Specifically, at 0 and 2 months of storage, speed of germination reached 16.72 and 13.15 plants/day under the sand test, and 15.29 and 12.50 plants/day under the top of paper covered with sand method, respectively (Table 4). Seed coating with *E. kobei* at 10^5 – 10^7 CFU/mL also significantly improved mean

germination time; MGT compared to uncoated seeds under both test methods. In particular, the 10^6 CFU/mL treatment consistently yielded the lowest MGT values across all storage durations, indicating its sustained efficacy in promoting rapid and uniform germination as shown in Table 5.

Under ambient conditions, germination percentages declined markedly across all treatments as storage duration increased. However, coating with *E. kobei* at 10^6 CFU/mL consistently resulted in significantly higher germination percentages than both uncoated seeds and those coated with polymer alone, particularly during the first 0–4 months, under both testing conditions as shown in Table 3. Similarly, the speed of germination decreased with extended storage in all treatments, with uncoated seeds showing the lowest rates at all time points. Nevertheless, seeds coated with *E. kobei* at 10^6 CFU/mL maintained significantly higher speed of germination, especially during months 0–4 under both test methods as shown in Table 4. MGT values increased progressively with storage time in all treatments; however, seeds coated with *E. kobei* at 10^6 CFU/mL consistently exhibited shorter MGT values across multiple time points, reflecting enhanced germination speed and temporal stability as shown in Table 5.

Table 3 Germination percentage of vegetable soybean seeds coated with *Enterobacter kobei* and stored under controlled and ambient conditions was evaluated under both sand and top of paper covered with sand testing methods

Treatment ¹	Germination (%)							
	Sand				Top of paper covered with sand			
Controlled condition								
	0 ⁴	2	4	6	0	2	4	6
T1	76 ^{b2, 3}	80 ^{bc}	65 ^b	68 ^b	70 ^b	53 ^b	68 ^b	65 ^b
T2	79 ^{ab}	59 ^d	64 ^b	70 ^{ab}	73 ^{ab}	65 ^{ab}	74 ^{ab}	68 ^b
T3	82 ^a	74 ^c	76 ^{ab}	73 ^a	79 ^{ab}	84 ^a	71 ^{ab}	73 ^{ab}
T4	83 ^a	91 ^a	82 ^a	74 ^a	84 ^a	80 ^a	77 ^a	78 ^a
T5	81 ^a	86 ^{ab}	80 ^a	74 ^a	79 ^{ab}	82 ^a	76 ^a	69 ^b
F-test	**	**	**	**	**	**	**	**
CV.%	5.74	7.75	9.50	8.50	9.62	16.54	9.85	6.56

Table 3 (Continue)

Treatment ¹		Germination (%)						
		Sand			Top of paper covered with sand			
Ambient condition								
T1	76 ^b	57 ^b	50 ^c	36 ^b	70 ^b	56 ^b	49 ^{ab}	35 ^b
T2	79 ^{ab}	49 ^b	52 ^c	38 ^b	73 ^{ab}	44 ^c	40 ^b	53 ^{ab}
T3	82 ^a	64 ^{ab}	59 ^{bc}	40 ^{ab}	79 ^{ab}	61 ^b	55 ^a	49 ^{ab}
T4	83 ^a	76 ^a	68 ^a	54 ^a	84 ^a	73 ^a	54 ^a	67 ^a
T5	81 ^a	64 ^{ab}	64 ^{ab}	41 ^{ab}	79 ^{ab}	67 ^{ab}	56 ^a	53 ^{ab}
F-test	**	**	**	**	**	**	**	**
CV.%	5.74	12.08	9.75	14.06	9.52	8.57	9.09	15.83

** : significantly different $P \leq 0.01$

¹T1=uncoated seed, T2=coated seed, T3= coated seed + *E. kobei* 10⁵ CFU/ml, T4=coated seed + *E. kobei* 10⁶ CFU/ml, T5=coated seed + *E. kobei* 10⁷ CFU/ml

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

³Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

⁴Months of seed storage

Table 4 Speed of germination of vegetable soybean seeds coated with *Enterobacter kobei* and stored under controlled and ambient conditions was evaluated under both sand and top of paper covered with sand testing methods

