JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 947-957

Antagonistic *Streptomyces*-PR87 Application, Improves Significantly Growth, Yield, and Fruit Qualities of Greenhouse Tomatoes

Assiri Klangsawad[1] and Petcharat Thummabenjapone[1,2]*

[1]Plant Pathology Division, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen - 40002, Thailand.
[2]Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University, Khon Kaen - 40002, Thailand.

(Received: 02 February 2016; accepted: 30 March 2016)

***Streptomyces*-PR87 has potential to control plant pathogens and promote plant-growth. This research aims to, develop the application of *Streptomyces*-PR87 for tomato-growth enhancement and determine plant-growth promoting mechanisms. Results, the efficient protocol was weekly interval cell suspension spraying OD_{600} = 1 volume 200 mL / tray 4 times. Seedling was showed the increasing of plant height, number of true leaf, SPAD value, root area and biomass. Continuous spraying OD_{600} = 1, 20 mL/plant at after transplanting, 12 weeks was promoted plant high, SPAD value, number of inflorescence, fruit set inflorescence and also increased yield up to 50-60 percent, flesh thickness 16-20 percent, fruit firmness 24-66 percent and percentage of Brix. Response of 6 tomatoes varieties on spraying application was showed well growth and non-appeared negative effected. *Streptomyces*- PR87 has mechanisms involved plant-growth promotion including biosynthesized indole-3-acetic acid via tryptophan dependent pathway with the highest level was 3.07 μg/ mL, the highest phosphate solubility index was 2.0 and presented of siderophore activity.**

Keywords: Plant-growth promoting bacteria (PGPBs), Indole-3-acetic acid (IAA), phosphate-solubilizing microorganism (PSMs), siderophore, *Lycopersicon esculentum*.

Tomato is a one of the widely consumed vegetable, rich source of minerals, vitamin A, fiber, proteins, and bioactive anticancer[1]. In the past decade, total world productivity was ongoing increased, approximated to 163 million tons in 2013[2]. The improper using of chemical pesticides and fertilizers in agriculture are serious issues. Plant-beneficial microbe utilization is an alternative strategy for agriculture worldwide[3].

Plant growth-promoting bacteria (PGPBs) are among such microbes greatly contributing to enhance plant growth and productivity[4]. The various advantages, safety for human, reduce environmental damage, quickly decompose, can be able to multiply themselves, and capable apply in conventional or integrated pest management practices[3]. PGPBs inhabit in rhizosphere, phyllosphere or entophyte. The divers mechanisms through direct or indirect mechanisms, enhancing nitrogen or phosphate uptake, decrease soil toxicity[5], release phytohormones thus directly modulate plant growth[3], modulate plant hormone biosynthesis[5], and plant-disease suppression[3].

PGPBs screening and applications are important steps for successfully keys to utilizing[6]. Seeds and plants direct non-formulation bacterization was pioneering procedure for PGPBs application, still obtain nowadays, which demonstrated to the large number of publications[7].

* To whom all correspondence should be addressed.
TeleFax: +66-43-343114;
E-mail: petsir@kku.ac.th

In tomato, drenching of seedling root in IAA biosynthesized *Azospirillum brasilense* FT326 was increased shoot and root fresh weight, root length, and root surface[8]. Also seed soaking in siderophores produced fluorescent *Pseudomonas* Psf5 was significantly improved the level of root dry weight and reduced damage of damping-off disease[9]. Seed bacterization of phosphate solubilizing *Bacillus circulans* CB7 was promoted shoot, root length, and plant dry biomass[10].

Several of actinobacteria have capability to suppress plant pathogens and promote plant growth[11]. *Streptomyces* are filamentous gram-positive actinobacteria, has DNA G+C content of 69-78 mol%[12], important source of antibiotics[13], and wide range of hydrolytic enzymes[14]. *Streptomyces* well known as soil microbe and a few species are plant pathogen[15]. Several of plant-beneficial *Streptomyces* were proposed as biological control and plant-growth promoting agents. The first commercial *S. griseoviridis* (Mycostop®)[11] , *S. toxytricini* vh6, *S. flavotricini* vh8, *S. toxytricini* vh22, *S. avidinii* vh32, *S. tricolor* vh85[16], *S. mutabilis* NBRC 12800, *S. cyaneofuscatus* JCM 4364[17]. The several of difference applications of *Streptomyces* strains for promote tomato growth were proposed such as soil infestation spore[18] or seed soaking[17].

Antagonistic *Streptomyces*-PR87 is broad spectrum bio-control agent *in vitro*[19-24]. The plant-disease suppression mechanisms were proved, production of cellulase, avicellase, chitinase, amylase and source of antibiotics[19]**.** In some evidences *Streptomyces*-PR87 act as plant-growth promoting agent, induced growth of chili pepper[23] and cucumber[21]. On the other hand, high level of inoculums application was made necrotic symptom on cucumber leaf [21]. In tomato, cell suspension seed coating was showed reduction of shoot height and root length[25] as well as overnight seed submerged before sowing was reduced growth of tomato seedlings[26].

The development of standard protocol is one of an important step for field utilization. Although, previous reports suggested *Streptomyces*-PR87 is an effective antagonist and plant-growth promoting agent. Nevertheless, a few of evidences were indicated the limitation of application in both lab- and field-trials, and no report involve plant growth promoting mechanisms of this strain. The aims of this study were, to 1) establish the application protocol of *Streptomyces*-PR87 for improves tomato-growth, yield, and fruit qualities in natural greenhouse condition, and 2) to prove plant-growth promoting mechanisms including IAA biosynthesis, phosphate-solubilizing activity and siderophore biosynthesis in *vitro*.

