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Seed coating application of endophytic and rhizosphere bacteria for germination enhancement and seedling growth promotion in soybeans

Kangsopa, J.* **and Atnaseo, C.**

Division of Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand.

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Abstract *Stenotrophomonas* sp. and *Bacillus* sp. isolated from rhizosphere and seed of kale, respectively, were found to have IAA producing activity but lack ability to solubilize phosphate. When used as an active ingratient for soybean seed coating, it was apparent that under laboratory environment, soybean seeds coated with $1x10^7$ CFU/mL *Stenotrophomonas* spp. resulted in higher rate of germination and higher speed of germination compared to uncoated seeds. However, under greenhouse condition, coating soybean seed with bacteria did not show differences in germination and speed of germination. In term of growth, seed coated with $1x10^7$ CFU/mL *Bacillus* sp. resulted in higher fresh and dry weight of seedlings when examined in laboratory conditions and better fresh and dry weight of shoot in greenhouse conditions although it did not enhance seedling or shoot length. Therefore, application of *Bacillus* sp. at the concentration of $1x10^7$ CFU/mL as an active ingradient in soybean seed coating was an appropriate method for enhancing soybean production.

Keywords: Seed enhancement, Microorganism, Organic seed treatments

Introduction

Soybean is a significant crop in Thailand due to its high demand. It is used for different purposes, such as for consumption and processing into different kinds of products, including soymilk, tofu, and soybean paste. However, at present, the areas used to grow soybeans have decreased dramatically due to environmental fructuation, which could affect yield and quality of soybean seeds (Agricultural Research Development Agency, 2016; Office of Agricultural Economics, 2016). Changes in levels of rain fall and humidity could lead to low seed germination and vigor, as a result of damge from infection or poor seed development (Taweekul and Sangla, 2010).

* **Corresponding Author:** Kangsopa, J.; **Email:** jakkrapong_ks@mju.ac.th

Using poor quality seeds for cultivation will result in uneven germination and unhealthy seedlings. Recently, the use of chemicals in crop cultivation was not encouraged due to possible long-term effects on soil conditions and the environment. Therefore, agricultural practices became more focused on biological methods to be more environmentally friendly as well as to reduce production costs (Kesan, 2007). Biological method, particularly, the use of microorganisms to promote plant growth in conjunction with modern seed condition improvement technology, such as seed coating, was preferred.

Seed coating is a method of using thin polymers to evenly cover the surface of the seeds (Pedrini *et al*., 2017). This is done so the polymer functions as a medium for attachment of microorganism to the seeds. Preparing seeds by coating them with microorganisms before cultivation is a method that can enhance seed quality (Siri, 2015; Koohakan *et al*., 2020).

Plant growth promoting bacteria (PGPB) can be found both in the rhizosphere and within plant tissues as an endophyte. Myriad of plant growth promoting activities have been associated with PGPB, such as facilitating nutrient availability and uptake, enhancing growth through phytohormone production or manipulation, and increasing level of disease resistance in plants (Glick, 2012). In particular, *Bacillus subtilis* has the ability to produce plant hormones as well as enhancing nutrients uptakes using various mechanisms. Such mechanisms include atmospheric nitrogen fixation (Lakshminarayana *et al*., 1992), solubilization of phosphates (Kundu and Gaur, 1980), production of siderophores (Glick *et al*., 2007), and production of indole-3-acetic acid (IAA) that improves root and stem growth (Oteino *et al*., 2015). According to a report by Junges *et al*. (2013), coating seeds with *B. subtilis* increased seed germination. In addition, coating seeds with *B. subtilis* SL-13 increased cotton seed germination by 28.74% (Tu *et al*., 2016).

Stenotrophomonas is a genus of gram-negative bacteria that belongs to the Xanthomonadaceae family (Alexander *et al*., 2019). There are few reports of seed treatments using this bacterial genus. It has mainly been reported as being utilized for nitrogen fixation to effectively increase yield in sugarcane (Xing *et al*. 2016; Singh *et al*., 2020). *Stenotrophomonas* sp. has been found to have abiltity to promote nitrogen fixation through the production of enzyme nitrogenase and is also effective in promoting phosphorus uptake from the soil making it more useful for seedling growth (Raja *et al*., 2006; Mehnaz *et al*., 2010). An experiment conducted on *Arachis hypogea* in a hydroponics system showed that when growing under nitrogen deficiency, supplementing the media with *Stenotrophomonas maltophilia* BJ01 could help promoting better growth (Alexander *et al*., 2019).