Treatment ¹	Speed of germination (plant/day)							
	Sand				Top of paper covered with sand			
Controlled condition								
	0 ³	2	4	6	0	2	4	6
T1	12.66 ^{b 2}	11.84 ^c	10.50 ^b	9.69 ^b	12.72 ^c	8.95 ^b	8.03 ^b	7.50 ^b
T2	13.77 ^{ab}	12.82 ^b	12.28 ^{ab}	10.82 ^{ab}	13.42 ^{bc}	10.81 ^b	7.01 ^b	7.23 ^b
T3	14.62 ^a	12.71 ^b	12.35 ^{ab}	10.36 ^{ab}	14.50 ^{ab}	15.57 ^a	10.31 ^{ab}	10.02 ^{ab}
T4	14.96 ^a	16.72 ^a	13.15 ^a	11.53 ^a	15.29 ^a	15.20 ^a	12.54 ^a	10.46 ^a
T5	13.64 ^{ab}	15.69 ^a	12.71 ^{ab}	10.94 ^{ab}	14.41 ^{ab}	15.19 ^a	12.48 ^a	10.07 ^{ab}
F–test	**	**	**	**	**	**	**	**
CV.%	7.35	10.12	12.76	9.96	11.39	21.08	12.76	8.23
Ambient condition								
T1	12.66 ^b	8.46 ^b	7.48 ^b	4.57 ^c	12.72 ^c	8.86 ^{bc}	5.72 ^b	4.23 ^b
T2	13.77 ^{ab}	9.70 ^b	7.92 ^{ab}	6.33 ^{bc}	13.42 ^{bc}	7.41 ^c	7.14 ^{ab}	5.93 ^{ab}
T3	14.62 ^a	10.98 ^{ab}	8.96 ^{ab}	7.57 ^{ab}	14.50 ^{ab}	10.03 ^{ab}	8.21 ^a	5.45 ^{ab}
T4	14.96 ^a	13.16 ^a	9.97 ^a	9.46 ^a	15.29 ^a	11.89 ^a	8.48 ^a	7.54 ^a
T5	13.64 ^{ab}	10.64 ^{ab}	9.84 ^a	9.17 ^a	14.41 ^{ab}	11.23 ^a	8.41 ^a	6.08 ^{ab}

Table 4 (Continue)

Treatment ¹	Speed of germination (plant/day)							
	Sand				Top of paper covered with sand			
F-test	**	**	**	**	**	**	**	**
CV.%	7.35	11.74	11.49	12.52	11.39	12.19	13.74	15.41

** : significantly different $P \leq 0.01$

¹T1=uncoated seed, T2=coated seed, T3= coated seed + *E. kobei* 10^5 CFU/ml, T4=coated seed + *E. kobei* 10^6 CFU/ml, T5=coated seed + *E. kobei* 10^7 CFU/ml

²Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

³ Months of seed storage

Table 5 Mean germination time of vegetable soybean seeds coated with *Enterobacter kobei* and stored under controlled and ambient conditions was evaluated under both sand and top of paper covered with sand testing methods

Treatment ¹	Mean germination time (day)							
	Sand				Top of paper covered with sand			
Controlled condition								
	0 ³	2	4	6	0	2	4	6
T1	5.62 ^{a 2}	6.28 ^b	6.50 ^a	7.37 ^a	5.78 ^a	6.86 ^a	6.27 ^a	6.85 ^a
T2	5.44 ^{ab}	6.88 ^a	6.17 ^b	6.95 ^{ab}	5.62 ^{ab}	6.32 ^a	6.71 ^{ab}	6.73 ^{ab}
T3	5.43 ^{ab}	5.43 ^c	6.22 ^{ab}	7.06 ^{ab}	5.52 ^{ab}	5.41 ^b	6.28 ^{ab}	6.70 ^{ab}
T4	5.24 ^b	5.36 ^c	6.25 ^{ab}	6.83 ^b	5.29 ^b	5.21 ^b	6.18 ^b	6.56 ^b
T5	5.28 ^b	5.47 ^c	6.20 ^{ab}	7.17 ^{ab}	5.46 ^b	5.45 ^b	6.30 ^{ab}	6.54 ^b
F-test	**	**	**	**	**	**	**	**
CV.%	5.75	8.41	3.09	2.85	4.27	6.63	5.09	5.18
Ambient condition								
T1	5.62 ^a	5.98 ^b	7.05 ^a	7.17 ^a	5.78 ^a	6.43 ^a	7.02 ^a	6.44 ^a
T2	5.44 ^{ab}	5.93 ^b	6.63 ^b	6.24 ^{ab}	5.62 ^{ab}	6.57 ^a	6.88 ^{ab}	5.82 ^b
T3	5.43 ^{ab}	5.48 ^{ab}	6.69 ^b	5.90 ^b	5.52 ^{ab}	6.15 ^b	6.74 ^{bc}	5.73 ^b
T4	5.24 ^b	5.36 ^b	6.56 ^b	5.90 ^b	5.29 ^b	6.12 ^b	6.60 ^c	5.90 ^b
T5	5.28 ^b	5.32 ^b	6.57 ^b	5.81 ^b	5.46 ^b	6.09 ^b	6.70 ^{bc}	5.85 ^b
F-test	**	**	**	**	**	**	**	**
CV.%	5.75	4.96	6.20	6.32	4.27	5.70	6.21	6.67

