

Effects of Commercial Seed Coating Formulations on Rice Germination, Seedling Growth, and Rhizobacteria Viability

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Abstract

The development of high-quality rice seeds is essential for food security and production efficiency; however, biological seed coating may negatively affect seed germination, vigor, and microbial viability during storage. This study aimed to identify an optimal rice seed-coating formulation that maintains seed quality and to evaluate the storage viability of bacterial isolates incorporated into the selected coating. The experiment was conducted using a completely randomized design (CRD), evaluating eight seed-coating formulations differing in polymer composition and bacterial inoculation. Seed quality parameters, including radicle emergence percentage, speed of radicle emergence, germination percentage, speed of germination, mean germination time, and seedling growth traits, were assessed under controlled laboratory conditions and greenhouse conditions. Bacterial viability was determined by colony-forming unit (CFU) counts during 30 days of storage at 4°C and 70% relative humidity. The results indicated that coating formulation 8 was the most suitable, as it maintained germination, seed vigor and seedling growth under both laboratory and greenhouse conditions. This formulation also supported balanced root and shoot development. In terms of bacterial viability, an initial reduction in viable cell counts was observed after incorporation into the coating matrix, followed by a gradual increase during storage. All bacterial isolates remained viable throughout the 30-day storage period, although differences in stability were observed among isolates. Notably, *Microbacterium proteolyticum* (isolate 3) exhibited the highest and most stable viability within the selected coating formulation. Therefore, coating-formulation 8 combined with *M. proteolyticum* (isolate 3) represents a promising approach for the development of biological seed coatings for rice production systems.

Keywords: Seed enhancement, seedling vigor, microbial carrier, cold storage stability

Introduction

Rice (*Oryza sativa* L.) is a staple food crop of global importance, particularly in Asia, where rice production underpins economic development, social stability, and ecosystem sustainability (Mohidem *et al.*, 2022). However, production efficiency during early developmental stages remains constrained by uneven germination, weak seedling growth, and high seedling losses under variable laboratory, greenhouse and field conditions, leading to poor stand establishment and reduced early crop competitiveness (Chaudhary *et al.*, 2022). These constraints directly affect plant population density, intra-specific competition, and long-term yield potential, particularly in systems where early emergence and seedling vigor represent major bottlenecks (Negi *et al.*, 2024). To compensate for uneven crop establishment, farmers often increase seeding rates and intensify post-sowing management, thereby increasing production costs without consistently improving early seedling performance under fluctuating moisture and soil aeration conditions (Feng *et al.*, 2024).

Seed coating technology is widely recognized as a key seed-enhancement strategy that improves the physical and physiological performance of seeds while serving as a delivery platform for active ingredients and beneficial microorganisms to the seed microenvironment during early germination (Rocha *et al.*, 2019; Javed *et al.*, 2022; Kangsopa *et al.*, 2025). Commercial seed coating formulations are widely used due to their ability to enhance seed handling, regulate water uptake, and protect seeds from mechanical damage during sowing, while

maintaining seed structural integrity (Kangsopa, 2019; Wang *et al.*, 2024). In addition, coating materials may influence water availability in the near-seed environment and support early metabolic activation, thereby facilitating reserve mobilization and embryo growth (Pedrini *et al.*, 2017; Paravar *et al.*, 2023). The integration of seed coating with plant growth-promoting bacteria has attracted increasing attention due to their roles in phytohormone-related responses, enhanced nutrient availability, improved root development, and increased tolerance to suboptimal early-season conditions (Rocha *et al.*, 2019).