MATERIALS AND METHODS

Inoculums preparation

Streptomyces-PR87 in 20% glycerol stock preserved at -20°C was activated on arginine glycerol mineral salt agar (AGMA)[27] (per litter contained; 1.0 g arginine, 12.5 mL glycerol, 1.0 g K_2HPO_4, 1.0 g NaCl, 0.5 g $MgSO_4$ $7H_2O$, 10.0 mg $Fe_2(SO_4)_3$ $6H_2O$, 0.1 mg $CuSO_4$ $5H_2O$, 0.1 g $ZnSO_4$ $7H_2O$, 0.1 g $MnSO_4$ H_2O, 15.0 g agar and final adjusted to pH 6.9-7.1), incubated at 28°C for 7 days. A piece of agar medium (sporulation stage) was transferred into arginine glycerol mineral salt broth (AGMB), contained 150 mL, incubated at room temperature (28-30°C) on 150 rpm running shaker for 7 days. The cell were harvested by filtering paper (Whatman®No.1) and homogenized at 24,000 rpm for 5 min, finally preserved at 4°C. The density was measured with spectrophotometer at 600 nm, and plating in AGMA for population counting.

Establishment of application protocols

The experimental field located at faculty of agriculture, Khon Kaen University, Thailand**.** The plant investigations were divided to 2 experiments of seedling and 1 experiment of after transplanting stage. Seedling experiments were applied planting mediam non-sterilized peat moss (Kekkila®). The seed were sown in 104 pots tray container, 1 seed per pot. For transplanting experiment, was applied non-sterilized artificial mixed soil contained 3 liter per pot. Non-sterilized water was applied when necessary. Insecticides, carbosulfan, imidacloprid, and acetamiprid and also fertigation were according application program. All of experiments were maintained under a greenhouse with natural condition.

Drenching vs. spraying applications in seedling

The experiment was used local commercial tomato seed sida type var. Sida (East-west seed®) and table type var. Lugthor (Chiatai®).

This experiment was conducted in late rainy season from September to November in 2013. The investigation were varied on (A) applications (drenching and spraying), inoculums concentration and (B) variety of tomatoes. For d0renching protocol (3D), drenched *Streptomyces*-PR87 cell suspension concentration at OD_{600}=3 (approx. 10^6 cfu mL), volume at 2 mL/pot, repeat weekly interval sprayed amount 4 times. For spraying applications, direct sprayed cell suspension concentration at OD_{600}=1 (approx. 10^5 cfu mL) or 3 (approx. 10^6 cfu mL) (1S or 3S), volume at 200 mL/104 pots tray and repeat similar to drenching method.

Responses of tomato varieties on Streptomyces-PR87 spraying

This experiment was conducted in dry season from January to February in 2014. For determine stability of spraying applications on more tomato varieties, using tomato 6 var.; cherry type var. LycoRed and Golden princess, table type var. Manee Siam 80, KKU-T11018, and sida type var. Sida (East-west seed®) and KKU-T24011, excepted var. Sida the seed were obtained from Plant breading research centre for sustainable agriculture, Khon Kaen university, Thailand. The application was according spraying protocol, using cell suspension at concentrations OD_{600}=1 or 3 (1S or 3S).

Transplanting stage application

Transplanting experiment was used var. Sida (East-west seed®) and Lugthor (Chiatai®). The investigation was conducted in rainy season on June-October, 2014. Four-week old tomato seedlings prepared from spraying protocols OD_{600}=1 or 3 (1S or 3S) were transplanted into potting soil. The application was sprayed cell suspension at concentration OD_{600}=1 or 3 covered shoot part and soil, volume at 20 mL per plant, and repeated every week total 12 times.

Growth, yield, and fruit qualities assessment

Seedling

Plant growth assessment following, seed germination (%) (*n*=3) at 7 and 14 days after sowing (DAS). Thirty samples per treatment (*n*=30) were collected shoot height (mm), number of true leaf at 21 and 28 DAS. Leaf chlorophyll content was determined by SPAD chlorophyll meter (SPAD®-502 Konica Minolta)[28] at 21 and 28 DAS. Root area assessment, 28 DAS roots were stained in methyl violet for 30 min and removed surplus staining solution with running water[29]. Finally, the roots were scanned with root length scanner (Delta-T scan®) and calculated to root area/plant (mm^2) with program DT-SCAN. For dry biomass measuring, shoot and root samples at 28 DAS were dried at 60°C for 3 days and recorded the weight (mg).

Transplanting stage

Growth measuring (*n*=60), were collected plant height (cm), SPAD® value at 0, 4, 8 week after transplanting (WAT), number of total inflorescent flower, and fruit set inflorescent flower at 8 WAT. Yield quantity and qualities assessment was recorded number of fruit/plant, total fruit weight/plant (mg), and weight/fruit (mg). For fruit quality tests, matured red fruits (*n*=60) were recorded the data of fruit weight (mg), and flesh thickness (mm). The concentration of total solid soluble sugar content in fruit pericarp tissue was determined by (%) Brix[30] using digital Brix meter (Atago®pocket refractometer PAL-1). Fruit firmness was determined using General ® FHT 801 fruit hardness tester averaged of 3 points per fruit[31].

Assay of plant-growth promoting mechanisms in *vitro*

Streptomyces–PR87 was compared with selected 6 strains of antagonistic *Streptomyces* spp. -PR13, -PR15, -PR22, -PR33, -PR78, and -PR84[19-21].

