The effects of IAA produced by *Bacillus pumilus* A1_YM_1 on growth of orchids under micropropagation

Kajohnpong Dasri[1*], Phukphon Munglue[2], Khwanduean Rattana[2] and Supavee Sangchanjiradet[2]

ABSTRACT: This research investigated the effects of Indole-3-acetic (IAA) produced by *Bacillus pumilus* A1_YM_1 (CF-B) on growth of orchids cultured on Murashige and Skoog (MS) under micropropagation for 4 weeks. The results showed that the most suitable media that provided 100 percent of *D. cumulatum* protocorms survival were MS supplemented with 0.25 and 0.5 mg l^{-1} IAA produced by CF-B combined with 1 mg l^{-1} BA. The highest average score of protocorm growth was 2.96, which induced by culturing on 0.5 mg l^{-1} IAA produced by CF-B added with 1 mg l^{-1} BA. The most suitable medium for plantlet development of *D. delacourii* was MS medium added with 0.5 mg l^{-1} IAA produced by CF-B and this medium can induce the highest average of the number of leaves, the leaf length and the shoot height which were 3.20 leaf/shoot, 7.80 mm. and 4.33 mm., respectively. The highest number of roots (3.20 roots/plants) was obtained when cultured on MS medium supplemented with 0.25 mg l^{-1} IAA produced by CF-B.
Keywords: *Bacillus pumilus*, plant growth regulators, *Dendrobium cumulatum*, *Dendrobium delacourii*, micropropagation

Introduction

Micropropagation is a technique for plant multiplication under sterilized conditions. By this technique, sterilized explants are cultivated on basal media (MS, VW, PM, KC) supplemented with plant growth regulators (PGRs) like auxins and cytokinins. IAA, IBA, NAA, 2,4-D BA and kinetin are generally used in micropropagation. Auxin hormones are commonly added to the media to order to induce plant elongation and root induction. For cytokinin, it usually promotes cell division and apical growth. The combination of the high level of cytokinin and low level of auxin can enhance shoot number and plant growth. In the case of plantlet culture, the use of auxin can stimulate the development of branch root and the differentiation of roots (Nhat and Dung, 2006; Roy et al., 2007; Kunakhonnuruk et al., 2010).

It is generally accepted that the use of chemical fertilizers or synthetic PGRs could lead to increase environmental pollution (Russo et al., 2008). Thus, search for novel biofertilizers or natural PGRs produced by beneficial bacteria may lead to support a sustainable agriculture. Many researchers have been studied the properties of IAA produced by bacteria which were isolated from soil, rhizosphere, manure and biofertilizer in order to use in the cultivation of plants or other purposes (Probanza et al., 2002; Russo et al., 2008; Tsavkelova et al., 2007). It was found that genera *Sphinogmonas*, *Rhizobium*, *Mycobacterium*, *Microbacterium*, *Bacillus* and *Pseudomonas* have the potential to produce IAA.

[1] Program of Microbiology, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000
[2] Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000
* Corresponding author: kajohnpong.d@ubru.ac.th

Whole cell and culture filtrate from those bacteria can promote seed germination, enhance plant growth and protect plants from several important diseases (Probanza et al., 2002; Tsavkelova et al., 2007; Choudhary and Johri, 2009; Lin et al., 2014). Interestingly, some bacterial species also produce other PGRs such as gibberellins-3-acid, salicylic acid, jasmonic acid as well as abscissic acid (Masciarelli et al., 2014).

As there is an urgent need to find better bacteria species that produce biofertilizers or PGRs and some species are discovered (Russo et al., 2008), thus, the aim of this research was to investigate culture filtrate of bacteria compared with auxin (IAA and NAA) to induce growth of orchid under micropropagation.

Materials and Methods

IAA production by *Bacillus pumilus* A1_YM_1

Bacterial strain *Bacillus pumilus* A1_YM_1 was cultured in Nutrient broth (NB) supplemented with 2.5 mM L-tryptophan, incubated at 30°C with shaking 150 rpm for 192 h (Dasri et al., 2013). Quantitative analysis of IAA was measured by Salkowski colorimetric technique (Glickmann and Dessaux, 1995). This experiment was conducted in the Program of Microbiology, Faculty of Science, Ubon Ratchathani Rajabhat University, Thailand.

