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PRIMARY RESEARCH

Correlation of growth and IAA production of *Lysinibacillus Fusiformis* UD 270

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Index Terms
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***Abstract*—** This study was investigated in IAA biosynthesis with different concentrations of tryptophan and bacterial cell number as well as observed the correlation between bacterial growth and IAA production. The bacteria were grown in nutrient broth supplemented with 0, 0.5, 1, 2, 3, 4, and 5 mg mL^{-1} of tryptophan at 30°C for 72h. Reasonable bacterial growth and IAA production were appreciably determined at 36h of incubation in 5 mg mL^{-1} of tryptophan. Meanwhile, the pH of the culture media was gradually increased at the beginning and made stable after 36h. Moreover, when the cell concentration was varied from 10^3 to 10^9 Colony-Forming Unit (CFU) mL^{-1}, the IAA production was significantly higher at high cell concentrations, concretely with 10^7 and 10^9 CFU mL^{-1}. Also, a positive correlation between growth and IAA production was indicated in the two experiments. The results have referred to the suitable uses of tryptophan and cell number for bacterial growth and IAA biosynthesis of *L. fusiformis* UD 270 to apply for further researches. Plant Growth Promoting Bacteria (PGPB) have been considered as beneficial microorganisms that can promote plant growth by their potential mechanism, particularly Indole-3-Acetic Acid (IAA) production. *Lysinibacillus fusiformis* UD 270 isolated from local plants in Khon Kaen, Thailand, displayed its capability to produce IAA from tryptophan, well-known as its precursor.

I. INTRODUCTION

Bacteria which can promote plant growth are called PGPB. This group includes either free-living form or endophytic form that has specific symbiotic relationships with plants [1]. PGPB are known as beneficial microorganisms and, when used in place of synthetic chemicals, are capable of improving plant growth through supply of plant nutrients. They may also help to sustain environment health and soil productivity [2, 3, 4]. Indeed, the plant growth promoting effect is mostly explained by the release of metabolites directly stimulating growth such as phosphate solubilization, nitrogen fixation, siderophore production and modulating phytohormone levels. The indirect promotion of plant growth is performed as biocontrol agents [1]. One of the mechanisms by which PGPB promote plant growth is the ability to produce plant hormones, such as auxins [5]. IAA is the most common natural auxin found in plants and has a positive effect on root growth [6]. IAA responds to cell division, root elongation, seed and tuber germination, rate of xylem and root development, and resistanace to stress conditons [1]. Up to 80% of rhizobacteria, both through colonization of the seed or root surfaces, can synthesize IAA and it is proposed that they act in conjunction with endogenous IAA in plants to stimulate cell proliferation and to enhance the host's uptake of minerals and nutrients from the soil [7] . Additionally, plant growth promoting bacteria can produce IAA from tryptophan, which is the main precursor molecule for IAA production in bacteria by tryptophan dependent biosynthetic pathways. The optimization of bacterial IAA production has been studied for several years, mainly in culture media with varying important factors as temperature, pH, precursor concentration [8, 9, 10] and inoculum sizes [11] . The optimal condition for IAA production is distinct of each bacterial specie. The previous

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researches showed that *Lysinibacillus fusiformis*, a gram-positive, rod-shaped bacterium of the genus *Lysinibacillus* is a Plant Growth Promoting Endophytic Bacteria (PGPEB). The endophyte *L. fusiformis* had positive traits for most of plant growth promotion [12]. Moreover, the capability for phytohormone biosynthesis of *L. fusiformis* has been observed in the recent years. This bacterium could synthesize IAA from tryptophan as the main precursor [13]. It was also reported that *L. fusiformis* strain Ps7 could synthesize ABA, GA3, IAA [14] . Nevertheless, it seems that there is not much information about a correlation between growth and IAA biosynthesis of *L. fusiformis* in culture media containing tryptophan in terms of using suitable amount of tryptophan and cell number. These two factors are considered more reasonable to determine the optimal condition for IAA production because they directly affect the amount of synthesized IAA like the increasing of precursor or inoculum size could account for the increasing of IAA production, and indirectly influence on practical application like the suitable cell number is good for preparing and applying to field. Therefore, the aim of this study was to investigate the effects of various concentrations of L-tryptophan and bacterial cells on growth and IAA production together with the correlation between growth and IAA production in culture medium.