** : significantly different $P \leq 0.01$

¹T1=uncoated seed, T2=coated seed, T3= coated seed + *E. kobei* 10^5 CFU/ml, T4=coated seed + *E. kobei* 10^6 CFU/ml, T5=coated seed + *E. kobei* 10^7 CFU/ml

²Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

³Months of seed storage

3.2 Seedling growth after storage

Under controlled conditions, seed coating with *E. kobei* at concentrations of 10^5 – 10^7 CFU/mL resulted in significantly greater shoot length compared to other treatments throughout the 0–6 month storage period. In particular, seeds coated with *E. kobei* at 10^6 and 10^7 CFU/mL consistently exhibited superior shoot elongation across all storage durations under both sand and top of paper covered with sand conditions. Notably, coating with *E. kobei* at 10^6 CFU/mL produced the highest shoot length of 21.01 cm at 0 months and remained significantly higher than uncoated seeds throughout the 6-month storage period as shown in Table 6. Under ambient conditions, shoot length gradually declined over time across all treatments, with uncoated seeds consistently showing the lowest values at every time point. Nevertheless, seeds coated with *E. kobei* at 10^6 and 10^7 CFU/mL maintained enhanced shoot growth, with significantly greater shoot length than uncoated seeds during the 0–4 month storage period under both sand and top of paper covered with sand testing conditions as shown in Table 6.

Table 6 Shoot length of vegetable soybean seeds coated with *Enterobacter kobei* and stored under controlled and ambient conditions was evaluated under both sand and top of paper covered with sand testing methods.

Treatment	Shoot length (cm)							
	Sand				Top of paper covered with sand			
Controlled condition								
	0	2	4	6	0	2	4	6
T1	18.84 b ^{1, 2}	11.98 c	9.75 b	10.66 b	15.92 c	11.26 c	11.18 b	9.36 b
T2	21.76 a	14.45 b	10.94 ab	10.57 b	17.32 bc	13.97 bc	11.81 ab	9.17 b
T3	22.87 a	16.62 ab	11.07 ab	11.39 ab	19.38 ab	18.73 ab	11.88 ab	11.44 a
T4	21.53 a	18.91 a	11.78 a	11.50 ab	21.18 a	19.57 ab	12.37 a	11.49 a
T5	21.53 a	17.84 a	11.65 a	11.71 a	21.01 a	20.36 a	12.15 ab	11.30 a
F-test	**	**	**	**	**	**	**	**
CV.%	4.79	9.53	8.80	5.37	9.05	12.36	8.80	7.59
Ambient condition								
T1	18.84 b	9.61 c	8.82 b	7.43 b	15.92 c	10.09 c	9.44 b	8.50 b
T2	21.76 a	12.00 bc	10.34 a	10.07 a	17.32 bc	14.16 bc	11.64 ab	9.28 ab
T3	22.87 a	13.90 ab	9.84 a	10.94 a	19.38 ab	15.78 ab	12.12 a	9.77 ab
T4	21.53 a	14.98 a	9.82 a	11.13 a	21.18 a	15.50 ab	12.25 a	11.11 a
T5	21.53 a	15.63 a	10.03 a	11.54 a	21.01 a	16.46 a	12.80 a	10.50 ab
F-test	**	**	**	**	**	**	**	**
CV.%	4.79	7.30	6.12	11.50	9.05	6.66	15.08	12.74

**: significantly different $P \leq 0.01$

¹ T1=uncoated seed, T2=coated seed, T3= coated seed + *E. kobei* 10^5 CFU/ml, T4=coated seed + *E. kobei* 10^6 CFU/ml, T5=coated seed + *E. kobei* 10^7 CFU/ml

² Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT

3.3 Viability of bacteria

As shown in Figure 1, the number of bacterial cells declined progressively over the storage period under both storage conditions. However, under controlled conditions, bacterial populations remained substantially higher compared to those stored under ambient conditions.