Indole-3-acetic acid (IAA) biosynthesis

Bacterial each strains were activated on AGMA medium and incubated at 28°C for 7 days. The medium dish 5 mm diameter was transferred in 5 mL of AGMB medium containing 2 mM L-tryptophan or without (*n*=6), after that incubated at 28°C on a 150 rpm running shaker for 7 days. The amount of IAA concentration was quantified via Salkowski's reagent[32]. Briefly, broth culture was centrifuged at 10,000 rpm for 2 min, 2mL of supernatant was added 2 drops of 85% orthophosphoric acid followed 2 mL of Salkowski's reagent (100 ml; 2mL $FeCl_3$ in 98 mL 35%$HClO_4$), handing mixed, and incubated in dark at room temperature for 30 min. Development to a pink color of solution indicated positive IAA production. The solutions were read with spectrophotometer at wave length 530 nm (OD_{530}). IAA concentration of each sample was calculated with an IAA standard curve.

Phosphate-solubilizing activity

Each isolate were triplicate cultured (*n*=3)

in Pikovskaya's agar medium containing 0.5% of insoluble tricalcium phosphate [$Ca_3(PO_4)_2$] (per litter contained; 0.5 g yeast extract, 10.0 g dextrose, 5.0 g $Ca_3(PO_4)_2$, 0.5g $(NH_4)2SO4$,0.2g KCl, 0.1 g $MgSO_4$, 0.0001g $MnSO_4$, 0.1 mg , 0.1 mg $FeSO_4$) and incubated at 28°C for 7days[33]. The halo zone around bacterial colony and colony diameter were recorded, and then calculated to solubilization index (S.I.) following below formula[34].

S.I. = (colony diameter + halo zone diameter)/colony diameter

Siderophore biosynthesis

Siderophore biosysthesis assessment was used standard chrome azurol S agar (CAS)[35]. Each antagonists were 4 points spot inoculated into CAS agar[36] triplicate (*n*=3). The plates were incubated at 28°C, for 7-10 days. The changing of color surround colony edge from blue to pink or orange was indicative of the presence of siderophore production[35].

Statistical analysis

In planta experiments were factorial experiment in completely randomized design (CRD). Statistical significance of means was performed using *Least Significant Difference* (LSD) $P<0.05$. Data were analyzed using SPSS v.19 software.

RESULTS

***Streptomyces*-PR87 applications**

Seedling experiments, drenching and spraying of *Streptomyces* –PR87 all of concentrations (OD_{600}=1 or 3) not made necrotic symptom on leaf and non-appear negative effect on tomato seed germination (Table 1-2). Drenching and spraying applications, the growth level of treated seedlings was significantly (P <0.05) increased higher than untreated plants, in growth parameters of plant height, number of true leaf, leaf chlorophyll density (SPAD value), root area and dried biomass. In group of drenched and sprayed seedlings, were exhibited closely response on growth between drenching and spraying treatment of 2 concentrations and showed correlation of application and var. (Table 1-4). As well as spraying suspension on 6 tomatoes varieties, the spraying applications (OD_{600}=1 or 3) were well increased (P <0.05) plant height, number of true leaf, SPAD value, root area and dried biomass (Table 2).

Continuous spraying *Streptomyces* –PR87 sell suspension both of concentrations (OD_{600}=1 or 3) at transplanting stage were significantly (P <0.05) promoted the average of height, SPAD value, number of total inflorescent flower, fruit set inflorescent flower in var. Sida and Lugthor. In addition, assessment of yield, was showed effective of *Streptomyces* –PR87 to enhance fruit quantity and qualities. The increasing of number of fruit/plant, total fruit weight/plant, and weight/fruit average were appeared in treated tomato. Average number of fruit/plant was increased to 22.04-59.30, 29.21-61.89, and total fruit weight/plant 9.20-67.78, 35.87-61.89 percentage. (Table 3). For fruit quality tests, spraying applied tomatoes were indicated the increasing of flesh thickness 16.79-17.11 and 20.03-29.74, fruit firmness 24.52-37.24 and 66.24-77.38 percentage of var. Sida and Lugthor respectively and also increase fruit weight and content of total soluble sugar in fruit pericarp (Table 4).

Plant-growth promoting mechanisms determination

The results of IAA, siderophore production and phosphate-solubilizing activity was showed in (Table 5). IAA biosynthesis was non-observed in a medium without tryptophan supplemented all of *Streptomyces* spp. 7 isolates. Differently, the IAA was detected in medium with 2mM tryptophan supplemented, which highest were *Streptomyces* –PR87 and –PR84, the concentrations at 3.07, 0.49 µg/ml respectively. The maximum S.I. value of Ca-phosphate solubilizing activity was 2.00 observed in *Streptomyces*–PR87 followed by -PR-84, -PR15, -PR13, and -P33 respectively excepted -PR13 and -PR22 not found this activity. *Streptomyces* -PR33, -PR78, -PR84, and -PR87 were able detected siderophore biosynthesis. The positive isolates showed orange halo surrounded colonies when cultured on CAS medium.