II. LITERATURE REVIEW

A. Plant Growth Promoting Bacteria

Soil is full of microscopic life forms including bacteria, fungi, actinomycetes, algae and protozoa. Among different microorganisms, bacteria are the most common (i.e., approximately 95%) [1]. Soil bacteria have capabilities to survive and use a wide range of different substances as sources of nutrients. The bacterial distribution is around the roots of plants, i.e in the rhizosphere which is typically much greater than in the rest of the soil [15]. It can be explained that the presence of high levels of nutrients including amino acids, sugars, organic acids and other small molecules that are exuded from plant roots leads to the high bacterial concentration around the roots of plants. As rhizosphere bacteria use the exudates for their growth and metabolism [15, 16]. The interaction between bacteria in soil and plants may be beneficial, harmful or neutral [15, 17]. The bacteria that can promote plant growth, are called plant growth promoting bacteria, including either free-living form or endophytic form that has specific symbiotic interaction with plants. There are different types of bacteria-plant interaction such as rhizospheric way (bacteria bind to the roots or seed surfaces), phyllospheric way (bacteria bind to leaf or stem surfaces), symbiotic way (bacteria are typically found in root nodules), or endophytic interaction (bacteria colonize inside the plant tissue) [18, 19, 20, 21]. Although the differences between these bacteria, they still use the same mechanisms in promoting plant growth. PGPB may promote plant growth either through indirect mechanisms via antibiotics and lytic enzymes, competition, ethylene regulation, induced systemic resistance or direct mechanisms via facilitating resource acquisition including fixed nitrogen, iron and phosphate, and specifically modulating phytohormone levels such as auxin, cytokinin, gibberellin and ethylene [1].

L. fusiformis or *Aerobacillus fusiformis*, is a gram positive, rod-shaped, and non-motile bacterium. A bacterial cell is approximately 2.5-3.0 micrometers in length, and 0.5-0.9 micrometers in width. This bacterium grows well in range of temperature from 17-37°C, pH from 6-9.5, and NaCl concentration of 2-7%. It can produce inactive spherical endospores that resist to unfavourable living conditions [22]. The research of Vendan et al. [12] showed that the endophyte, *L. fusiformis*, had positive traits for most of plant growth promotion. This organism has also been the beneficial bacterium that is associated with Citrus roots, and could significantly reduce the population of viable *Candidatus Liberibacter Asiaticus* in Huanglongbing symptomatic on leaves [23]. In branch of Cacao plants, *L. fusiformis* was found as endophytic endospore-forming bacteria which have the potential biological control of cacao diseases [24]. Moreover, the capability for phytohormone biosynthesis of *L. fusiformis* has been observed in the recent years. It was reported that *L. fusiformis* strain Ps7 could synthesize ABA, GA3, IAA [14] while *L. fusiformis* strain SW13 could produce IAA via indole-3-acetonitrile pathway using tryptophan as a precursor for conversion [13] including *L. fusiformis* UD270 that could produce high amount of IAA in culture media supplemented with tryptophan [25].

B. Study Condition for IAA Production in Culture Media of Some Bacteria

Mohite [10] isolated and identified 5 IAA producing bacteria from banana, cotton maize and wheat rhizosphere as *B. megaterium, Lactobacillus casei, B. subtilis, B. cereus and Lactobacillus acidophilus*. The test for optimization of physical factors for IAA production was conducted in pH

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range of 5 to 9, temperature range of 20 to 40°C, tryptophan concentration range of 0.05 % to 1.5 %. Maximum IAA production was found in the medium supplemented with 0.1% tryptophan for *B. megaterium, Lactobacillus casei, B. subtilis,*1.5% for *Lactobacillus acidophilus*, and 0.05% *B. cereus*. Each strain had different optimal concentration of precursor for IAA production. Indeed, Bharucha et al. [9] found the suitable concentrations of L-tryptophan for IAA production of *Pseudomonas putida* UB1 was 0.2 mg mL^{-1} in range of 0.05 to 0.25 mg mL^{-1}, and noticed that the IAA production was gradually increase with the increased of L-tryptophan concentration.

In another research, Tallapragada *et al*. [11] performed a study of IAA producing *Burkholderia seminalis* and its effect on tomato. The A response surface methodology was utilized to find the relationships between reasonable factor. The results showed that IAA production increased together with increasing of tryptophan concentration and inoculum sizes.