Currently, Thailand still lacks research on the application of microorganisms in coating of soybean seeds to promote plant growth. Therefore, this research aimed to use plant growth promoting bacteria, *Stenotrophomonas* spp. and *Bacillus* spp., as an active ingradient for soybean seed coating process in order to enhance seed germination, and seedling growth.

Materials and methods

The seeds of soybean var. Sor Jor 5 provided by Chiang Mai Seed Research and Development Center, Seed Research and Development Division, Department of Agriculture. The experiments were conducted at the Seed Technology Laboratory and Biotechnology Laboratory, Field Crop Program, Faculty of Agricultural Production, Maejo University between October 2020 and April 2021.

Microbes preparation

Bacteria used in this study were isolated from kale rhizosphere and seed. They were identified based on DNA sequence comparison of the 16s rRNA gene to the National Center for Biotechnology Information (NCBI) database. Isolates obtained from kale rhizosphere was found to match *Stenotrophomonas* sp. isolate obtained from kale seed was found to match *Bacillus* sp. Each bacterial isolate was cultured in 50% TSA (trypticase soy agar) (HiMedia®) for 24 hours before being suspended in 0.85% NaCl solution and adjusted concentration to $1x10^8$, $1x10^7$, $1x10^6$, and $1x10^5$ CFU/mL. Then, 1 mL of the bacterial culture was mixed with 99 mL of 0.1% CMC (carboxymethyl cellulose), which served as a coating agent.

Determination of phosphate solubilization

Phosphate solubility of both isolates were assessed on Pikovskaya's agar (Himedia®, India) by observing the formation of clear zone around colony after a 7-day incubation at 30 ℃. The phosphate solubilization index (PSI) was calculated by dividing the diameter clear zone with that of the colony (Paul and Sinha, 2017).

Determination of IAA production

Bacteria were cultured in liquid medium containing 1% peptone, 0.5% NaCl, 0.6% yeast extract, and 0.1% L-tryptophan (pH 7.6) on the 150 rpm

shaker for 48 hours at room temperature. Bacterial cells were collected by centrifugation at 8,000 rpm for 5 min, then 1 mL of Salkowski's reagent (0.5 M $FeCl_3$ in 35% of $HClO_4$, with a ratio of 1:50 (V:V)) was added to 1 mL culture media and incubated in the dark at 28 ℃ for 20 minutes. IAA production was assessed by spectrophotometry at a wavelength of 530 nm and IAA concentration was evaluated by comparison to IAA standards (Jomkhame and Atnaseo, 2021).

Coating soybean seeds with microorganisms that promote plant growth

Soybean seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 minute and washed with sterilized distilled water three times before being dried with sterilized tissue paper. Then, soybean seeds were coated with each of the bacterial isolate prepared previously. This experiment consisted of 10 treatments, which were without coating (T1), coating with CMC only (T2), coating with *Stenotrophomonas* sp. at concentrations of $1x10^8$, $1x10^7$, $1x10^6$, and $1x10^5$ CFU/mL (T3–T6, respectively), and coating with *Bacillus* sp. at concentrations of $1x10^8$, $1x10^7$, $1x10^6$, and $1x10^5$ CFU/mL (T7–T10, respectively). Coated seeds were then placed at room temperature for 48 hours to reduce seed moisture to 7%.

Seed evaluation

Seed quality examination in laboratory conditions

Fifty seeds from each treatment per replication for the total of four replications were tested for germination using the between paper (BP) method. Germinating seeds were kept in a transparent plastic box (110 × 110 × 30 mm., length × width × height) and placed in the germination incubator under continuous light at 25 ℃, with 80% relative humidity and 180 μE light intensity. Speed of germination was evaluated by counting the number of seeds that grew into normal seedlings on the 4^{th} day up until the 8^{th} day based on the method of AOSA (1983). Radicle emergence was evaluated by counting seed with radical appearance of at least 2 mm long 1 and 3 days after incubation. Ten seedlings per treatment per replication were randomly selected for seedling shoot and root length evaluation at 8 days after incubation (Klarod *et al*., 2021). Shoot length was measured from the joint between the shoot and the root to the tip of the foliage leaf. Root length was measured from the tip of the root to the joint between the root and shoot of the seedling (Baki and Anderson, 1973). Seedling length was measured from the tip of the root to the end of true leaf.