However, the performance of microbial seed coatings depends on both microbial strain selection and coating composition, as formulations must maintain microbial viability and functional activity during preparation, storage, and post-sowing phases (Gong *et al.*, 2025). Despite reported benefits for germination and early growth, mechanistic understanding remains limited regarding the interactions among coating physicochemical properties, radicle emergence dynamics, early seedling growth, and microbial survival during storage (Gong *et al.*, 2025). In this context, the use of indigenous bacterial isolates represents a promising approach, as microorganisms originating from local agroecosystems are often better adapted to prevailing environmental conditions, including soil properties, temperature fluctuations, and moisture regimes. Such adaptation may enhance their survival, colonization efficiency, and functional performance when applied as seed coating agents under field conditions. In addition, indigenous

isolates may exhibit specific plant growth-promoting traits that are compatible with the target crop and local cultivation systems, thereby improving the reliability and consistency of seed enhancement technologies. Therefore, bacterial isolates used in this study were obtained from rice fields in Northeastern Thailand, where environmental conditions are representative of major rice production areas, providing a biologically relevant basis for evaluating their performance in seed coating formulations. This knowledge gap is particularly critical for rice production systems, especially under direct-seeded and mechanized cultivation, where establishment uniformity and early seedling stability strongly determine stand density, crop competitiveness, and yield formation under variable environment conditions (Chaudhary *et al.*, 2022; Negi *et al.*, 2024).

Therefore, this study evaluated polymer-based microbial seed coatings for improving rice seed quality, seedling growth, and plant biomass, while assessing microbial survival during storage to support scalable biofunctional seed-enhancement technologies.

Materials and Methods

Experimental location, seed quality, and duration

The experiment was conducted at the Seed Technology Laboratory, the Modern Seed Technology Research Center of the Agronomy Program, and the Soil and Advanced Fertilizer Laboratory, Faculty of Agricultural Production, Maejo University. The KDML105 rice seeds used in this study had an initial germination percentage of 94% and moisture content of 14%, and were

produced in 2025. The experiment was performed during the period from May to November 2025. This study was divided into two experiments as follows:

Experiment 1: Evaluation of seed coating formulations affecting the enhancement of rice seed quality

Information on rhizobacteria isolates

Bacterial isolates were obtained from rhizosphere soil collected from rice fields in northeastern Thailand using standard rhizosphere sampling and serial dilution techniques (Somasegaran and Hoben, 1994). Soil samples were collected at a depth of 10 - 15 cm from the rhizosphere surrounding rice roots in Nong Mek, Nong Song Hong district, Khon Kaen province, and Non Daeng district, Nakhon Ratchasima province. Soil samples were air-dried, homogenized and serially diluted with sterile distilled water. Aliquots of diluted suspensions were plated on potato dextrose agar (PDA) and incubated at room temperature for 7 days. Distinct colonies were selected based on morphology and purified by repeated streaking on nutrient agar (NA) to obtain single colonies. Eleven isolates were initially obtained, of which four representative isolates with stable growth were selected for further analysis and seed coating experiments. Bacterial identification was performed using 16S rDNA sequencing (Weisburg *et al.*, 1991). Sequence similarity was determined by comparison with reference sequences in the GenBank database using BLAST. Isolates 1, 2 and 4 were identified as *Bacillus megaterium* with 99.65 - 99.73% sequence similarity, whereas isolate 3 was identified as *Microbacterium proteolyticum* with 99.15% sequence similarity.

Seed coating process

Commercial seed-coating formulations were evaluated for their effects on the quality of KDML105 rice seeds. Eight ready-to-use seed-coating formulations were selected from the Modern Seed Technology Research Center, Faculty of Agricultural Production, Maejo University. Each coating formulation was applied to KDML105 rice seeds using a rotary pan seed coater (model KSC-02D). After coating, the seeds were dried using a forced-air drying system (model KCU 40-2) at 33°C for 24 h until the post-coating seed moisture content was reduced to 14% ± 2% (Figure 1).

Preparation of rhizobacterial inoculum and incorporation into seed-coating formulations

Four rhizobacterial isolates were selected and identified through 16S rRNA gene sequencing as *B. megaterium* (isolate 1), *B. megaterium* (isolate 2), *M. proteolyticum* (isolate 3), and *B. megaterium* (isolate 4). Each isolate was individually cultured in

nutrient broth (NB) and incubated at room temperature for 7 days under aerobic conditions to obtain actively growing bacterial cells. After incubation, bacterial cultures were harvested by centrifugation at 8,000 × g for 5 min. The resulting cell pellets were collected and resuspended in sterile saline solution (0.85% NaCl) to obtain bacterial suspensions with a uniform cell density (Prommayakul, 2024). The prepared bacterial suspensions were subsequently incorporated into seed-coating formulation 8. Liquid seed-coating formulations were prepared at a total volume of 50 mL. A volume of 1 mL of each bacterial suspension was aseptically added to the coating formulation using a micropipette, followed by thorough mixing using a vortex mixer to ensure homogeneous distribution of bacterial cells within the coating matrix.