III. METHODOLOGY

A. Preparation of Endophytic Bacterial Strain

Lysinibacillus fusiformis UD270 isolated from local plants in Khon Kaen, Thailand, was grown in the 2mL tube of nutrient broth medium (NB, 5g beef extract, 3g peptone in 1000 mL distilled water), incubated at 30°C with agitation at 150 rpm. After 24 hours, the culture was used as the starter for the next experiments. The growth of 1 mL 24-hour culture was determined at 600nm of optical density by a spectrophotometer. Cell number was aseptically determined by plate counting method. One milliliter of bacterial culture was added into 9 mL of 0.85% NaCl (8.5g NaCl in 1000mL distilled water) and 10-fold serial dilutions to 10-5 were done and spread on nutrient agar medium (NA, 5g beef extract, 3g peptone, 15g agar in 1000mL distilled water). The bacterial colonies on inoculated NA plates were counted after incubating at 30°C for 24h. Bacterial growth measurement and colony counting were performed in triplicate.

B. Determination of the Optimal Tryptophan Concentration for Bacterial IAA Biosynthesis

For finding the suitable concentration of L-tryptophan for IAA production in terms of its effective application in practices, 0.5 mL of starter (OD_{600} = 1) was inoculated in each 250mL flask containing 50mL of NB medium separately supplemented with 0, 0.5, 1, 2, 3, 4, and 5mg mL^{-1} of L-tryptophan (99%, ACROS Organics, USA)[26]. The inoculated flasks were incubated at 30°C with agitation at 150 rpm for 72h. 5mL of bacterial culture were adequately withdrawn for each sampling at 0, 12, 24, 36, 48, and 72h of incubation. The bacterial growth was measured at 600nm of optical density by using the spectrophotometer. For obtaining IAA production, 1.5mL of bacterial culture were centrifuged at 10,000 rpm for 10 min to discard bacterial cells. The IAA concentration was then quantified by the colorimetric method in which 1mL of supernatant was added into a 10 mL tube containing 1 mL of Salkowski reagent (12g $FeCl_3$, 1000g 7.9M H_2SO_4) in dark conditions for 30min at room temperature. The concentration of IAA was monitored by measuring the optical density at 530nm and calculated using a IAA standard curve. pH of 5mL culture samples was also determined by a pH meter. The experiment was done in triplicate.

C. Determination of the Optimal Cell Concentration for Bacterial IAA Biosynthesis

Five hundred microliters of starter in various cell concentrations at 10^3, 10^5, 10^7, 10^9 CFU mL^{-1} were added into several 250mL flasks containing 50mL of NB medium supplemented with 5 mg mL^{-1} L-tryptophan. The inoculated flasks were kept in the incubating shaker with 150 rpm, at 30°C, for 60h in each sampling, 5mL of bacterial culture was taken at 0, 12, 24, 36, 48, and 60h of incubation. The bacterial growth and IAA production were determined through the above-mentioned methods with three replications.

IV. RESULTS AND DISCUSSION

A. Optimization of IAA Production of Lysinibacillus Fusiformis UD270

Lysinibacillus fusiformis UD270 grew rapidly in the first 12h and gradually produced IAA over the incubated time. IAA was synthesized in stationary phase of growth as a secondary metabolite which was produced strongly after 24h. In various amount of tryptophan supplement, the growth and IAA production of the studied endophyte were increased at the high tryptophan concentrations (Figure 1: A, B; Table: 1, 2). Similarly, *Lysinibacillus fusiformis* showed the ability to synthesize IAA with the presence of trypto-

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phan. This bacterium could synthesize IAA from tryptophan as the main precursor [27] . The addition of tryptophan to the culture medium results in higher IAA production in all studied bacteria [13].