The same 10 seedlings were used for fresh weight measurement before drying in hot air oven at 60 ℃ for 72 hours and dry weight were assessed.

Seed quality examination in greenhouse conditions

For each treatment, fifty seeds were planted in nursery tray filled with peat moss for four replications and germination rate and speed of germination were evaluated as per laboratory condition. Cotyledon emergence rate was evaluated by counting number of visible cotyledons on the 1st and the 3rd days of planting. Speed of cotyledon emergence was evaluated by counting cotyledon emergence everyday from day1 to day 3 of planting and calculated according to ISTA (2006). Growth was assessed on 10 randomly selected plants 8 days after planting by measuring shoot length, shoot fresh and dry weights. Shoot were cut at the base of the shoot close to seedling media and measured shoot length and weighed for fresh weight. Then, fresh shoots were oven dried and dry weight was taken as mentioned earlier.

Statistical analysis

The percentage of germination was arcsine-transformed to normalize the data before the statistical analysis. All data were analyzed by one-way ANOVA (Complete Randomized Design), and differences among treatments was tested by Duncan's Multiple Range Test (DMRT).

Results

Determination of phosphate solubilisation

Stenotrophomonas sp. and *Bacillus* sp. did not have ability to solubilize phosphate, while both were able to produce IAA.IAA produced by *Stenotrophomonas* sp. was 2.58 times more than *Bacillus* sp. (Table 1).

Table 1. Phosphate solubilization index and indole-3-acetic acid (IAA) productions (µg/mL) of *Stenotrophomonas* sp. and *Bacillus* sp.

Bacterium	Phosphate solubilization index	IAA production (µg/mL)
Stenotrophomonas sp.	-	10.78
Bacillus sp.	-	4.18

Seed quality

Radicle emergence and germination percentage in laboratory condition

Coating soybean seeds with $1x10^8$ and $1x10^7$ CFU/ml *Stenotrophomonas* sp. resulted in up to 98% radicle emergence and was statistically different compared to other methods (Figure 1A). Radicle emergence evaluated after 72 hours showed that coating with *Stenotrophomonas* sp. resulted in slightly better radicle emergence compared to coating with *Bacillus* sp. (Figure 2). The seeds coated with $1x10^7$ CFU/mL *Stenotrophomonas* sp. (T4) displayed the highest speed of radicle emergence, germination rate, and speed of germination and this were statistically different compared to other methods (Figure 1B, C, D).