Figure 1 Physical appearance of rice seeds after coating with eight different seed-coating formulations:

(A) formulation 1, (B) formulation 2, (C) formulation 3, (D) formulation 4, (E) formulation 5, (F) formulation 6, (G) formulation 7, and (H) formulation 8

Experiment 2: Evaluation of rhizobacteria viability in rice seed coating formulations

Assessment of bacterial survival in coating formulations

Rhizobacterial survival in the seed-coating formulations was evaluated using the drop plate method. Serial tenfold dilutions were prepared by transferring 1 mL of the bacterial suspension into 9 mL of sterile distilled water (Thomma and Sirithorn, 2012) followed by vortexing for approximately 8 s. This dilution procedure was repeated sequentially to obtain dilution levels ranging from 10^{-2} to 10^{-9} , following standard microbiological protocols (Herigstad *et al.*, 2001). Aliquots from appropriate dilutions were drop-plated onto nutrient agar plates and incubated at room temperature. The coated samples were stored under controlled conditions at 4°C and 70% relative humidity throughout the experimental period. Bacterial viability in the coating formulations was assessed at 0 5 10 15 20 25 and 30 days after preparation. Colony-forming units (CFU) were counted after incubation, and bacterial populations were expressed as CFU/mL according to established enumeration methods (Griffith and Wolf Jr, 2002).

Data collection

Laboratory testing conditions

In the seed quality test, 50 coated and non-coated seeds were evaluated using the between paper (BP) method with four replications. The seeds were incubated in a germination chamber at 25 °C, 80 % relative humidity, a light intensity of $180 \mu\text{E m}^{-2} \text{s}^{-1}$ and continuous 24-h illumination. Subsequently, radicle emergence percentage, speed of radicle emergence, germination percentage,

speed of germination and mean germination time were determined.

1.1 Radicle emergence percentage (RE) was evaluated in all treatments with four replications, each consisting of 50 seeds. Observations were conducted daily starting on day 4 after sowing, and seeds were recorded as having emerged when the radicle reached a minimum length of 2 mm (Kangsopa and Atnaseo, 2022).

1.2 Speed of radicle emergence was (SRE) determined by daily recording, from day 1 to day 4 after sowing, of the number of seeds exhibiting radicles of at least 2 mm in length in each treatment, with four replications of 50 seeds per replication (Thawong and Kangsopa, 2025).

1.3 Germination percentage (GE) was assessed using randomly selected seeds, with four replicates of 50 seeds per treatment. The proportion of normal seedlings was recorded at 5 and 14 days after sowing, following the procedures recommended by the International Seed Testing Association (ISTA, 2023).

1.4 Speed of germination (SGE) was determined by monitoring the emergence of normal seedlings from the initial count on day 5 to the final count on day 14 after sowing. Each treatment was conducted with four replications, each consisting of 50 seeds, in accordance with the guidelines of the Association of Official Seed Analysts (AOSA, 1983).

1.5 Mean germination time (MGT) was determined by recording the number of normal seedlings that emerged each day from day 1 to day 14 after sowing (Ellis and Roberts, 1980).

1.6 Seedling growth, including shoot lengths (SHL) and root lengths (ROL), was evaluated 14 days after germination using 10 randomly selected seedlings from each of the four replications. Shoot length was determined from the base of the coleoptile to the tip of the longest leaf, whereas root length was measured from the root base to the apex of the primary root. Total seedling length was calculated as the distance from the root tip to the leaf tip, following the method described by Abdul - Baki and Anderson (1973). The same set of seedlings was subsequently used to determine shoot dry weights (SDW) and root dry weights (RDW) by oven-drying at 60°C for 72 h, after which the dry weights were recorded.