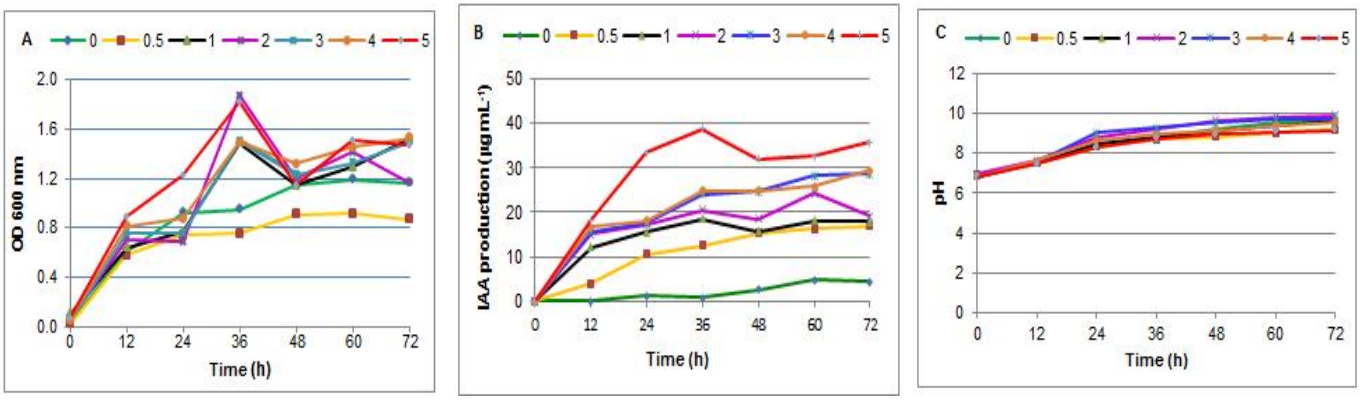

Fig. 1 . The growth (A), indole acetic acid production (B) and pH (C) of *L. fusiformis* UD270 in nutrient broth with various concentrations of L-tryptophan

TABLE 1
THE GROWTH (OD_{600}nm) OF *L. FUSIFORMIS* UD270 IN NUTRIENT BROTH WITH VARIOUS CONCENTRATIONS OF L-TRYPTOPHAN

Treatment	Time (h)	0	12	24	36	48	60	72
0		0.023 ± 0.002	0.609 ± 0.032	0.922 ± 0.064	0.946 ± 0.041	1.147 ± 0.104	1.191 ± 0.175	1.160 ± 0.144
0.5		0.021 ± 0.005	0.578 ± 0.080	0.740 ± 0.089	0.749 ± 0.128	0.908 ± 0.073	0.917 ± 0.032	0.867 ± 0.064
1		0.099 ± 0.009	0.637 ± 0.008	0.765 ± 0.086	1.488 ± 0.221	1.143 ± 0.039	1.293 ± 0.115	1.520 ± 0.015
2		0.062 ± 0.015	0.705 ± 0.093	0.688 ± 0.004	1.870 ± 0.017	1.201 ± 0.040	1.411 ± 0.355	1.163 ± 0.045
3		0.046 ± 0.017	0.761 ± 0.090	0.750 ± 0.014	1.498 ± 0.012	1.230 ± 0.157	1.325 ± 0.195	1.496 ± 0.004
4		0.053 ± 0.016	0.815 ± 0.137	0.875 ± 0.073	1.493 ± 0.023	1.319 ± 0.053	1.451 ± 0.047	1.529 ± 0.039
5		0.064 ± 0.007	0.890 ± 0.106	1.221 ± 0.079	1.819 ± 0.080	1.142 ± 0.069	1.507 ± 0.190	1.469 ± 0.210

TABLE 2
INDOLE ACETIC ACID PRODUCTION (μg ml $^{-1}$) OF *L. FUSIFORMIS* UD270 IN NUTRIENT BROTH WITH VARIOUS CONCENTRATIONS OF L-TRYPTOPHAN

Treatments	Time (h)	0	12	24	36	48	60	72
0		0.00	0.00	1.21 ± 0.013	0.81 ± 0.019	2.69 ± 0.020	4.70 ± 0.015	4.29 ± 0.009
0.5		0.00	4.01 ± 0.008	10.47 ± 0.026	12.45 ± 0.050	15.40 ± 0.013	16.24 ± 0.008	16.84 ± 0.045
1		0.00	12.21 ± 0.049	15.46 ± 0.040	18.50 ± 0.039	15.65 ± 0.004	18.04 ± 0.020	17.88 ± 0.016
2		0.00	15.10 ± 0.007	17.20 ± 0.034	20.38 ± 0.006	18.41 ± 0.061	24.29 ± 0.038	19.09 ± 0.059
3		0.00	15.64 ± 0.005	17.42 ± 0.045	24.15 ± 0.037	24.74 ± 0.011	28.16 ± 0.010	28.63 ± 0.019
4		0.00	16.64 ± 0.010	18.14 ± 0.031	24.83 ± 0.024	24.89 ± 0.032	25.75 ± 0.063	29.42 ± 0.030
5		0.00	18.30 ± 0.069	33.44 ± 0.046	38.68 ± 0.111	32.03 ± 0.004	32.60 ± 0.147	35.77 ± 0.026

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TABLE 3
THE PH OF *L. FUSIFORMIS* UD270 IN NUTRIENT BROTH WITH VARIOUS CONCENTRATIONS OF L-TRYPTOPHAN