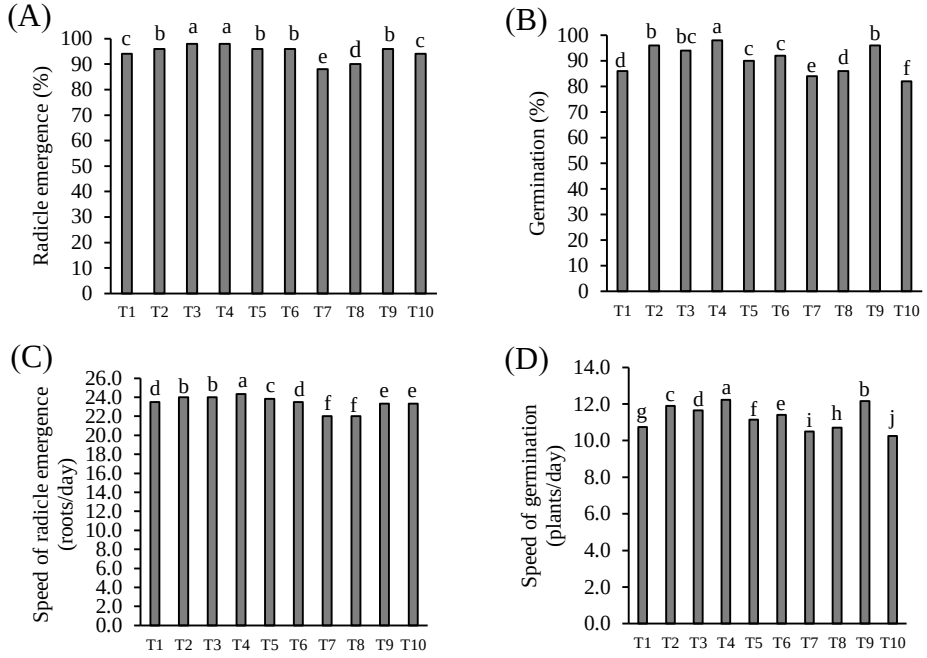

Figure 1. Radicle emergence percentage (A), germination percentage (B), speed of radicle emergence (C), and speed of germination (D) of soybean seeds after seed coating with different concentrations of *Stenotrophomonas* sp. and *Bacillus* sp. tested under laboratory conditions: T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. $1x10^8$ CFU/mL, T4 = coating + *Stenotrophomonas* sp. $1x10^7$ CFU/mL, T5 = coating + *Stenotrophomonas* sp. $1x10^6$ CFU/mL, T6 = coating + *Stenotrophomonas* sp. $1x10^5$ CFU/mL, T7 = coating + *Bacillus* sp. $1x10^8$ CFU/mL, T8 = coating + *Bacillus* sp. $1x10^7$ CFU/mL, T9 = coating + *Bacillus* sp. $1x10^6$ CFU/mL and T10 = coating + *Bacillus* sp. $1x10^5$ CFU/mL

Figure 2. Effect of seed coating with different concentrations of *Stenotrophomonas* sp. and *Bacillus* sp.; radicle emergence 72 hours after planting, tested under laboratory conditions: T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. $1x10^8$ CFU/mL, T4 = coating + *Stenotrophomonas* sp. $1x10^7$ CFU/mL, T5 = coating + *Stenotrophomonas* sp. $1x10^6$ CFU/mL, T6 = coating + *Stenotrophomonas* sp. $1x10^5$ CFU/mL, T7 = coating + *Bacillus* sp. $1x10^8$ CFU/mL, T8 = coating + *Bacillus* sp. $1x10^7$ CFU/mL, T9 = coating + *Bacillus* sp. $1x10^6$ CFU/mL and T10 = coating + *Bacillus* sp. $1x10^5$ CFU/mL

Cotyledon emergence and germination percentage in greenhouse condition

Under greenhouse conditions, seeds coated with $1x10^5$ CFU/mL *Bacillus* sp. (T10) had the higherst cotyledon emergence but not statistically differed from T1, T4, T8, and T9 (Figure 3A). T10 also resulted in the higherst speed of cotyledon emergence but not statistically differed from T4, T8, and T9 (Figure 3C).

Coating seeds with any bacterial treatments did not affect germination rate and speed of germination (Figure 3B and 3D).

Seedling growth

Under laboratory conditions, coating seeds with different concentrations of *Stenotrophomonas* sp. and *Bacillus* sp. had no effect on shoot length at all rates. Uncoated seeds had higher shoot length than seeds treated with bacteria. However, coating seeds with $1x10^6$ CFU/mL *Stenotrophomonas* sp. (T5) gave seedlings the highest root and seedling length at 19.00 and 34.44 cm (Table 2), respectively, and were statistically different compared to other seed coating methods (Figure 4). Seeds coated with $1x10^7$ CFU/mL *Bacillus* sp. (T8) had the highest seedling fresh and dry weights and were statistically different from other methods. In addition, T5 and T10 gave higher dry weight than controls (T1, T2) while other treatments gave lower dry weight (Table 3). Seeds treated with treatments, other than T8, had the same or lower seedling fresh weight than control (T1).

Under greenhouse conditions, soybean seed coated with $1x10^6$ CFU/mL *Bacillus* sp. (T9) had longer shoot length and was different from other methods,

but no statistical difference was found with T1, T2, T3, and T10 (Table 2). Seeds coated with 1x10^7 CFU/mL *Bacillus* sp. (T8) had the highest shoot fresh weights but was not statistically different from controls (Table 3). T8 also resulted in the highest shoot dry weight and statically different from control but not different from T9 and T10 (Table 3).

