Chapter 2

PGPR (*Plant Growth Promoting Rizobacteria*) Benefits in Spurring Germination, Growth and Increase the Yield of Tomato Plants

I Ketut Widnyana

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78776

Abstract

There are microbes that are beneficial to plants. Among these, rhizobacteria, which functions as *plant growth promoting rhizobacteria* (PGPR) such as *Pseudomonas* spp. and *Bacillus* sp., can serve as fertilizer. These organisms have proven to accelerate germination and improve the yield of tomato plants. Colonization of rhizosphere by PGPR results in acceleration of plant growth and protection against plant pathogens. Soaking tomato seeds with *Pseudomonas* spp. and *Bacillus* sp. suspension accelerated germination by 2–3 days than the control without immersion with both bacteria. Soaking tomato seeds for 10–30 min in the suspension of *Pseudomonas* spp. yielded the same effect in tomato germination. Soaking in *Bacillus* sp. tends to cause faster growth as compared to immersion in *Pseudomonas* spp. suspension. Mixing these two bacterial suspensions had no significant effect in accelerating the germination of tomato seeds. Soaking tomato seeds for 20 min with a suspension of *Pseudomonas* spp. and *Bacillus* sp. at densities of 4×10^5 CFU and 8×10^5 CFU showed significant differences ($p < 0.05$) in plant height, leaf number, root length, number, and weight of tomato fruits. The highest fruit weight using *Pseudomonas* spp. and *Bacillus* sp. at 8×10^5 CFU was 491.7 g tomato plant^{-1} while the control average fruits weight was 100.0 g tomato plant^{-1}.

Keywords: *PGPR*, *Pseudomonas* spp., *Bacillus* sp., soaking, germination, yield

1. Tomato and plant growth promoting rhizobacteria

Tomato is a potential horticultural crop for cultivation due to its high economic value. The production of the crop in Indonesia was 864,798 t/ha in 2008–2011, with an average productivity of 21.5 t/ha, which is below production levels of 100 t/ha in the United States and Europe.

Rhizobacteria of *Pseudomonas* spp. group are beneficial for plants, improving soil fertility, and function as biological control agents for plant pathogens and have the potential of increasing plant resistance (induced systemic resistance; ISR) [1]. Rhizobacteria plays an indirect role as a biological fertilizer and biological stimulant through the production of plant growth hormones, such as indole acetic acid (IAA), gibberellins, cytokinins, ethylene, and solubilizing minerals. These organisms also indirectly function to inhibit pathogenic microorganisms, through the formation of siderophores and antibiotics [1, 2].

Rhizobacteria, such as *P. fluorescens*, *P. putida*, and *P. aeruginosa*, are beneficial to plants as plant growth promoting rhizobacteria (PGPR), with the ability to control plant diseases [3, 4]. Research on the benefits of *Pseudomonas* spp. still continues to better understand its mechanism in spurring plant growth.

Bacillus sp. is a Gram-positive bacteria used in controlling root disease. These bacteria produce spores that can be stored for long periods and are easily inoculated into the soil. Previous research has shown that the bacteria *Bacillus* strains PRBS-1 and AP-3 proved to inhibit the growth of pathogenic fungi (*Rhizoctonia solani*, *Colletotrichum truncatum*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, and *Phomopsis* sp.) in soybean seeds and enhanced the growth of plants [5].

Rhizobacteria can be used as a bioprotectant that can suppress the development of plant pests/diseases, as a biostimulant that for production of indole acetic acid (IAA), cytokines, and gibberellin, and as a biofertilizer for increasing nutrient availability to plants [6].

2. Concentration levels of *Pseudomonas* spp. and *Bacillus* sp. in germination of tomato seeds

Soaking of tomato seeds in *Pseudomonas* spp. at a concentration of 8×10^8 CFU produced the highest germination percentage that of 91.7%, while germination in distilled water was at 41.6%. Concentrations of *Pseudomonas* spp. and *Bacillus* sp. significantly influenced tomato seed germination (**Figure 1**).