DISCUSSION

Both of drenching and spraying applications are efficient protocol for promote tomatoes growth. However, drenching protocol was consumed labor and time more than spraying protocol. Concentration of cell suspension in spraying applications, OD_{600}=1 or 3 are well closely

Table 1. Comparison on drenching and spraying applications of *Streptomyces* -PR87 for tomatoes seedling growth enhancement

Applications	Var.	Germination (%)		Height (cm)	No. of true leaf	SPAD® value	Root area (mm^2)	Dry biomass weight (mg) 28DAS		
		7DAS	14DAS	28DAS	28DAS	28DAS	28DAS	Shoot	Root	Total
Control	Lugthor	92.94±2.00	92.94±2.00	8.18±0.87	3.13±0.63	26.66±5.11	1110.92±301.10	107.57±17.76	18.83±4.62	126.40±20.60
	Sida	95.15±2.88	96.47±1.15	10.48±1024	4.60±0.50	25.92±2.55	1256.66±247.68	102.08±12.83	26.48±4.01	128.57±15.30
3D	Lugthor	92.30±0.55	95.50±0.57	9.37±1.17	3.56±0.50	30.84±3.99	1097.09±344.37	123.72±22.70	22.54±5.22	146.27±27.18
	Sida	93.56±0.57	97.43±2.08	12.44±1.26	4.96±0.41	27.37±3.33	1179.70±326.76	116.57±15.12	25.84±4.95	142.41±16.40
1S	Lugthor	90.38±1.52	91.34±1.52	10.08±1.27	3.56±0.50	31.69±4.93	1026.22±296.49	132.23±22.16	23.76±4.76	156.00±25.43
	Sida	97.11±2.00	97.43±2.01	11.43±1.36	4.96±0.32	28.76±2.46	1235.75±298.72	116.53±18.91	25.99±5.44	142.53±19.42
3S	Lugthor	93.26±1.73	94.23±2.88	9.68±1.10	3.70±0.60	33.25±3.09	1133.02±170.79	135.73±19.69	25.00±4.23	160.74±22.76
	Sida	96.15±1.00	97.11±3.46	11.91±1.57	5.06±0.52	28.68±3.39	1390.94±298.95	119.27±27.19	27.58±6.81	146.86±32.63
Applications (A)		ns	ns	**	**	**	ns	**	**	**
Var. (B)		**	**	**	**	**	**	**	**	*
A*B		ns	ns	*	ns	*	ns	ns	*	ns
LSD		0.98	1.24	2.28	0.92	0.68	52.76	3.65	0.92	4.22

Note: DAS = days after sowing, ns=non-significant, Means within the same column with a common letter are not significantly different by LSD ($P < 0.05$). Applications: 3D = drenched cell suspension concentration at OD_{600}=3, 1S or 3S = direct sprayed suspension concentration at OD_{600}=1 or 3

Table 2. Growth response of tomatoes 6 varieties on *Streptomyces* -PR87 spraying applications at difference concentrations

Applications	Var.	Germination (%)		Height (cm.)	No. of true leaf	SPAD® value	Root area (mm^2)	Dry biomass weight (mg) 28DAS		
		7DAS	14DAS	28DAS	28DAS	28DAS	28DAS	Shoot	Root	Total
Control	Manee Siam-80	95.65±4.35	97.10±1.25	10.86±2.15	4.33±0.54	21.82±1.61	965.42±182.21	110.96±18.62	20.56±4.38	131.53±22.51
	KKU-T11018	95.65±2.17	97.822.17	9.69±1.86	4.33±0.47	25.03±2.35	1147.26±200.62	97.62±16.42	21.88±4.03	119.51±19.94
	LycoRed	94.20±3.32	94.92±3.32	8.23±0.95	4.73±0.44	37.30±3.21	785.77±148.28	141.32±18.22	18.17±2.50	159.50±19.65
	Golden princess	76.81±6.98	89.12±3.76	8.16±1.07	4.73±0.44	32.26±4.24	1013.03±326.76	105.58±25.40	22.62±6.19	128.20±30.77
	Sida	89.85±4.52	95.65±2.17	10.66±0.97	4.90±0.30	27.21±2.96	920.66±265.48	135.17±30.25	25.46±6.34	160.64±33.64
	KKU-T24011	22.46±8.23	40.58±7.63	6.75±0.61	4.73±0.44	36.56±4.03	873.53±212.33	134.54±29.52	25.10±4.54	158.71±33.47
1S	Manee Siam-80	88.40±1.25	92.75±1.25	12.29±1.48	4.93±0.44	24.53±1.98	1142.81±315.86	147.38±28.79	26.53±5.53	173.91±32.63
	KKU-T11018	99.27±1.25	99.27±1.25	12.83±1.65	5.03±0.51	27.47±2.06	1098.58±222.65	142.19±23.24	29.16±5.47	171.35±27.33
	LycoRed	92.02±1.25	95.65±2.17	9.22±1.35	5.20±0.40	39.12±2.71	804.94±170.21	158.59±27.34	19.89±3.24	178.48±29.07
	Golden princess	78.98±1.25	89.12±3.76	9.03±0.95	5.10±0.40	35.85±2.48	1280.27±387.47	132.02±33.39	27.72±6.76	159.74±37.93
	Sida	92.75±5.47	97.82±2.17	9.98±0.92	5.10±0.30	28.94±2.40	981.18±223.37	137.75±20.82	25.98±5.05	163.73±24.92
	KKU-T24011	15.94±4.52	36.96±0.00	7.09±0.84	5.00±0.00	37.89±7.14	1030.51±237.88	156.29±27.70	26.29±4.74	182.59±28.23
3S	Manee Siam-80	94.92±1.25	97.10±2.51	11.72±0.98	4.60±0.49	22.86±1.79	1020.40±328.46	133.60±20.11	24.51±4.38	158.11±20.32
	KKU-T11018	97.82±2.17	97.82±2.17	11.74±1.37	4.80±0.48	25.80±1.86	1017.77±217.76	117.73±16.28	26.13±4.14	143.87±19.48
	LycoRed	87.68±2.51	93.47±3.76	9.25±1.12	5.00±0.00	39.62±2.59	901.13±236.01	141.32±18.22	20.42±3.14	193.37±30.10
	Golden princess	74.64±8.78	89.85±1.25	8.52±1.81	5.00±0.00	35.70±6.49	1213.45±461.59	127.95±41.48	26.99±6.15	154.94±43.65
	Sida	93.47±3.76	96.37±3.32	10.39±1.25	5.10±0.30	29.11±2.61	1180.67±359.62	149.64±16.79	29.69±5.25	179.33±16.59
	KKU-T24011	22.46±1.25	44.20±6.98	6.75±0.59	5.00±0.00	40.02±3.64	895.95±222.09	160.50±25.11	26.72±3.86	187.23±26.11
Applications (A)		ns	ns	**	**	**	**	**	**	**
Var. (B)		*	*	**	**	**	**	**	**	**
A*B		ns	ns	**	*	ns	**	**	**	**
LSD		2.48	1.96	1.36	0.056	0.51	40.90	3.75	0.73	4.24