Greenhouse testing conditions

Seed quality was assessed in a greenhouse using peat moss as the growth medium and seedling trays with 104 compartments. The temperature was maintained at $27 \pm 2^\circ\text{C}$, with a relative humidity of $70 \pm 5\%$. Each treatment was replicated four times, with 50 seeds per replication.

2.1 The emergence percentage was recorded 5 days after sowing based on the number of coleoptiles visibly emerging from the peat moss (Kangsopa and Atnaseo, 2022).

2.2 The speed of emergence was evaluated daily from 1 to 4 days after sowing, following the same criterion as coleoptiles emergence (Kangsopa and Atnaseo, 2022).

2.3 The germination percentage, speed of germination, and mean germination time were evaluated using the same procedures as those under laboratory conditions.

2.4 Shoot length was measured 14 days after germination using 10 randomly selected seedlings from each of the four replications. Shoot

length was determined from the base of the coleoptile to the tip of the first leaf. The same set of shoots was subsequently used to determine shoot dry weight by oven-drying at 60°C for 72 h, after which the dry weight was recorded.

Statistical analysis

The experiment was arranged using a Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA). Percentage data were transformed using the arcsine transformation, while percentage values equal to zero were transformed using the $(x + 0.5)^{1/2}$ method prior to analysis. Mean comparisons among treatments were performed using Duncan's New Multiple Range Test (DMRT). All statistical analyses were conducted using a standard statistical analysis software package.

Results and Discussion

Effect of seed quality after coating with different coating formulations

The results showed that uncoated seeds achieved the highest germination initiation and post-sowing establishment, particularly under greenhouse conditions, where emergence percentage and speed of emergence were maximized (Bewley *et al.*, 2013) (Table 1). Under greenhouse conditions, seeds were exposed to natural light fluctuations, with temperatures ranging from $27 \pm 2^\circ\text{C}$ and relative humidity of $70 \pm 5\%$, resulting in variable environmental conditions compared to the controlled laboratory environment. In contrast, laboratory conditions provided a more stable environment with constant temperature, humidity, and light intensity, which are optimal for uniform germination and early metabolic activation. Without a coating layer, seeds maintained balanced water uptake,

gas exchange, and respiration, promoting rapid membrane repair, reduced electrolyte leakage, and efficient activation of mitochondrial respiration and reserve-mobilizing enzymes (Finch-Savage and Bassel, 2016). This enabled embryos to generate sufficient growth force to overcome mechanical resistance of the growth medium and achieve effective root and shoot emergence (Bradford, 2005). Under laboratory conditions, uncoated seeds and coating formula 8 exhibited similarly high radicle emergence, although uncoated seeds showed the fastest emergence rate, reflecting higher inherent vigor (Reed *et al.*, 2022) (Table 1). This supports the concept that

germination rate and uniformity are stronger predictors of establishment success than final germination percentage alone (Finch-Savage and Bassel, 2016). Among the eight commercial coatings tested, performance differences primarily reflected variation in physical properties such as density, porosity, water-holding capacity, wettability and film strength, which influence seed water relations and oxygen diffusion (Wang *et al.*, 2025). Coating formula 8 achieved the highest germination percentage, fastest germination rate, and shortest mean germination time under laboratory conditions, indicating effective regulation of water uptake and rapid reserve mobilization (Paparella *et al.*, 2015).

Table 1 Radicle emergence percentage (RE), speed of radicle emergence (SRE), emergence percentage (EMG) and speed of emergence (SEMG) of rice seeds coated with different formulations under laboratory and greenhouse conditions

Treatment	Laboratory condition		Greenhouse condition	
	RE ¹ (%)	SRE (roots/day)	EMG (%)	SEMG (plants/day)
Uncoated seeds	94 a ²	14.65 a	72 a	9.04 a
Coated-formula 1	85 ab	11.34 bcd	41 b	5.13 b
Coated-formula 2	87 ab	12.81 abc	52 ab	6.44 ab
Coated-formula 3	73 bc	10.77 cde	43 ab	5.38 b
Coated-formula 4	59 c	8.69 e	61 ab	7.56 ab
Coated-formula 5	81 ab	12.29 bcd	69 ab	8.63 ab
Coated-formula 6	83 ab	13.35 ab	62 ab	7.69 ab
Coated-formula 7	71 cb	10.48 de	5 c	0.63 c
Coated-formula 8	91 a	13.59 ab	61 ab	6.86 ab
F-test	**	**	**	**
C.V. (%)	11.35	11.80	26.14	36.40