Treatments	Time (h)	0	12	24	36	48	60	72
0		6.93	7.48	8.45	8.83	9.2	9.57	9.69
0.5		6.92	7.49	8.34	8.68	8.81	9.03	9.18
1		6.85	7.58	8.5	8.8	9.14	9.34	9.59
2		6.96	7.62	8.76	9.21	9.64	9.83	9.90
3		6.82	7.57	9.04	9.26	9.54	9.69	9.71
4		6.84	7.62	8.64	8.96	9.17	9.41	9.55
5		6.82	7.46	8.29	8.74	8.96	9.05	9.15

Tryptophan concentrations were set to apply to the practices, varied from 0.5 to 5mg mL^{-1}. It has been reported that the IAA produced by fluorescent *Pseudomonas* isolates increased with an increase in concentration of tryptophan from 1 to 5mg mL^{-1} [8]. However, IAA production of different *L. fusiformis* endophytic strains was varied from each other. In this present study, the highest level of biosynthetic IAA of *L. fusiformis* UD270 was obtained in 5mg mL^{-1} tryptophan at 36h of incubation, reaching up to 38.68 μg mL^{-1}, followed by 4mg mL^{-1} and 3mg mL^{-1} tryptophan with 24.83 μg mL^{-1} and 24.15 μg mL^{-1} IAA, respectively (Figure 1B, Table 2). In previous study, *L. fusiformis* strain ANA81 could significantly produce IAA at 32.12 μg mL^{-1} [23]. In the study of Park *et al*. [28] IAA production from *L. fusiformis* isolate PM-5 and PM-24 was 140.9 μg mL^{-1} and 255 μg mL^{-1}, respectively, in culture that contained 100 mg L^{-1} L-tryptophan. Thus, in a comparison to others, *L. fusiformis* UD270 may become a strong candidate for bacterial IAA biosynthesis to function in plant-bacteria interaction. This bacterium was able to produce IAA without tryptophan in an amount of 4.29 μg mL^{-1} at 72h, while *L. fusiformis* isolate Ps7 was found as the endophytic plant growth promoting in *Prosoposis strombulifera* and could produce a small amount of IAA (0.02 μg mL^{-1}) in defined media without tryptophan [14]. The pH of culture was slightly increasing to be alkaline (Figure 1C, Table 3).

It was likely to be the optimum pH for IAA producing Bacillus spp. which could synthesize the maximum amount of IAA at pH range in 8-9 [10]. Bootkotr and Mongkolthanaruk [25] have found that *L. fusiformis* UD270 could produce esterase and protease during its growth. The protease of *L. fusiformis* C250R was alkaline and stable in the wide pH range of 5-10. It might be considered that *L. fusiformis* tended to adjust pH of culture to be alkaline for growing and releasing some reasonable metabolites during their growth mechanisms. The alkaline protease might also hydrolyze proteins in the culture and release amino acids, leading to the increase of pH. Moreover, IAA is an uncharged weak acid [29]. Therefore its production might not much affect pH of culture, causing the pH of culture depending on growth mechanisms.

The same trend of growth and IAA synthesis was clearly observed when the different cell number was added into culture media containing 5mg mL^{-1} L-tryptophan (Figure 2, Tables 4, 5). The cells were varied from 10^3 to 10^9 CFU mL^{-1}. The bacterial growth in all treatments was rapidly increased at the early incubation, and tendentiously stable after 24h. The IAA could be synthesized immediately after incubation, particularly with high cell number. It indicated that IAA production increased in the case of increasing tryptophan levels and increasing inoculum size when *Burkholderia seminalis* was applied to tomato seeds [11]. In present study, the IAA production was increased orderly with increasing cell concentrations in that 10^7 and 10^9 CFU mL^{-1} treatments could synthesize IAA faster and in greater quantities than 10^3 and 10^5 CFU mL^{-1} treatments. IAA was produced quickly and abundantly within 24h and stably reached highest amount until 48h when it was decreased in all treatments. The biosynthetic IAA was highest at 49.39 μg mL^{-1} in 10^9 CFU mL^{-1} experiment (Table 5). As the secondary metabolite, IAA was then increased during the stationary phase and gradually decreased in the later incubation period. The decrease in IAA production might be due to the release of IAA-degrading enzymes such as indole acetic acid oxidase and peroxidase [30].