Note: DAS = days after sowing, ns=non-significant, Means within the same column with a common letter are not significantly different by LSD ($P < 0.05$). Applications: 1S or 3S = direct sprayed suspension concentration at OD_{600}=1 or 3

Table 3. Growth and development of tomatoes at after transplanting stage on *Streptomyces* -PR87 spraying applications at difference concentration.

App..	Var.	Height (cm)			SPAD value			No. of inflorescence flower	No. of fruit set inflorescence	No. of fruit	Total fruit weight /plant	Weight /fruit
		0WAT	4WAT	8WAT	0WAT	4WAT	8WAT	8WAT	8WAT	12WAT	12WAT	12WAT
Control	Lugthor	11.83±1.16	115.00±7.61	160.39±12.15	28.25±3.09	37.27±5.88	40.45±2.44	9.07±1.91	1.92±0.64	4.32±0.76	109.08±21.18	25.85±6.29
	Sida	7.70±1.28	99.81±7.58	138.04±9.27	35.49±2.64	42.26±2.01	41.72±5.68	14.91±2.43	7.48±2.73	13.54±5.43	217.15±78.86	16.53±3.87
1S	Lugthor	12.15±0.79	125.61±6.55	166.34±12.76	33.27±2.18	43.09±2.83	42.63±2.67	10.86±1.97	2.60±0.91	5.43±1.16	176.60±25.94	33.43±6.99
	Sida	12.06±1.55	99.45±12.77	148.25±8.40	42.28±2.26	46.77±2.42	45.93±2.46	18.50±3.22	11.37±4.05	21.57±6.31	364.36±117.85	16.83±2.70
3S	Lugthor	12.29±0.71	125.73±4.94	171.30±15.14	33.16±2.12	44.07±3.26	42.77±2.10	10.95±2.12	2.43±0.76	5.50±1.10	168.04±32.37	30.98±4.62
	Sida	11.78±0.93	118.48±12.00	150.46±8.00	42.04±2.08	44.68±5.13	45.39±5.71	19.04±3.49	11.84±3.46	21.18±4.89	364.24±90.27	17.33±2.71
Applications (A)		**	**	**	**	**	**	**	**	**	**	**
Var. (B)		**	**	**	**	**	**	**	**	**	**	**
A*B		**	**	ns	**	**	ns	**	**	**	**	**
LSD		0.64	1.26	1.62	0.31	0.55	0.55	0.37	0.37	0.66	11.72	0.72

Note: WAT= week after transplanting, ns=non-significant, Means within the same column with a common letter are not significantly different by LSD ($P < 0.05$). Applications: 1S or 3S = direct sprayed suspension concentration at OD_{600}=1 or 3

response on several growth parameters. This comparisons was showed spraying cell suspension OD_{600}=1 was high possibility for large field utilizing than other protocols. In addition, this report proposed stability of spraying protocol on growth enhancement of 6 tomatoes varieties representative of 3 types. This evidence was indicated capability of *Streptomyces*–PR87 OD_{600}=1 spraying protocol may be broad spectrum for using with wide range of tomato varieties.

The effective utilizing of *Streptomyces*–PR87 should be considerate on plant species, concentration of inoculums, and application. Previous reports[21,25-26] were proposed the disadvantages of application. *Streptomyces*–PR87 (10^5 cfu mL) spraying on 1-week old melon seedling was produced light-brown necrotic spot on leaf[25]. On the other hand, also applied similar concentration not made abnormality symptom on chili leaf[23]. Like a chili, non-appeared necrotic symptom on tomatoes when applied suspension at range 10^3-10^7 cfu mL[26]. Nevertheless methods of inoculation is important factor, direct seed coating[25] and overnight seeds submerged methods were reduced tomato seedling germination and growth[23]. Differ from this study, direct drenching and spraying method were showed the increasing of tomatoes growth and development. This evidence should be involved with mechanisms between tomato and *Streptomyces*–PR87 on regulate plant growth and development. Root exudate is one of explanation, he content of root exudate is depends on plant species and growth stage. Each plant species or plant varieties were produced difference level of organic acids, sugar, and L-tryptophan, and it effect on microbe colonization and phytostimulating activity[37-38]. Previous report[38], the difference content of tomato root exudates was affected on *P. chlororaphis* SPB1217 and *P. fluorescens* SPB2137 growth and also effected on tomatoes growth. This evidences suggested the important role of root exudates on PGPBs-plant association and plant available. The mechanisms of plant growth promotion understanding is important point for suitable selection type of bacteria to use with a plant[39].