Remarks:**: significantly difference at $p \leq 0.01$

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

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

However, under greenhouse conditions, coating formula 8 did not match the emergence performance of uncoated seeds, demonstrating that high laboratory germination does not necessarily translate into successful emergence in soil or substrate environments (Pedrini *et al.*, 2017) (Table 2). Emergence requires sustained aerobic respiration under mechanical impedance, and coating layers may increase friction or restrict gas diffusion, thereby reducing emergence success (Phillips *et al.*, 2026). Similarly, coating formula 7

showed low greenhouse emergence despite high laboratory germination, suggesting that emergence failure was mainly due to coating-induced physical constraints rather than reduced seed viability (Halmer, 2008). Such constraints may involve coating swelling, increased adhesion to substrate particles or localized water saturation that limits oxygen diffusion, effects that are intensified under heterogeneous greenhouse conditions (Bradford, 2005; Wang *et al.*, 2025).

Table 2 Germination percentage (GE), speed of germination (SGE) and mean germination time (MGT) of rice seeds coated with different formulations under laboratory and greenhouse conditions

Treatment	Laboratory condition			Greenhouse condition		
	GE ¹ (%)	SGE (plants/day)	MGT (day)	GE (%)	SGE (plants/day)	MGT (day)
Uncoated seeds	94 ab ²	7.28 ab	6.42 cd	97 a	7.96 a	5.99 bc
Coated-formula 1	93 ab	6.43 b	6.96 bc	91 b	7.04 b	5.98 bc
Coated-formula 2	94 ab	6.60 b	6.79 bcd	94 ab	7.90 a	5.67 bc
Coated-formula 3	94 ab	6.11 b	7.39 ab	91 b	7.37 ab	5.63 c
Coated-formula 4	94 ab	6.69 b	6.84 bc	94 ab	7.83 a	5.68 bc
Coated-formula 5	91 b	6.69 b	7.61 a	92 b	7.06 b	6.01 bc
Coated-formula 6	94 ab	6.66 b	6.86 bc	92 b	7.31 ab	6.22 ab
Coated-formula 7	91 b	6.59 b	6.63 cd	91 b	6.26 c	6.59 a
Coated-formula 8	98 a	7.63 a	6.19 d	96 a	7.79 a	5.90 bc
F-test	**	**	**	**	**	*
C.V. (%)	6.01	5.99	2.94	5.20	6.57	5.74

Remarks: *, ** : significantly difference at $p \leq 0.05$ and $p \leq 0.01$, respectively

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

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

Differences among coatings were also evident in biomass allocation. Under laboratory conditions, coating formula 8 enhanced root length and root and shoot dry weights, indicating efficient reserve mobilization and early vigor (Bewley *et al.*, 2013) (Table 3). Under greenhouse conditions, coating formula 2 and coating formulas 4, 5, and 8 promoted shoot growth without consistently improving emergence, suggesting that some coatings enhance post-emergence growth rather than alleviating the emergence bottleneck (Rocha *et al.*, 2019). Consequently, when emergence is constrained, subsequent improvements in seedling growth are limited by reduced stand establishment (Taylor *et al.*, 1998).

Thus, these findings demonstrate that commercial seed coatings can exert both beneficial and detrimental effects depending on film properties

and environmental interactions (Javed *et al.*, 2022). While coatings that reduced mean germination time and enhanced seedling biomass promoted reserve mobilization, even minor increases in viscosity, water retention, or reductions in gas diffusion substantially decreased emergence under practical conditions (Wang *et al.*, 2025). In contrast, uncoated seeds maintained balanced water and gas exchange and therefore achieved superior establishment under greenhouse conditions (Finch-Savage and Bassel, 2016). These results emphasize that selection of seed coatings should prioritize consistency between laboratory vigor assessments and actual emergence performance, as high germination under controlled conditions alone may be insufficient if coatings impose physical or gaseous constraints during emergence (Reed *et al.*, 2022).