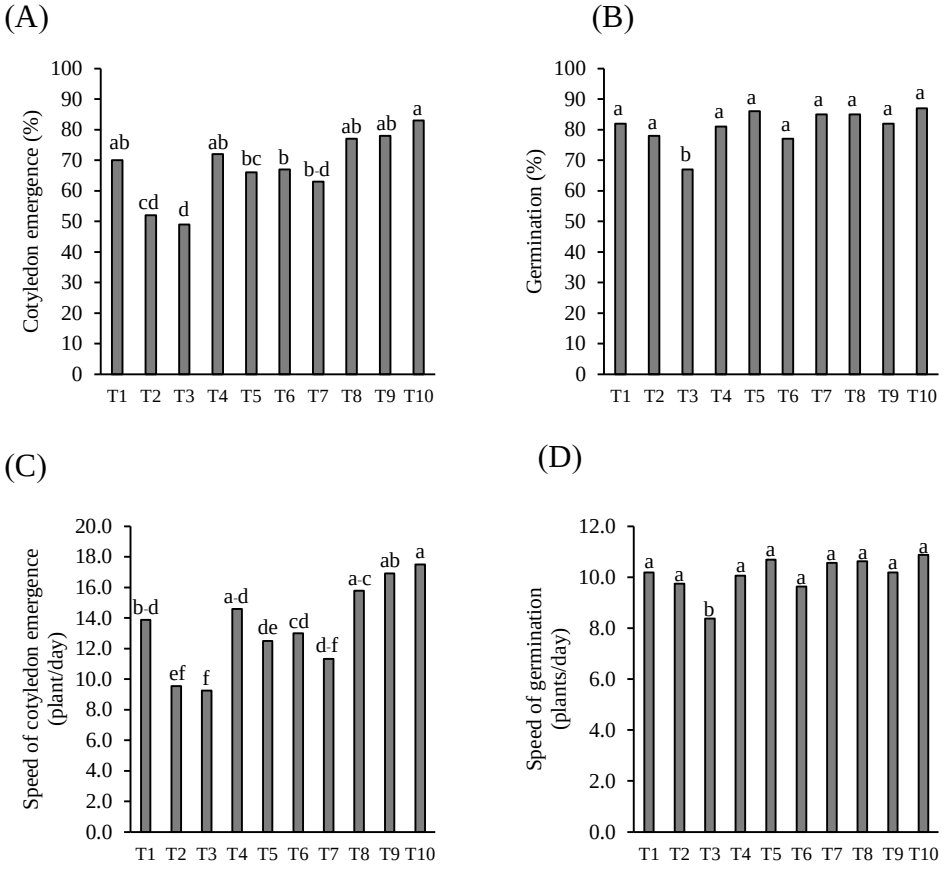

Figure 3. Cotyledon emergence percentage (A), germination percentage (B), speed of cotyledon emergence (C), and speed of germination (D) of soybean seeds after coating with different concentrations of *Stenotrophomonas* sp. and *Bacillus* sp., tested under greenhouse conditions: T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. 1x10^8 CFU/mL, T4 = coating + *Stenotrophomonas* sp. 1x10^7 CFU/mL, T5 = coating + *Stenotrophomonas* sp. 1x10^6 CFU/mL, T6 = coating + *Stenotrophomonas* sp.1x10^5 CFU/mL, T7 = coating + *Bacillus* sp. 1x10^8 CFU/mL, T8 = coating + *Bacillus* sp. 1x10^7 CFU/mL, T9 = coating + *Bacillus* sp. 1x10^6 CFU/mL and T10 = coating + *Bacillus* sp. 1x10^5 CFU/mL

Table 2. Shoot length, root length, and total seedling length of soybeans after coating seed with *Stenotrophomonas* sp. and *Bacillus* sp., tested under laboratory and greenhouse conditions

Treatment[1/]	Laboratory condition			Greenhouse condition
	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Shoot length (cm)
T1	17.00 a[2/]	15.78 f	32.78 bc	12.29 a-d
T2	14.43 e	16.43 d	30.86 f	12.45 a-c
T3	14.90 d	16.68 cd	31.58 e	12.49 a-c
T4	14.38 e	16.80 c	31.18 f	11.56 cd
T5	15.44 c	19.00 a	34.44 a	11.82 b-d
T6	15.45 c	16.77 cd	32.22 c	11.19 d
T7	16.38 b	17.46 b	33.84 b	11.45 cd
T8	16.47 b	16.19 e	32.66 bc	11.48 cd
T9	14.86 d	16.99 c	31.85 d	13.21 a
T10	15.98 c	15.64 f	31.62 d	12.85 ab
F-test	**	**	**	**
CV.(%)	3.27	16.77	32.30	6.25

**: Significantly different at P≤0.01.

[1/] T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. $1x10^8$ CFU/mL, T4 = coating + *Stenotrophomonas* sp. $1x10^7$ CFU/mL, T5 = coating + *Stenotrophomonas* sp.$1x10^6$ CFU/mL, T6 = coating + *Stenotrophomonas* sp.$1x10^5$ CFU/mL, T7 = coating + *Bacillus* sp. $1x10^8$ CFU/mL, T8 = coating + *Bacillus* sp. $1x10^7$ CFU/mL, T9 = coating + *Bacillus* sp. $1x10^6$ CFU/mL and T10 = coating + *Bacillus* sp. $1x10^5$ CFU/mL.