Soaking tomato seeds with bacterial suspension *Pseudomonas* spp. and *Bacillus sp*. gives a significant effect when soaked for 10–20 min at a concentration from 4×10^5 CFU, 8×10^5, and 12×10^5 CFU (**Figure 2**). Tomato seeds soaked in a mixture of bacterial suspension of *Pseudomonas* spp. and *Bacillus* sp. showed significant effect when compared to distilled water. A previous study conducted by Widnyana et al. [7] involving the soaking of swamp cabbage (*Ipomoea reptans* Poir) seeds for 20 min with suspension of *P. alcaligenes* TrN2 resulted in 25% faster germination and increased fresh weight of stems up to 67.07%, compared to soaking of seeds in distilled water.

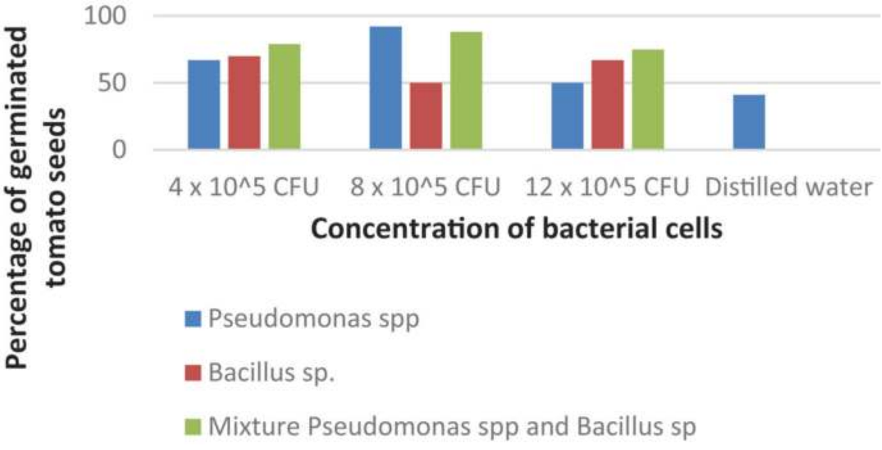

Figure 1. Percentage of tomato seed germination at different concentrations of *Pseudomonas* spp. and *Bacillus* sp.

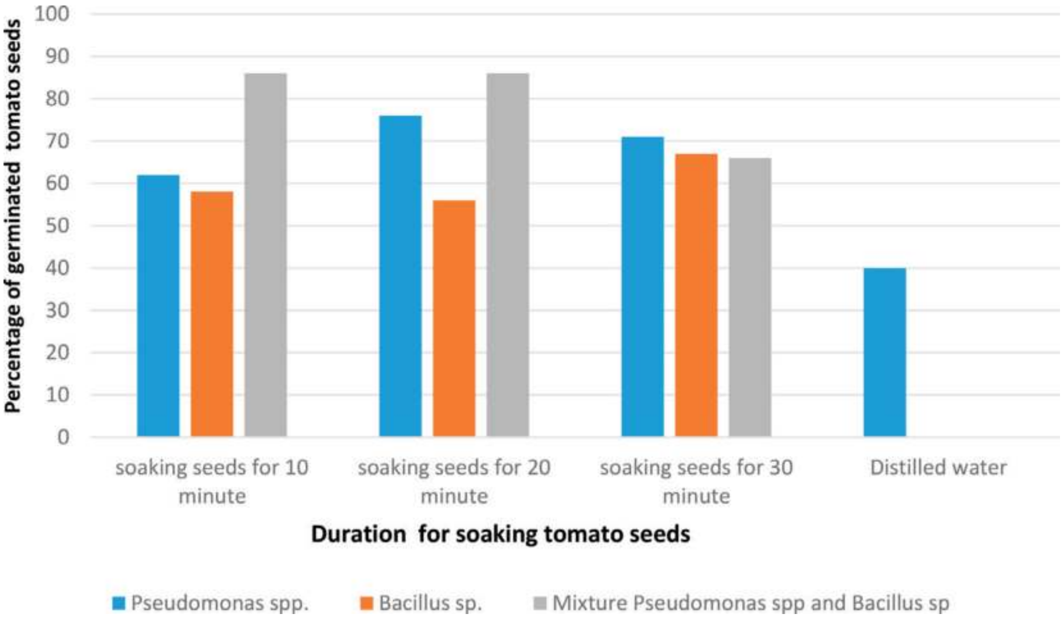

Figure 2. The percentage of tomato seeds germinated after soaking in bacterial suspensions of *Pseudomonas* spp. and *Bacillus* sp.