Plant materials

Protocorms preparation: Capsules of *Dendrobium cumulatum* were washed by 10% detergent and running tap water for 30 min. The capsules were surface sterilized by soaking in 0.05% sodium hypochlorite solution for 15 min and washed three times with sterile distilled water. The capsules were dipped in 95% ethanol for 30 s followed by flaming for 3-4 s. The sterilized capsules were cut open vertically with a sterile scalpel blade and seeds were cultured on Murashige and Skoog medium (MS) (Murashige and Skoog, 1962).

Plantlets preparation: Capsules of *Dendrobium delacourii* were surface sterilized and performed with the same technique as indicated in the protocorms preparation. However, they were inoculated on half strength MS medium for 4 weeks and subcultured intervals onto the same medium. Plantlets containing 2-3 leaves and 3-4 mm in height were used to study the effects of PGRs on plantlets growth.

Protocorms proliferation and plantlets growth

Protocorms of *D. crystallinum* and plantlets of *D. delacourii* were cultured on MS medium supplemented with 0, 0.25, 0.50 and 1.0 mg l^{-1} IAA produced by CF-B compared to synthetic IAA and combination with 1 mg/l BA. The media were adjusted pH to 5.8 and autoclaved at 121°C (15 psi) for 20 min, then cool down media for 45-50°C and added PGRs, mixed well and rinsed into petri dish. Inoculation of protocorms and plantlets on the surface of MS medium was performed and cultured at 25°C with 14-h photoperiod (45 µmol m^{-2} s^{-1}) under cool white fluorescent lamp. Survival rate and proliferation rate of protocorms were noted and scored. The score was designed and ranged between 1-3 due to the number of shoots and leaf development as follows. If protocorms contained 1 shoot and the leaf did not develop, the score was 1. If protocorms contained 2-3 shoots and leaves were gradually developed, the

score was 2. If protocorms contained more than 3 shoots and leaves were permanently developed, the score was 3. Plantlets were then cultivated and the number of shoots, leaves and roots, length of leaves and high of stem were studied after 4 weeks. This experiment was conducted in the Tissue Culture Laboratory, Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, Thailand.

Chemicals

All chemicals were purchased from Sigma, Singapore.

Data analysis

The experiments were designed by completely randomized design (CRD) and the results were analyzed by using analysis of variance (ANOVA). To compare the differences between the test groups, DMRT (Duncan's Multiple Range Test) was used. If $P<0.05$, it was considered statistically significant.

Results and Discussion

The survival of *D. cumulatum* protocorms was 100.0±0.00% when cultured on the basal MS medium supplemented with IAA at the concentration of 1 mg l^{-1} (**Table 1**). The combinations of CF-B (0.25 and 0.50 mg l^{-1}) and BA (1.0 mg l^{-1}) promoted the survival rate of protocorms. Additionally, the combination of CF-B at 0.50 mg l^{-1} and BA at 1.0 mg l^{-1} enhanced proliferation rate of protocorms (2.96±0.09). The protocorms were developed to plantlets and the number of shoots and leaves were increased as shown in Figure 1.

CF-B significantly improved the growth of *D. delacourii* plantlets, when cultured on MS medium supplemented with 0.25 or 0.50 mg l^{-1} CF-B. The number of leaves and roots, the length of leaves and the height of stem were higher than those of other PGRs (**Table 2**).

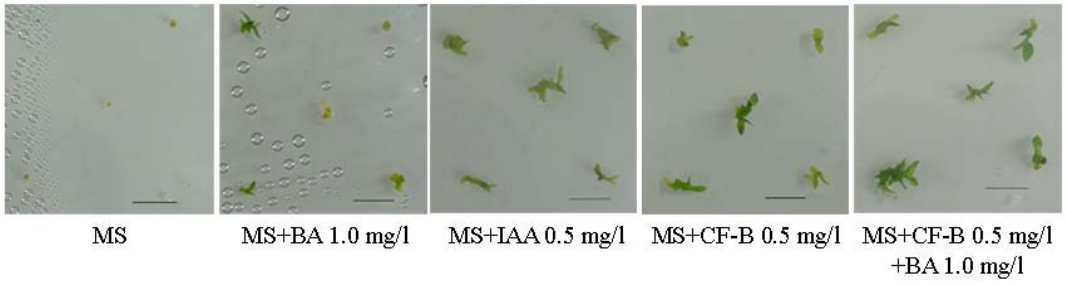

Figure 1 The development of *D. cumulatum* protocorms cultured on MS medium combined with CF-B compared to MS medium combined with the synthetic IAA (Scale bar = 1 cm). MS=Murashige and Skoog (1962), CF-B = culture filtrate of *B. pumilus* A1_YM_1, IAA = indole-3-acetic acid, NAA = α-naphthalene acetic acid, BA = 6-benzylaminopurine.