IAA has the most importance roles in higher plants to regulation of growth and development processes[40]. This research work was explained the IAA biosynthesis depends on

bacterial strains, the highest IAA biosynthesis was *Streptomyces*-PR87. Total 4 of 7 antagonistic-*Streptomyces* produced IAA at the range of 0.49-3.07 µg/mL only in a medium with presented of tryptophan. Similar to other genus member, 30 isolates of *Streptomyces* sp. from medical plant rhizospheres were biosynthesized IAA in a medium presented of 0.2% tryptophan with the range of 5.5-144 µg mL. Like other PGPBs, the IAA concentration was involved with concentration of tryptophan[32]. Some evidence were showed critical level of tryptophan for IAA biosynthesis, highest IAA concentration of *Streptomyces viridisi*-CMU H009 observed in a medium supplement with 2 mg mL tryptophan. In conditions of lower or higher level of tryptophan the IAA level was decreased. Furthermore, temperature and pH was effected factors on IAA biosynthesis[41]. Tomato root endophytic *Streptomyces*-PT2, was produced IAA at highest level in medium pH=7, supplemented of 5 mg mL tryptophan, at 25°C[17]. This research was resulted the antagonistic-*Streptomyces* spp. produced IAA via only tryptophan-dependent pathway similar to *S. scabies* potato-scab diseased agent[42], and *Streptomyces*-En-1 were showed IAM pathway[43].

Not only phytohormones, plant mineral nutrition's uptake improvement are direct mechanisms to promote plant growth by PGPBs. Phosphorus or phosphate (PO_4^{3-}) play an important

Table 4. Fruit qualities of *Streptomyces* -PR87 spraying application tomatoes at difference concentration

Applications	Var.	Fruit weight (mg)	Fruit length (mm)	Fruit width (mm)	Flesh thickness (mm)	Fruit firmness (N)	% Brix
Control	Lugthor	36.63±12.94	40.85±6.14	40.27±4.08	54.20±4.82	12.56±2.22	5.81±0.75
	Sida	17.12±3.83	38.03±3.27	28.30±2.27	37.58±5.15	11.09±2.56	6.14±0.73
1S	Lugthor	42.38±8.75	45.73±6.11	39.78±3.03	65.06±4.05	20.88±2.89	5.58±0.54
	Sida	18.82±4.64	38.62±3.11	29.01±2.87	43.89±5.66	13.81±2.51	6.12±1.05
3S	Lugthor	41.93±10.20	43.57±4.96	41.40±2.96	70.32±4.09	22.28±2.36	5.83±0.49
	Sida	18.26±3.33	38.73±2.93	28.62±2.54	44.01±5.29	15.22±3.26	6.41±0.73
Applications (A)		**	**	ns	**	**	*
Var.(B)		**	**	**	**	**	**
A*B		ns	**	*	**	**	ns
LSD		1.05	0.59	0.38	0.63	0.34	0.09

Note: Means within the same column with a common letter are not significantly different by LSD ($P < 0.05$), Applications: 1S or 3S = direct sprayed suspension concentration at OD_{600}=1 or 3

Table 5. Indole-3-acetic acid (IAA), phosphate-solubilizing, and siderophore biosynthesis estimation of antagonistic *Streptomyces* -PR87, -PR13, -PR15, -PR22, -PR33, -PR78, and -PR84 in vitro

Isolates/plant -growth promoting mechanisms	IAA concentration		Phosphate-solubilizig activity solubilizing index (S.I. index)	Siderophore biosynthesis
	AGMB Without Tryptophan	AGMB+ 2mM Tryptophan		
PR13	-	-	-	-
PR15	-	0.10	1.04	-
PR22	-	-	-	-
PR33	-	-	1.03	+
PR78	-	0.10	1.04	+
PP84	-	0.49	1.44	+
PR87	-	3.07	2.00	+

Note: (+) positive, (-) negative

role on various plat-growth and development processes[40]. Phosphate in soil usually bonded with calcium, aluminum, or iron. Phosphate-solubilizing micro-organisms (PSMs) have capability to soluble phosphate to plant-beneficial forms with several mechanisms[44]. Our study was presented *Streptomyces* -PR15, -PR33, -PR78, -PR84, and -PR87 have capable to soluble tri-calcium phosphate (Ca-P) and *Streptomyces* –PR87 showed highly solubilizing index.

Bacteria obtained iron through iron chelator called siderophore[45] and over 500 siderophore species have been determined[46]. Siderophores play a role on plant-diseases suppression and plant-microbe interaction [47]. Fluorescent *Pseudomonas* Psf5 siderophore produced strain was suppressed damping-off disease of tomato[48]. Bacterial strain was synthesized more one siderophore species, *S. coelicolor* produced coelichelin and desferrioxamine, *S. tendae* produced desferrioxamine and enterobactin[45]. In iron-deficiency condition, the siderophores synthesis was increased and decreased in iron added condition[49]. Siderophore well know play a role on plant-diseased antagonistic agent, in addition to the role on direct plant-growth promoting was enlarge proposed. On the other hand, siderophore play role on plant-microbe interaction, drenching of *Streptomyces* sp. GMKU3100 siderophore produced strain was increased mung bean root length, shoot length, and also dried biomass than mutant strain *Streptomyces* sp. GMKU3100 lack of siderophore produced gene[50]. Tomato and maize seeds treated with pure siderophore isolated from *Pseudomonas auroginosa* were showed the increasing of 3-4 times of seedling shoot length[51]. The promotion of tomatoes growth and fruit qualities of this research was involved with several mechanisms. This reason was supported the suggestionsuggestionËs, PGPBs have divers mechanisms to promote the growth and it is very difficult to separate the influence of any mechanisms[3].

Furthermore, the investigation necessary to develop formulations for improve product stability and shelf life. The inoculants can be liquid, slurry, granular, or powder formulations[7]. This research suggested ACC deaminase may be one of influence mechanism on plant-growth regulation and alternative using for promote plant growth in drought and salinity conditions[11].