3. Effect of immersion of tomato seeds in *Pseudomonas* spp. and *Bacillus* sp. on plant height and number of leaves

Soaking tomato seeds with *Pseudomonas* spp. suspension and *Bacillus* sp. can increase the growth of tomato plants. This is evidenced in **Table 1**, with the increase in plant height followed by the increase in number of tomato plant leaves. The positive effect of soaking the tomato seeds is obtained on population density of *Bacillus* sp. and *Pseudomonas* spp. which is a minimum of 4 × 10^5 to 12 × 10^5 CFU. The application of *Pseudomonas* spp. suspension with concentration of 5 × 10^5 CFU through seed immersion showed significant difference in tomato plant height, with average tomato height in the first and fourth week at 2.7 cm and 8.5 cm, respectively [8] (**Table 2**).

Tomato plants treated with rhizobacteria have higher productivity caused by the ability of PGPR in spurring plant growth and inhibiting the growth of pathogens. This is in accordance with Hatayama et al.'s [9] study that plants treated with PGPR bacteria have higher yields than controls. One of the PGPR product compounds that inhibit the growth of pathogens is siderophore. Siderophore serves as a systemic booster of plant resistance by inducing plants to form salicylic acid at higher level. Mukaromah [10] stated that salicylic acid acts as a signal transduction gene that activates the systemic inducing receptor in plant tissue. *Bacillus* sp. and *Pseudomonas* sp. are antagonistic microorganisms that are able to suppress soil pathogens by forming antibiotic compounds such as chitinase enzymes that can hydrolyze fungal cell walls and form siderophores and other antibiotics [11, 12].

The growth of tomato seedlings after the soaking treatment with suspensions of *Pseudomonas* spp. bacteria, *Bacillus* sp., and suspense mixture of both types of bacteria with different soaking time for 10, 20, and 30 min are presented in **Figures 3–5**. It appears that immersion with sterile water provides the smallest seed growth as compared to other treatments. Soaking tomato seeds for 20–30 min in the suspensions gives better growth for

Seedling height	Control average	Treatment average	95.00% confidence	t	df	p-value	Significance
1st week	0.5	2.7	1.8	8.589	49.714	0.000	Significant
2nd week	3.0	5.0	1.6	8.596	41.209	0.000	Significant
3rd week	4.3	6.1	1.2	5.612	27.993	0.000	Significant
4th week	7.8	8.4	0.2	2.363	30.688	0.012	Significant

Table 1. T-test results of the higher tomato seedlings on control and soaking treatment with suspensions of *Pseudomonas* spp. and *Bacillus* sp.

Leaves of seedlings	Control average	Treatment average	95.00% confidence bound	t	df	p-value	Significance
1st week	0.5	1.5	0.6	4.923	32.166	0.000	Significant
1st week	2.5	2.7	−0.2	0.740	25.716	0.233	Nonsignificant
1st week	3.9	3.9	−0.2	−0.204	32.195	0.580	Nonsignificant
1st week	5.2	5.1	−0.2	−0.437	30.990	0.667	Nonsignificant

Table 2. T-test results of the number of leaves of tomato seedlings on control and soaking treatment with suspensions of *Pseudomonas* spp. and *Bacillus* sp.

tomato germination. This indicates that the soaking of tomato seeds with suspensions of bacterium *Pseudomonas* spp. and *Bacillus* sp., or suspense mixture of both types of bacteria is very useful in spurring the growth of tomato seeds when the soaking treatment lasts 20–30 min.