Our results are agreed with the study of Russo et al. (2008) who studied the application of *Azospirillum brasilense* Sp245 to MS medium combined with IBA to propagate *Prunus cerasifera* L. clone Mr.S 2/5 significantly increased root number, compared with the control group. Our study also showed that CF-B can induce the growth of all parts of cultured orchids and this could be due to the bacterial extract contains not only IAA, the important phytohormone in the regulation of plant growth and plant development (cell division and cell elongation) with a critical role in root initiation, but the extract also contains other PGRs such as gibberellins-3-acid, salicylic acid, jasmonic acid and abscissic acid (Masciarelli et al., 2014). It was found that IAA can potentially protect plants from several diseases, resulting an increase in plant survival when transferred to environmental conditions (Sharaf and Farrag, 2004). Thus, our results support the use of IAA produced by *B. pumilus* A1_YM_1 as a growth promoter to enhance the growth of orchids under micropropagation.

Table 1 Comparative effects of PGRs on survival and proliferation rate of *D. cumulatum* protocorms cultivated on basal MS medium for 4 weeks.

PGRs (mg l^{-1})[1/]				Percentage of survival	Proliferation rate
CF-B	IAA	NAA	BA	(mean±SEM)	(mean±SEM)
control				30.00 ± 34.64^{c}	1.00 ± 0.00^{h}
			1.00	84.00 ± 16.73^{ab}	1.88 ± 0.21^{ef}
0.25				66.67 ± 23.09^{b}	2.08 ± 0.38^{cdef}
0.50				88.00 ± 10.95^{ab}	2.09 ± 0.12^{cdef}
1.00				80.00 ± 20.00^{ab}	2.21 ± 0.20^{bced}
	0.25			92.00 ± 10.95^{a}	2.39 ± 0.30^{bcd}
	0.50			95.00 ± 10.00^{a}	2.53 ± 0.25^{abc}
	1.00			100.00 ± 0.00^{a}	2.10 ± 0.20^{cdef}
		0.25		86.67 ± 11.55^{ab}	1.97 ± 0.63^{def}
		0.50		80.00 ± 0.00^{ab}	1.88 ± 0.14^{ef}
		1.00		84.00 ± 16.73^{ab}	1.43 ± 0.26^{g}
0.25			1.00	100.00 ± 0.00^{a}	2.60 ± 0.23^{ab}
0.50			1.00	100.00 ± 0.00^{a}	2.96 ± 0.09^{a}
1.00			1.00	88.00 ± 17.89^{ab}	2.59 ± 0.25^{ab}
	0.25		1.00	95.00 ± 10.00^{a}	2.40 ± 0.28^{bcd}
	0.50		1.00	95.00 ± 10.00^{a}	2.45 ± 0.34^{bc}
	1.00		1.00	90.00 ± 20.00^{ab}	2.38 ± 0.29^{bcd}
		0.25	1.00	88.00 ± 17.89^{ab}	2.57 ± 0.28^{ab}
		0.50	1.00	92.00 ± 10.95^{a}	2.54 ± 0.45^{abc}
		1.00	1.00	95.00 ± 10.00^{a}	1.73 ± 0.15^{fg}

[1/]PGRs = plant growth regulators, MS=Murashige and Skoog (1962), CF-B = culture filtrate of *B. pumilus* A1_YM_1, IAA = indole-3-acetic acid, NAA = α-naphthalene acetic acid, BA = 6-benzylaminopurine; Mean values within a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test; n = 5 per treatment with 5 replications each.

Table 2 Comparative effects of PGRs on plantlet of *D. delacourii* cultured on basal MS medium for 4 weeks.