Under controlled storage, seeds coated with *E. kobei* at 10^7 CFU/mL maintained the highest bacterial counts throughout the 6-month period, with viable cell numbers remaining within the range of 6–7 log CFU/mL until month 5 before decreasing in month 6. In contrast, seeds coated with *E. kobei* at 10^6 CFU/mL exhibited stable bacterial viability until month 4, with a gradual decline observed in months 5 and 6. Coating with *E. kobei* at 10^5 CFU/mL resulted in a continuous decline in viable cell numbers from month 1 onward, reaching near zero by month 6 as shown in Figure 1(a). Under ambient conditions, bacterial viability declined more rapidly across all concentrations compared to controlled storage. In particular, despite a high initial bacterial count, seeds coated with *E. kobei* at 10^7 CFU/mL exhibited a sharp decline after month 4 and showed no detectable viability by month 6. Similarly, coatings with 10^6 and 10^5 CFU/mL showed a continuous reduction in cell numbers, with no viable bacteria detected at the end of the storage period as shown in Figure 1(b).

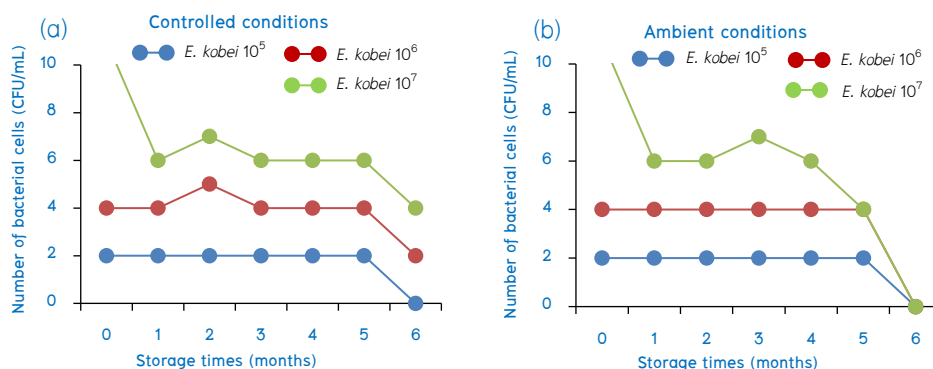


Figure 1 Seed viability of vegetable soybean after coating with *Enterobacter kobei* at 10^5 , 10^6 , and 10^7 CFU/mL during a 6-month storage period under (a) controlled and (b) ambient conditions

Discussion

The results from seed coating with *Enterobacter kobei* at varying concentrations, followed by quality and storability assessments, revealed significant differences among treatments. Specifically, coating vegetable soybean seeds with *E. kobei* at 10^5 , 10^6 , and 10^7 CFU/mL significantly improved germination percentage, speed of germination, and reduced mean germination time compared to uncoated seeds. According to the International Seed Testing Association (ISTA, 2023), the minimum standard germination percentage for soybean seeds is 80%. In this study, all *E. kobei* coated treatments achieved germination percentages meeting or exceeding this standard, demonstrating the potential of microbial seed coating to produce high quality seeds that comply with international seed quality requirements. In this study, carboxymethyl cellulose; CMC was used as the carrier polymer for bacterial delivery. CMC, a natural polymer with high water-holding capacity, creates a humid microenvironment favorable for bacterial survival (Ma, 2019; Rahman et al., 2021). Its molecular structure contains negatively charged carboxylate; $-\text{COO}^-$ groups that facilitate electrostatic and hydrogen bonding with bacterial cell surfaces, enabling stable adhesion to the seed surface after coating (Rocha et al., 2019). Moreover, seed quality indicators such as germination and seedling vigor showed clear improvement following coating with *E. kobei* at 10^5 – 10^7 CFU/mL. This can be attributed to *E. kobei*'s capacity to produce plant growth-promoting compounds, particularly indole-3-acetic acid; IAA, which plays a crucial role in cell elongation and root

development (Ma, 2019; Rocha et al., 2019; Paravar et al., 2023). Enhanced root initiation facilitates early water and nutrient uptake, promoting rapid and uniform germination. Additionally, *E. kobei* is capable of synthesizing hydrolytic enzymes such as cellulase and protease, which degrade seed coat barriers, thereby accelerating radicle protrusion (Sahu et al., 2025). The bacterial coating also creates a semi-moist microenvironment around the seed, which reduces desiccation stress and offers protection from seed-borne pathogens during the germination stage (Qiu et al., 2020). These combined effects contribute to increased germination rates, faster germination, and shorter mean germination time.