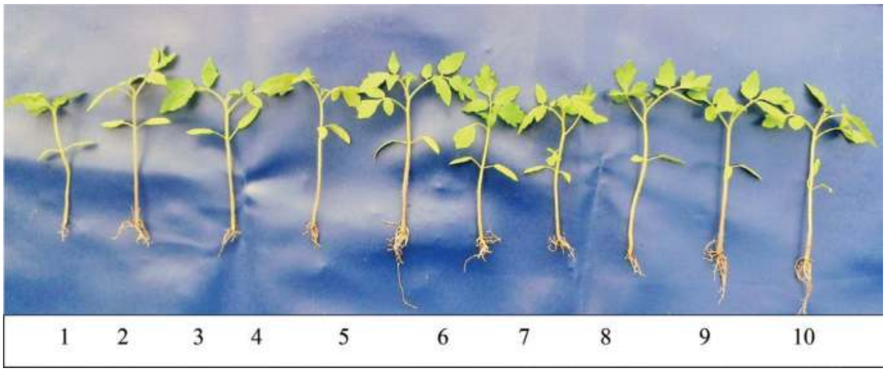

Figure 3. Growth of tomato seeds with *Bacillus* sp. suspense at different seed soaking time periods. *Note*: 1: seeds soaked in distilled water for 20 min; 2–4: seed soaked in *Bacillus* sp. suspension for 10 min; 5–7: seed soaked in *Bacillus* sp. suspension for 20 min; 8–10: seed soaked in *Bacillus* sp. suspension for 30 min.

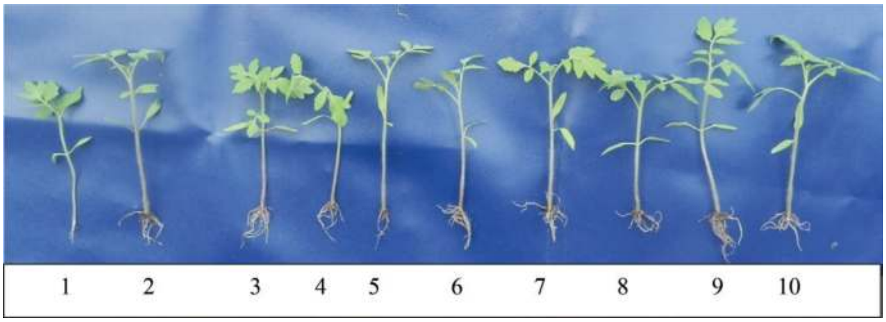

Figure 4. Growth of tomato seeds with *Pseudomonas* spp. suspension at different seed-soaking time periods. *Note*: 1: seed soaked with distilled water for 20 min; 2–4: seed soaked *Pseudomonas* spp. suspension for 10 min; 5–7: seed soaked *Pseudomonas* spp. suspension for 20 min; 8–10: seed soaked *Pseudomonas* spp. suspension for 30 min.

Figure 5. Growth of tomato seeds with a suspense mixture of *Bacillus* sp. and *Pseudomonas* spp. in different seed soaking times. *Note*: 1: seed soaked with distilled water for 20 min; 2–4: seed soaked in *Bacillus* sp. + *Pseudomonas* spp. suspension for 10 min; 5–7: seed soaked in *Bacillus* sp. + *Pseudomonas* spp. suspension for 20 min; 8–10: seed soaked in *Bacillus* sp. + *Pseudomonas* spp. suspension for 30 min.

4. Tomato seed immersion treatment on growth and yield of tomato plants

Treatment of tomato seeds with *Pseudomonas* spp. bacterial suspension in addition to spurring the germination of tomato seeds also has an impact on the growth and yield of tomato fruit [13]. Significant differences were observed ($P \leq 0.01$) among plant height and leaf numbers for *P. alcaligenes* bacteria isolate and the application method used (**Table 3**). Also significant differences were observed ($P \leq 0.01$) among fruit number, total fruit weight per plant, and weight per tomato fruit for *P. alcaligenes* bacteria isolate and the application method used (**Table 4**).