PGRs (mg l^{-1})[1/]				No. shoots /plant	No. Leaves/plant	No. Roots/plant	Length of leave (mm.)	Height of stem (mm.)
CF-B	IAA	NAA	BA	(mean±SEM)	(mean±SEM)	(mean±SEM)	(mean±SEM)	(mean±SEM)
control				1.00 ± 0.00	3.11 ± 0.60^{a}	2.33 ± 1.66^{a-d}	7.56 ± 2.65^{ab}	4.22 ± 1.79^{ab}
			1.00	1.22 ± 0.44	2.67 ± 1.00^{ab}	1.89 ± 1.05^{b-f}	7.00 ± 2.69^{a-d}	3.22 ± 1.20^{a-d}
0.25				1.00 ± 0.00	2.60 ± 0.70^{ab}	3.20 ± 1.14^{a}	7.40 ± 2.01^{abc}	4.00 ± 1.25^{ab}
0.50				1.10 ± 0.32	3.20 ± 1.14^{a}	2.80 ± 1.03^{ab}	7.80 ± 2.44^{a}	4.30 ± 1.25^{a}
1.00				1.20 ± 0.42	2.90 ± 0.32^{ab}	1.30 ± 0.67^{ef}	5.50 ± 1.35^{b-e}	3.10 ± 0.32^{bcd}
	0.25			1.20 ± 0.42	2.60 ± 0.70^{ab}	2.10 ± 1.10^{b-e}	5.90 ± 2.02^{a-e}	3.10 ± 1.10^{bcd}
	0.50			1.10 ± 0.32	2.70 ± 1.34^{ab}	3.20 ± 1.48^{a}	7.20 ± 2.53^{a-d}	3.60 ± 1.71^{abc}
	1.00			1.20 ± 0.42	2.90 ± 0.99^{ab}	2.70 ± 1.25^{abc}	6.50 ± 2.17^{a-e}	4.20 ± 1.23^{ab}
		0.25		1.20 ± 0.42	2.80 ± 0.42^{ab}	1.70 ± 0.67^{c-f}	5.70 ± 1.64^{a-e}	2.80 ± 1.03^{cd}
		0.50		1.00 ± 0.00	3.10 ± 0.57^{a}	1.90 ± 0.74^{b-f}	6.30 ± 1.57^{a-e}	3.10 ± 0.74^{bcd}
		1.00		1.00 ± 0.00	2.40 ± 0.70^{ab}	1.50 ± 0.71^{def}	5.30 ± 1.83^{cde}	2.80 ± 0.63^{cd}
0.25			1.00	1.00 ± 0.00	2.20 ± 0.42^{b}	1.70 ± 1.06^{c-f}	4.70 ± 1.70^{e}	3.50 ± 0.97^{a-d}
0.50			1.00	1.00 ± 0.00	2.40 ± 0.84^{ab}	1.40 ± 0.52^{def}	6.80 ± 1.32^{a-e}	3.40 ± 1.07^{a-d}
1.00			1.00	1.10 ± 0.32	2.70 ± 1.25^{ab}	1.80 ± 0.63^{c-f}	6.00 ± 2.00^{a-e}	3.60 ± 0.97^{abc}
	0.25		1.00	1.10 ± 0.32	2.40 ± 0.70^{ab}	1.80 ± 0.79^{c-f}	5.70 ± 2.11^{a-e}	3.30 ± 0.67^{a-d}
	0.50		1.00	1.00 ± 0.00	3.20 ± 0.63^{a}	2.70 ± 1.25^{abc}	6.80 ± 1.87^{a-e}	4.20 ± 1.03^{ab}
	1.00		1.00	1.30 ± 0.67	2.20 ± 0.79^{b}	1.40 ± 0.70^{def}	5.10 ± 2.13^{de}	2.80 ± 0.63^{cd}
		0.25	1.00	1.20 ± 0.42	2.80 ± 0.63^{ab}	1.50 ± 0.71^{def}	6.40 ± 2.01^{a-e}	3.20 ± 0.92^{a-d}
		0.50	1.00	1.00 ± 0.00	2.70 ± 0.82^{ab}	1.40 ± 0.52^{def}	6.40 ± 2.67^{a-e}	3.40 ± 0.97^{abcd}
		1.00	1.00	1.00 ± 0.00	1.73 ± 0.15^{fg}	1.00 ± 0.00^{f}	5.60 ± 1.17^{a-e}	2.40 ± 0.52^{d}

[1/]PGRs = plant growth regulators, MS = Murashige and Skoog (1962), CF-B = culture filtrate of *B. pumilus* A1_YM_1, IAA = indole-3-acetic acid, NAA = α-naphthalene acetic acid, BA = 6-benzylaminopurine; Mean values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test; n = 10 per treatment with 5 replications each.

Conclusion

We found that *B. pumilus* A1_YM_1 is the potential bacterial stain which could be useful for producing IAA in order to improve micropropagation of orchids. Additionally, IAA produced by *B. pumilus* A1_YM_1 can enhance plant growth which was the same as synthetic PGRs produced. Thus, our results support the application of culture filtrate of some bacterial species in culture media to promote plant growth under micropropagation.

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