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

Figure 4. Effect of seed coating with different concentrations of *Stenotrophomonas* sp. and *Bacillus* sp.; seedling length 8 days after planting, tested under laboratory conditions: T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. $1x10^8$ CFU/mL, T4 = coating + *Stenotrophomonas* sp. $1x10^7$ CFU/mL, T5 = coating + *Stenotrophomonas* sp.$1x10^6$ CFU/mL, T6 = coating + *Stenotrophomonas* sp.$1x10^5$ CFU/mL, T7 = coating + *Bacillus* sp. $1x10^8$ CFU/mL, T8 = coating + *Bacillus* sp. $1x10^7$ CFU/mL, T9 = coating + *Bacillus* sp. $1x10^6$ CFU/mL and T10 = coating + *Bacillus* sp. $1x10^5$ CFU/mL

Table 3. Seedling fresh weight, seedling dry weight, shoot fresh weight, and shoot dry weight of soybean seedlings after coating seed with *Stenotrophomonas* sp.and *Bacillus* sp., tested under laboratory and greenhouse conditions

Treatment[1/]	Laboratory condition		Greenhouse condition	
	Seedling fresh weight (mg)	Seedling dry weight (mg)	Shoot fresh weight (mg)	Shoot dry weight (mg)
T1	8240 b[2/]	620 c	4108 a-c	565 b
T2	6190 cd	600 e	4343 ab	578b
T3	7830 bc	605 e	3488 c	495 d
T4	5850 d	535 h	3730 a-c	538 bc
T5	7760 bc	630 b	3635 bc	545 bc
T6	7120 c	570 f	3670 bc	538 bc
T7	6830 c	610 d	3745 a-c	528 c
T8	9060 a	640 a	4420 a	613 a
T9	6840 c	550 g	4010 a-c	583 ab
T10	7630 bc	630 b	4205 a-c	580 ab
F-test	**	**	*	*
CV.(%)	37.19	5.99	11.10	7.02

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

[1/] T1 = Control, T2 = coating + CMC, T3 = coating + *Stenotrophomonas* sp. $1x10^8$ CFU/mL, T4 = coating + *Stenotrophomonas* sp. $1x10^7$ CFU/mL, T5 = coating + *Stenotrophomonas* sp.$1x10^6$ CFU/mL, T6 = coating + *Stenotrophomonas* sp.$1x10^5$ CFU/mL, T7 = coating + *Bacillus* sp. $1x10^8$ CFU/mL, T8 = coating + *Bacillus* sp. $1x10^7$ CFU/mL, T9 = coating + *Bacillus* sp. $1x10^6$ CFU/mL and T10 = coating + *Bacillus* sp. $1x10^5$ CFU/mL.

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

Discussion

Microorganisms that are beneficial for plant growth are an option to reduce chemical contamination in cropping systems and postharvest crops. It also helps restore soil fertility and reduce problems arising from abiotic and biotic stress (Malusá *et al*., 2012; Nadeem *et al*., 2014). This experiment applied plant growth promoting microbes as active agents in a soybean seed coating process using carboxymethyl cellulose (CMC) as a coating substance. CMC is a naturally extracted cellulose derivative that can form of a film that can cling tightly to the surface of the seeds. In addition, it can easily dissolve in water allowing for a quick release of active ingredients (Khorasani and Shojaosadati, 2016). For these reason, CMC is widely used as a binding agent in seed coating (Ashraf and Foolad, 2005), especially when microbes are used as it has been reported to be a good medium for seed coating with microbes (Sharma *et al*., 2003; Roesti *et al*., 2006; Nawar, 2007; Zhou *et al*., 2017).