CONCLUSIONS

This research proposed broad-spectrum application for tomatoes growth enhancement, the spraying of *Streptomyces*–PR87 cell suspension at concentration OD_{600}=1, volume at 200 mL/ tray (104 pots) every week total 4 times for seedling and continuously spraying 15-20 mL/pot for transplanting tomatoes was most effective application. The results, *Streptomyces*-PR87 application was promoted tomatoes growth as well as improves yield and fruit qualities. Plant-growth promoting mechanisms of *Streptomyces*-PR87 was showed IAA, siderophore biosynthesis and phosphate solubilizing activity.

ACKNOWLEDGEMENTS

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Commission, through the Food and Functional Food Research Cluster of Khon Kaen University (code: M-54104). Thailand Research Fund (TRF) Project code: IRG5780003)Khon Kaen University, KKU) and the Faculty of Agriculture KKU for providing financial support for manuscript preparation activities.

REFERENCES

1. Tan, H.L., Thomas-Ahner, J.M., Grainger, E.M., Wan, L., Francis, D.M., Schwartz, S.J., Erdman J.W., Clinton S. K.. Tomato-based food products for prostate cancer prevention: what have we learned?. *Cancer Metastasis Rev.*, 2010; **29**: 553–568.
2. FAO, "FAOSTAT", 2015, http:// faostat3.fao.org/browse/Q/QC/E (Accessed: January 2015).
3. Berg, G. Plant-microbe interactions promoting plant growth and health:perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biot.*, 2009, **84**; 11-18.
4. Miransari, M. Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Appl. Microbiol. Biotechnol.*, 2011; **89**: 917–930.
5. Francis I., Holsters M., Vereeche D. The Gram-positive side of plant–microbe interactions.

Environ. Microbiol., 2010; **12**(1): 1–12.
6. Schoebitz, M., López, M.D., Roldán, A. Bioencapsulation of microbial inoculants for better soil–plant fertilization a review. *Agron. Sustain. Dev.*, 2013; **33**: 751–765.
7. Bashan, Y., Luz, E.D., Prabhu, S.R., Hernandez, J. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil*, 2014; **378**: 1–33.
8. Ribaudo, C.M., Krumpholz, E.M., Cassán, F.D., Bottini, R., Cantore M.L., Curá, J.A. *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. *J. Plant Growth Regul*., 2006; **24**: 175–185.
9. Hammami, I., Ben, H. A., Hamdi, N., Gdoura, R., Triki M.A. Isolation and characterization of rhizosphere bacteria for the biocontrol of the damping-off disease of tomatoes in Tunisia. *C. R. Biologies*, 2013; **336**: 557–564.
10. Mehta, P., Walia, A., Kulshrestha, S., Chauhan, A., Shirkot, C. K. Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for enhancement of tomato growth under net house conditions. *J. Basic Microbiol.*, 2015; **55**: 33–44.
11. Palaniyandi, S.A., Damodharan, K., Yang, S.H., Suh J.W. *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. *J. Appl. Micobiol*., 2013; **117**: 766—773.
12. Anderson, A.S., Wellington, E.M.H. The taxonomy of *Streptomyces* and related genera. *Int. J. Syst. Evol. Micr*., 2001; **51**: 797–814.
13. Chater, K.F. *Streptomyces* inside-out: a new perspective on the bacteria that provide us with antibiotics. *Philos. T. Roy. Soc. B.*, 2006; **361**: 761–768.
14. Connell, N.D. Expression systems for use in actinomycetes and related organisms. *Curr. Opin. Biotech.*, 2001; **12**: 446–449.
15. Loria, R., Bukhalid, R.A., Fry, B.A., King, R.R. Plant pathogenicity in the genus *Streptomyces*. *Plant Dis.*, 1997; **81**: 836-846.
16. Patil, H.J., Srivastava, A.K., Singh, D.P., Chaudhari, B.L., Arora, D.K. Actinomycetes mediated biochemical responses in tomato (*Solanum lycopersicum*) enhances bioprotection against *Rhizoctonia solani*. *Crop Prot*., 2011; **30**: 1269-1273.
17. Goudjal, Y., Toumatia, O., Sabaou, N., Barakate, M., Mathieu, F., Zitouni A., Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. *World J. Microbiol. Biotechnol.*, 2013; **29**: 1821–1829.
18. Palaniyandi, S.A., Yang, S.H., Zhang, L., Suh, J. Effects of actinobacteria on plant disease suppression and growth promotion. *Appl. Microbiol. Biotechnol.*, 2014; **97**: 9621–9636.
19. Purichinawut, P. Molecular genetics of *Streptomyces* spp. which inhibits the growth of bacteria *Acidovorax avenae* subsp. *citrulli* and fungus *Didymella bryoniae*. *Ms Thesis*, 2005, Khon Kaen University, Thailand.
20. Wanking, B. Identification of the *Streptomyces* spp. antagonist of *Acidovorax avenae* subsp. *citrulli*,causal agent of bacterial fruit blotch disease. *Ms Thesis*, 2006, Khon Kaen University, Thailand.
21. Yaeram, C. Development of SCAR-PCR based tagging system for antagonist *Streptomyces* spp. of watermelon fruit blotch disease and evaluation their potential to control disease in green house condition. *PhD Thesis*, 2009, Khon Kaen University, Thailand.
22. Thimas, M. Potential of *Streptomyces* spp. to control bacterial wilt and root knot disease in tomato and the relationship between phenotype and genotype for antagonistic capability against *Ralstonia solanacearum*. *Ms Thesis*, 2009, Khon Kaen University, Thailand.
23. Saengsai, W. Role of antagonistic *Streptomyces* for plant growth enhancement and induced systemic resistance for TMV in Chilli. *Ms Thesis*, 2014, Khon Kaen University, Thailand.
24. Rattikan, Y., Thummabenjapone, P., Hiransalee, A. The potential of antagonistic *Streptomyces*-PR87 and application method to control root knot nematode (*Meloidogyne incognita*) in green house condition. *Khon Kaen Agr. J*., 2013; **41** suppl.: 213-219.
25. Chudpimai, W. Development of antagonistic *Streptomyces* products for controlling bacterial wilt of tomato. *Ms Thesis*, 2012, Khon Kaen University, Thailand.
26. Klangsawad, A., Thummabenjapone, P. Application of Streptomyces-PR87 for growth enhancement of tomato. *Khon Kaen Agr. J*., 2013; **41** suppl.: 205-212.
27. Dhingra, O.D., Sinclair, J.B. Basic plant pathology medthods. U.S.A: Lewis publishers, 1995; pp. 390.
28. Guler, S., Buyuk G. Relationships among chlorophyll-meter reading value, leaf n and yield of cucumber and tomatoes. *Acta. Hortic*., 2007; **729**: 307-311.
29. Bennet, R.J., Breen, C.M., Fey, M.V. Aluminium uptake sites in the primary root of *Zea mays* L. *S. Afr. J. Plant Soil*, 1985; **2**(1): 1-7.