Soaking tomato seeds with *P. alcaligenes* suspension yielded a significant effect on the number of tomato leaves, where the number of leaves reached 192.11 strands on immersion with *P. alcaligenes* TmA1, followed by *P. alcaligenes* TrN2 where the number of leaves reached 182.4 strands. There were 161.6 strands on soaking the seeds with *P. alcaligenes* KtS1, whereas in soaking the seeds with distilled water, the number of leaves was only 78.6 strands. Soaking tomato seeds with *P. alcaligenes* suspension also yields a significant effect on tomato plant height. The highest tomato plant reached 120.4 cm in tomato seed immersion with suspension *P. alcaligenes* TmA1, followed by 116.3 cm with *P. alcaligenes* TrN2, and 114.1 cm with *P. alcaligenes* KtS1, while in soaking the seeds with distilled water, tomato plant height was

Treatment	Application method	Plant height (cm)	Leaf number (leaf)
Distilled water (control)	Root dipping	36.1d	78.6f
	Seed soaking	36.1d	78.6f
	Seedling watering	36.1d	78.6f
P. alcaligenes KtS1	Root dipping	87.7c	109.2e
	Seed soaking	114.1ab	167.6b
	Seedling watering	98.5c	150.1bc
P. alcaligenes TrN2	Root dipping	97.6c	118.4de
	Seed soaking	116.3a	182.4a
	Seedling watering	104.5bc	149.8bc
P. alcaligenes TmA1	Root dipping	98.3c	129.4cd
	Seed soaking	120.4a	192.1a
	Seedling watering	105.5bc	157.7b

Notes: Values followed by the same letter in the same column are not significantly different at 5% DMRT.

Table 3. *Pseudomonas alcaligenes* isolate treatment and the application method on plant height and leaf number of tomato plants.

Treatment	Application methods	Fruit number	Fruit weight/plant (g)	Average weight per fruit (g)	Fruit weight/ha (tons)
Distilled water (control)	Root dipping	30.6c	84.0e	2.8d	3.8e
	Seed soaking	30.6c	84.0e	2.8d	3.8e
	Seedling watering	30.6c	84.0e	2.8d	3.8e
P. alcaligenes KtS1	Root dipping	41.9b	231.6e	5.1bc	10.4e
	Seed soaking	55.0a	278.3d	5.1bc	12.5d
	Seedling watering	48.8b	241.0d	5.0bc	10.8d
P. alcaligenes TrN2	Root dipping	58.8a	237.2d	4.1cd	10.7d
	Seed soaking	70.7a	393.1b	5.9ab	17.7b
	Seedling watering	62.9a	330.4c	5.3bc	14.9c
P. alcaligenes TmA1	Root dipping	54.9b	259.4d	4.8bc	11.7d
	Seed soaking	64.3a	451.9a	7.2a	20.3a
	Seedling watering	58.9a	376.3b	6.7ab	16.9b

Notes: Values followed by the same letter in the same column are not significantly different at 5% DMRT.

Table 4. *Pseudomonas alcaligenes* isolates and the application method on yield of tomato plants.

only 36.1 cm. The abovementioned data indicate that the seed-soaking treatment is the best application method when compared to soaking the roots of the seedlings or watering the tomato seeds (**Table 3**)

Soaking tomato seeds with *P. alcaligenes* suspension has a significant effect on the number of fruits per plant, fruit weight per plant, average weight per fruit unit, and fruit weight in hectare. On the weight parameters of tomato per plant, the average weight per fruit unit, and the weight of tomato per hectare, it was found that soaking the tomato seeds with a suspension of *P. alcaligenes* TmA1 had a significant effect and was significantly different with all other treatments. The highest weight of tomatoes per plant, weight per fruit unit, and fruit weight per hectare was found in tomato seed immersion treatment with *P. alcaligenes* TmA1 suspension that are 451.9, 7.2, and 20.3 tons, respectively. This value differs significantly with all other treatments (**Table 4**).