This study utilized two isolates of bacteria belongin to *Stenotrophomonas* sp. and *Bacillus* sp. according to their 16S rDNA sequences. They have been tested for two growth promoting activities and both lack the ability to sololubilize phosphate, while able to synthesize IAA at 10.78 µg/mL for *Stenotrophomonas* sp. and 4.18 µg/mL *Bacillus* sp. These levels of IAA production are in line with previous reports which indicate that *Bacillus* spp. could produce 10-44 µg/mL of IAA (Datta *et al*., 2011), while bacteria from rhizosphere of wheat, corn and cotton can produce 15-65 µg/ml of IAA (Mohite, 2013). In addition to the two-growth promoting activity tested, these bacteria may have prosessed other types of plant growth promoting activies not tested in this study. For example, members of *Stenotrophomonas* spp. have been shown to be able to fix nitrogen (Ramos *et al*., 2011; Wang *et al*., 2018), while *Bacillus* spp. have been shown to have abilities to increase plant nutrients availability, as well as to produce ACC deaminase, antagonistic compounds, and other plant growth hormones (Radhakrishnan *et al*., 2017; Misra and Chauhan, 2020).

Soybean seed coated with *Stenotrophomonas* sp. at $1x10^6$ CFU/mL gave the highest germination rate, and highest root and seedling lengths in a laboratory condition. This maybe explained by activity of IAA in inducing seed germination and plant cell division (Glick, 2012; Ahemad and Kibret, 2014). Similarly, study on mung bean found that inoculation of seeds with IAA producing bacteria resulted in higher germination rate which was a result of increasing glyoxalase I activity (Thornalley, 1990; Hentrich *et al*., 2013). In addition, members of *Stenotrophomonas* spp.has nitrogen fixing ability which were associate with enhancing of germination efficiency (Osuna *et al*., 2015). *Stenotrophomonas* is a genus of gram-negative bacteria that belongs to the Xanthomonadaceae family (Alexander *et al*., 2019). There are few reports of seed treatments using this bacterial genus. It has mainly been reported as being utilized for nitrogen fixation to effectively increase yield in sugarcane (Xing *et al*., 2016; Singh *et al*., 2020).

On the other hand, while coating seed with *Bacillus* sp. did not result in distinct enhancement of seed germination or seedling sizes, it did have noticeable effect on an accumulation of seedling biomass. Soybean seeds coated with *Bacillus* sp. $1x10^7$ CFU/mL had the highest seedling of shoot dry weights in the laboratory and greenhouse conditions, respectively. Effect of IAA producing bacteria on promoting biomass and secondary metabolite accumulation has been reported (Bhattacharyya and Jha, 2012). Inoculation of *Brassica juncea* and *B. oxyrrhina* with *Bacillus* sp. SN9 lead to increasing plant biomass and accumulation of nitrogen in plant tissues (Ma *et al*., 2009). As well, *Bacillus amyloliquefaciens* KPS46 capable of producing IAA has been

shown to enhance soybean growth (Buensanteai *et al*., 2008). Moreover, tomato plant inoculated with PGPR gave higher root biomass (Ribaudo *et al*., 2006).

Different effects of these two bacterial isolates on soybean may have been a result of differences in level of IAA production in that different plants reponses differently to different levels of IAA (Miransari and Smith, 2014). Also, different pathways maybe involved in the production of IAA in different bacteria (Ahemad and Kibret, 2014). Higher level of IAA produced by *Stenotrophomonas* sp. maybe suitable for the induction of seed germination and shoot elongation but lower level of IAA produced by *Bacillus* sp. cannot induce the same level of germination and shoot elongation but can induce different metabolic reaction that lead to higher accumulation of biomass. Study on Chinese fir (*Cunninghamia lanceolata* (Lamb) Hook) indicated that level of IAA could influence outcomes of seed germination and growth because exogenous IAA application could alter endogenous IAA and gibberellins (GAs) levels and yielded different outcomes (Zhao and Zhong, 2013). Therefore, appropriate IAA application is important for promoting germination rate and seedling growth.

In addition, different groups of bacteria contain a different set of growth promoting activities which in all could lead to different outcomes, especially when related to ability to produce and modulate phytohormone, which as to be balanced between exogenous and endogenous level. Such that different results from different types of bacteria is possible. Moreover, production of IAA by a bacterium can be altered by growing conditions, such as pH and type of growth medium (Malhotra and Srivastava, 2009). In seed coating, these conditions are largly unknown and therefore actual IAA production is not known and may not reflect those examined on bacterial culture medium. More detail examination of physiological changes within the seed upon bacterial application is necessary for further explation.

This study showed that seed coating with $1x10^7$ CFU/mL *Bacillus* sp. is the most promising method for soybean seed coating because it produces the best results in terms of promoting germination and biomass accumulation of the soybean seedlings.

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