Table 3 Root length (ROL), shoot length (SHL), root dry weight (RDW) and shoot dry weight (SDW) of rice seeds coated with different formulations under laboratory and greenhouse conditions

Treatment	Laboratory condition				Greenhouse condition	
	ROL	SHL	RDW	SDW	SHL	SDW
	(cm/root)	(cm/root)	(mg/plant)	(mg/plant)	(cm/root)	(mg/plant)
Uncoated seeds	10.13 b ¹	6.73 bc	2.8 b	4.5 ab	13.36 bc	6.8 bc
Coated-formula 1	5.25 c	6.83 bc	2.9 b	4.6 ab	11.18 de	6.4 c
Coated-formula 2	4.15 d	6.31 c	2.4 cd	4.7 ab	15.48 a	7.9 a
Coated-formula 3	5.24 c	6.24 c	2.5 cd	4.3 bc	10.68 e	6.7 bc
Coated-formula 4	5.13 c	6.03 c	2.1 d	3.7 c	15.10 ab	8.0 a
Coated-formula 5	4.61 cd	6.53 bc	2.1 d	4.5 bc	14.28 abc	7.7 a
Coated-formula 6	4.48 cd	6.65 bc	2.6 bc	4.8 ab	12.89 cd	6.6 bc
Coated-formula 7	4.55 cd	7.85 a	2.6 bc	4.4 bc	10.99 de	6.8 bc
Coated-formula 8	11.13 a	7.53 ab	3.9 a	5.3 a	14.85 ab	7.8 a
F-test	**	**	**	**	**	**
CV. (%)	9.72	9.93	9.91	14.54	9.78	12.76

Remarks: ** : significantly difference at $p \leq 0.01$

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

Bacterial viability in seed coating formulation

Following the selection of a seed coating formulation that did not adversely affect rice seed quality, coating formula 8 was identified as the optimal matrix due to its positive effects on seed germination, vigor, and early seedling growth. This formulation was therefore used to assess the storage viability of four bacterial isolates. All isolates maintained viability throughout the storage period, with bacterial populations either increasing or remaining consistently high over time. Although initial bacterial counts differed among isolates after coating preparation, all isolates adapted to the coating environment

within approximately 15 - 20 days, resulting in sustained population increases. These results indicate strong compatibility between the selected coating formulation and the incorporated microorganisms, particularly *M. proteolyticum* (isolate 3) and *B. megaterium* (isolate 1), which consistently exhibited the highest and most stable viability during storage. While the remaining isolates showed lower initial viability, their populations increased to levels comparable to those of the dominant isolates after 30 days of storage at room temperature (approximately 4°C and 70 ± 5% relative humidity) (Figure 2).