5. Conclusion

1. Soaking tomato seeds in a suspension of *Pseudomonas* spp. and *Bacillus* sp. can accelerate germination by 2–3 days than when not being immersed in both bacterial suspensions.

2. Soaking the tomato seed for 10–30 min in *Pseudomonas* spp. suspension yields the same effect on the speed of germination of tomato seeds.

3. Soaking of tomato seeds in *Bacillus* sp. tends to cause tomato growth faster than soaking in *Pseudomonas* spp. suspension.

4. Soaking the tomato seed for 20 min with *Pseudomonas* spp. suspension and Bacillus sp. at a population density of 8×10^5 CFU can increase the weight of tomatoes to 490% compared to controls.

Author details

I Ketut Widnyana

Address all correspondence to: widnyanaketut@gmail.com

Faculty of Agriculture, Laboratory of Agrotechnology, Mahasaraswati University of Denpasar, Denpasar, Bali, Indonesia

References

[1] McMilan S. Promoting Growth with PGPR. The Canadian Organic Grower. Soil Food Web Canada Ltd. Soil Biology Lab. & Learning Centre; 2007

[2] Sarma MV, Saharan RK, Prakash K, Bisaria A, Sahai V. Application of fluorescent pseudomonads inoculant formulations on *Vignamungo* through field trial. International Journal of Biological and Life Sciences. 2009;**7**:514-525

[3] Van Peer R, Schippers B. Plant growth responses to bacterization with selected *Pseudomonas* Spp. strains and rhizosphere microbial development in hydroponic cultures. Canadian Journal of Microbiology. 1988;**35**:456-463

[4] Weller DM, Cook RJ. Increased growth of wheat by seed treatments with fluorescens pseudomonads, and implications of phytium control. Canadian Journal of Plant Pathology. 1986;**8**:328-334

[5] Araújo FF, Henning AA, Hungria M. Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. World Journal of Microbiology and Biotechnology. 2005;**21**(8):1639-1645

[6] Tenuta M. Plant PGPR. Prospects for Increasing Nutrient Acquisition and Disease Control. Department of Soil Science. University of Manitoba; 2004

[7] Widnyana IK, Ngga M, Sapanca PLY. The effect of seed soaking with rhizobacteria *Pseudomonas alcaligenes* on the growth of swamp cabbage (*Ipomoea reptans* Poir). IOP Conference Series: Journal of Physics: Conference Series. 2017;**953**(2017):012007. DOI: 10.1088/1742-6596/953/1/012007

[8] Widnyana IK, Javandira C. Activities *Pseudomonas* spp. and *Bacillus* sp. to stimulate germination and seedling growth of tomato plants. Agriculture and Agricultural Science Procedia. 2016, 2016;**9**:419-423

[9] Hatayama K, Kawai S, Shoun H, Ueda Y, Nakamura A. *Pseudomonas azotifigens* sp. nov., a novel nitrogen-fixing bacterium isolated from a compost pile. International Journal of Systematic and Evolutionary Microbiology. 2005;**55**:1539-1544

[10] Mukaromah F. Relationship between population aphids with CMV disease incidence in the H382 tobacco introduced bacterium *Pseudomonas aeruginosa*, red worms (*Lumbricus rubellus*) and virus CMV-48. Essay. Faculty of Agriculture Universitas Jember; 2005 (in bahasa)

[11] Habazar T, Yaherwandi. Biological Control of Plant Pests and Diseases. Padang: Andalas Press; 2006 (in Bahasa)

[12] Wang SL, Chang WT. Purification and characterization of two bifungsional chitinases/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab Shell powder medium. Applied and Environmental Microbiology. 1997;**63**(2):380-386

[13] Widnyana IK, Suprapta DN, Sudana IM, Temaja IGRM. *Pseudomonas alcaligenes*, potential antagonist against *Fusarium oxysporum* f. sp. *lycopersicum* the cause of fusarium wilt disease on tomato. Journal of Biology, Agriculture and Healthcare. 2013;**3**(7):163-169