Seedling development was also enhanced by *E. kobei* treatment at 10^5 – 10^7 CFU/mL, as evidenced by increased shoot and root lengths compared to untreated seeds. This is likely due to *E. kobei*'s functional traits, such as nitrogen fixation and phosphate solubilization, which improve nutrient availability essential for cell division and tissue expansion (Ortega-Ortega et al., 2024). These results are consistent with studies by Ghosh et al. (2015) and Park et al. (2015), which reported that *Enterobacter* spp. producing IAA and other phytohormones play critical roles in promoting root cell expansion, enhancing early-stage water and nutrient absorption, and reducing oxidative damage during germination.

Based on these promising results, seed coating with *E. kobei* at 10^5 – 10^7 CFU/mL was further evaluated under controlled and ambient conditions. The findings demonstrated positive effects on seed quality both before and after storage under both conditions. Seeds coated with *E. kobei* in combination with CMC showed extended bacterial viability due to CMC's negatively charged carboxylate groups, which form stable hydrogen or electrostatic interactions with bacterial cells (Rahman et al., 2021). This interaction stabilizes bacterial adhesion and survival, creating a semi-moist matrix that reduces water loss, minimizes desiccation stress, and sustains bacterial function over time (Mnasri et al., 2017). As shown in Figure 1, *E. kobei* at all tested concentrations remained viable throughout the 6-month period under controlled conditions. However, under ambient conditions, bacterial viability declined drastically, approaching zero by the sixth month. These results confirm that storage environment strongly influences seed viability and microbial persistence. In particular, uncontrolled temperature and relative humidity under ambient conditions accelerate deterioration of both seeds and associated microbes, while controlled environments effectively maintain seed quality and bacterial viability over time (Deaker et al., 2007). Moreover, seed coating with *E. kobei* at a concentration of 10^6 CFU/mL

proved more effective in enhancing seed quality than either 10^5 or 10^7 CFU/mL. This is likely due to its optimal balance between viable microbial cell density and the ability to survive during storage (Ma, 2019). At 10^5 CFU/mL, the microbial population may be insufficient to exert significant biological effects, while at 10^7 CFU/mL, overcrowding may occur, leading to intra-species competition within the microbial niche on the seed surface (Gemell et al., 2005). Such high densities also increase susceptibility to environmental stressors, such as fluctuating moisture and temperature conditions during storage, which may compromise long-term bacterial viability and functional efficacy (Venturi & Keel, 2016).

Post-storage assessments further indicated that *E. kobei* at 10^6 CFU/mL yielded the most favorable results, outperforming other treatments in terms of preserving seed vigor and enhancing germination-related parameters. This may be explained by the strain's ability to produce IAA and cytokinins, which support germination and promote early-stage shoot elongation and root system development (Bailly, 2004; Glick, 2012; Ortega-Ortega et al., 2024). Therefore, seed coating with *E. kobei* presents a promising strategy for improving both immediate and long-term quality of vegetable soybean seeds, offering a biologically based enhancement to conventional seed technologies.

Conclusions

Seed coating with *E. kobei* at 10^5 – 10^7 CFU/mL significantly enhanced germination percentage, speed of germination, and reduced mean germination time of vegetable soybean seeds. The 10^6 CFU/mL concentration consistently produced the best results across both sand and top of paper covered with sand tests compared to uncoated seeds and polymer-only treatments. Under controlled storage, seeds coated with 10^6 CFU/mL maintained high germination and shoot length over six months. In contrast, seeds stored under ambient conditions showed a notable decline in quality, particularly after four months. Bacterial viability under controlled storage remained highest at 10^7 CFU/mL until month five, while 10^6 CFU/mL remained stable through month four. Under ambient conditions, viability dropped rapidly across all concentrations, with viable cells nearly undetectable after month four. Thus, seed coating with *E. kobei* at 10^6 CFU/mL can be considered a promising biological strategy for enhancing seed quality and vigor of vegetable soybean both prior to and following storage. The treatment shows strong promise for future application in commercial seed technology and production systems.

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