30. Javanmardi, J., Kubota, C. Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest Biol. Tec.*, 2006; **41**: 151–155.
31. Assi, N. E., Huber, D.J., Brecht, J.K. Irradiation-induced changes in tomato fruit and pericarp firmness, electrolyte efflux, and cell wall enzyme activity as influenced by ripening stage. *J. Am. Soc. Hortic. Sci.*, 1997; **112**: 100-106.
32. Khamna, S., YoKota, A., Lumyong, S. Actinomycetes isolated from medicinal plant rhizosphere soil: diversity and screening of antifungal compound, indole-3-acetic acid and siderophore production. *World J. Microb. Biot.*, 2009; **25**: 649-655.
33. Pikovskaya, R.I. Mobilization of phosphorus in soil in connection with vital activity of some microbial species", *Mikrobiologiya*, 1948; **17**: 363-370.
34. Babu, A.N., Jogaiah, S., Ito, S., Nagaraj, A.K., Tran, L.P. Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Sci.*, 2015; **231**: 62–73.
35. Schwyn, B., Neilands, B. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, 1987; **160**: 47-57.
36. Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L.S., Fernández, F.J. O-CAS, a fast and universal method for siderophore detection. *J. Microbiol.Meth.,* 2007; **70**: 127–131.
37. Kamilova, F., Kravchenko, L.V., Shaposhnikov, A.I., Azarova, T., Makarova, N., Lugtenberg, B. Organic acids, sugars, and L-Tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. *MPMI*, 2006; **19**(3): 250–256.
38. Kravchenko, L.V., Azarova, T.S., Makarova, N.M., Tikhonovich, I. A. The effect of tryptophan present in plant root exudates on the phytostimulating activity of rhizobacteria. *Microbiology*, 2004; **73**(2): 156–158.
39. Lucy, M., Reed, E., Glick, B.R. Applications of free living plant growth-promoting rhizobacteria. *A. V. Leeuw.*, 2004; **86**: 1–25.
40. Taiz, L., Zeiger, E. Plant physiology", 4th Edn., Massachusetts, USA; Sinauer associates Inc Publishers, 2006.
41. Khamna, S., Yokota, A. Peberdy, J. F., Lumyong, S. Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *Eurasia. J. Biosci*., 2010: **4**: 23-32.
42. Hsu, S. IAA production by *Streptomyces scabies* and its role in plant microbe interaction. *Ms Thesis*, 2010, Cornell University, U.S.A.
43. Lin, L., Xu, X. Indole-3-acetic acid production by endophytic *Streptomyces* sp. En-1 isolated from medicinal plants. *Curr. Microbiol.*, 2013; **67**: 209–217.
44. Gyaneshwar, P., Naresh, K., Parekhand, L.J., Poole, P.S. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil*, 2002; **245**: 83-93.
45. Chater, K.F., Biro, S., Lee, K.J., Palmer, T., Schrempf, H. The complex extracellular biology of *Streptomyces*. *FEMS Microbiol. Rev*., 2010; **34**: 171-198.
46. Miethke, M., Marahiel, M.K. Siderophore-based iron acquisition and pathogen control. *Microbiol. Mol. Biol. R*., 2007; **73**: 413-451.
47. Hammami, I., Hsouna, A.B., Hamdi, N., Gdoura, R., Triki, M.A. Isolation and characterization of rhizosphere bacteria for the biocontrol of the damping-off disease of tomatoes in Tunisia. *C. R. Biologies*, 2013; **336**; 557–564.
48. Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann. Microbiol.*, 2010; **60**: 579-598.
49. Rachid, D., Ahmed, B. Effect of iron and growth inhibitors on siderophores production by *Pseudomonas fluorescens*. *Afr. J. Biotechnol*., 2005; **4**: 697-702.
50. Rungin, S., Indananda, C., Suttiviriya, P., Kruasuwan, W., Jaemsaeng, R., Thamchaipenet, A. Plant growth enhancing effects by a siderophore-producing endophytic *Streptomycete* isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). *A. V. Leeuw.*, 2012; **102**: 463–472.
51. Rumpaneenil, O. Isolation process of siderophore and its application on seed germination-vigor and growth of some plants. *Ms Thesis*, 2000, Khon Kaen University, Thailand.