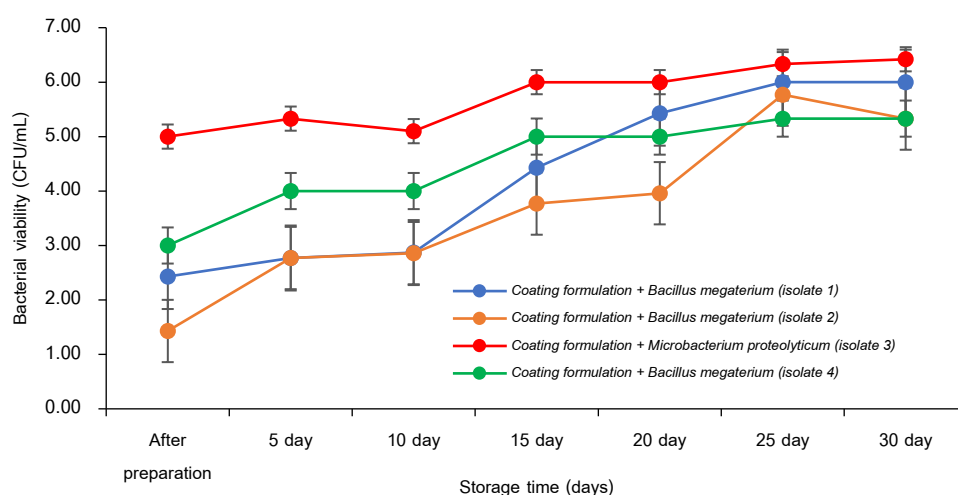


Figure 2 Changes in the viability of four bacterial isolates in seed-coating formulation 8 during storage at 4°C and 70 ± 5% relative humidity. Data are presented as mean ± standard deviation (SD) (n = 4)

The results showed that bacterial viability immediately after incorporation into coating formula 8 was lower than at later storage stages, likely due to acute stress and sublethal injury during mixing, dispersion within the coating matrix, and early storage. Under these conditions, some cells likely

remained viable but temporarily non-culturable, leading to CFU counts that underestimated the true number of living cells at early time points (Malusá *et al.*, 2012). Exposure to coating components, including polymers, binders, and wetting agents, can alter water potential, osmotic pressure, and cell

surface properties, causing transient membrane permeability changes and leakage of ions or metabolites, which require time-dependent membrane repair before growth resumes (Madigan *et al.*, 2018). In addition, formulation and mixing may impose shear stress and promote cell aggregation or uneven distribution, thereby limiting oxygen and nutrient availability and causing multiple cells to form single colonies during plating, thereby producing artificially low CFU counts at early sampling points (Bashan *et al.*, 2014).

During storage at 4°C, bacterial cells experienced cold shock, leading to reduced enzyme activity and membrane fluidity and an initial lag phase associated with physiological adaptation, including changes in membrane fatty acid composition, synthesis of cold-shock proteins, and reprogramming of transcriptional and translational processes (Madigan *et al.*, 2018). This adaptation phase is typically accompanied by reduced culturability, resulting in lower detectable CFU values during early storage. The subsequent increase in CFU counts indicates that coated-formula 8 acted as a supportive carrier matrix that facilitated bacterial survival and recovery. Over time, sublethally injured cells repaired membrane damage and restored enzymatic functions, regaining the ability to divide and form colonies, which was reflected as increased CFU values at later storage stages (Malusá *et al.*, 2012). In addition, the coating matrix likely mitigated localized desiccation and fluctuations in water activity and provided partial protection against temperature and oxidative stress, thereby promoting gradual

metabolic stabilization during cold storage (Bashan *et al.*, 2014).

The increase in CFU values may also reflect resuscitation from low-metabolic or dormant states induced by cold stress and nutrient limitation, as successful adaptation or exposure to favorable microenvironmental cues enhances culturability without substantial increases in growth rate (Oliver, 2005). For *Bacillus* spp., persistence as spores or spore-like forms and subsequent germination during CFU assessment may have further contributed to increased recoverability over time (Bashan *et al.*, 2014). Isolates with consistently higher viability, such as *M. proteolyticum*, may possess greater cold tolerance, membrane adaptability, or more efficient protein repair and oxidative stress defense systems, enabling sustained culturability during storage (Madigan *et al.*, 2018).

Conclusion

Coating formula 8 was identified as the most suitable seed-coating formulation, as it did not adversely affect rice seed quality and effectively functioned as a carrier for bacterial incorporation. Although relatively low bacterial viability was detected during the initial period following incorporation and storage, likely due to acute stress and sublethal cellular injury, bacterial populations increased markedly as storage duration at 4°C and 70% relative humidity progressed. This increase reflects the ability of bacterial cells to adapt to the coating environment, repair cellular structures, and restore metabolic activity, resulting in higher viability during the middle to late stages of

storage. All bacterial isolates remained viable throughout the 30-day storage period.

Accordingly, coating formula 8 was confirmed as the most appropriate formulation, and *M. proteolyticum* (isolate 3), which exhibited the highest and most consistent viability, was identified as the most suitable bacterial isolate for incorporation into this coating. This combination shows strong potential for the development of a biological seed coating for practical application in rice production systems.

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