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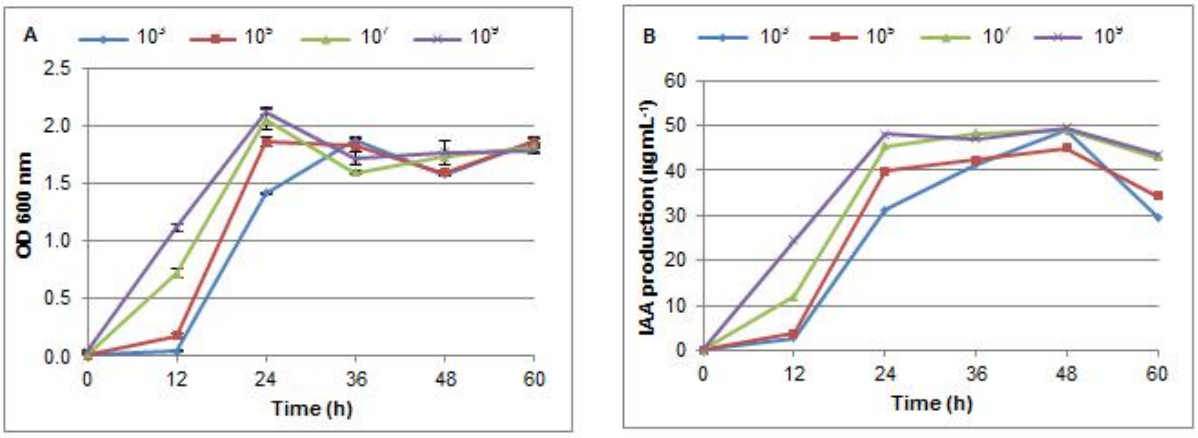

Fig. 2 . The growth (A), indole acetic acid production (B) of *L. fusiformis* UD270 in nutrient broth with various cell concentrations

TABLE 4
THE GROWTH (OD_{600}nm) OF *L. FUSIFORMIS* UD270 IN NUTRIENT BROTH WITH VARIOUS CELL CONCENTRATIONS

Treatment	Time (h)	0	12	24	36	48	60
10^3		0.007 ± 0.004	0.042 ± 0.012	1.416 ± 0.008	1.878 ± 0.017	1.580 ± 0.023	1.867 ± 0.036
10^5		0.012 ± 0.004	0.179 ± 0.011	1.862 ± 0.043	1.827 ± 0.053	1.593 ± 0.021	1.854 ± 0.027
10^7		0.012 ± 0.004	0.716 ± 0.041	2.052 ± 0.090	1.596 ± 0.013	1.727 ± 0.003	1.817 ± 0.038
10^9		0.041 ± 0.006	1.118 ± 0.034	2.126 ± 0.036	1.711 ± 0.056	1.766 ± 0.105	1.782 ± 0.026

TABLE 5
INDOLE ACETIC ACID PRODUCTION (μg ml $^{-1}$) OF *L. FUSIFORMIS* UD270 IN NUTRIENT BROTH WITH VARIOUS CELL CONCENTRATIONS

Treatments	Time (h)	0	12	24	36	48	60
10^3		0.000	2.7 ±0.012	31.3 ± 0.056	41.3 ± 0.017	48.8 ± 0.030	29.6 ± 0.033
10^5		0.000	3.8 ± 0.035	40.0 ± 0.042	42.3 ± 0.044	45.0 ± 0.029	34.4 ± 0.014
10^7		0.000	12.1 ± 0.020	45.3 ± 0.045	48.3 ± 0.022	49.2 ± 0.037	43.0 ± 0.038
10^9		0.000	24.4 ± 0.027	48.0 ± 0.014	47.0 ± 0.088	49.4 ± 0.083	43.5 ± 0.026

B. The Correlation of Growth and IAA Production of L. Fusiformis UD270 in Culture Media

Since the periodical samples were taken by time, this allowed the correlation between bacterial growth and IAA biosynthesis to be obtained. The correlation was displayed in Figure 3. In the two experiments in which tryptophan concentration and cell number were the main factors, a positive correlation between growth and IAA production was found. In detail, when varying the concentration of tryptophan added in culture media, the bacteria grew and synthesized IAA with the same tendency, showing the significant positive correlation between growth and IAA production with correlation coefficient r = 0.773 at p < 0.05 (Figure 3A, Table 6). The similar observation was indicated in treatments that had different cell number amounts. The positive relationship between these two experimental parameters was also strongly indicated with r = 0.974 at p < 0.05 (Figure 3B, Table 7).

There were three factors that can affect growth as well as IAA synthesis such as carbon limitation, reduction in growth rate and concentration of substrate for IAA production [31]. In the present study, although the concentration of tryptophan was varied from lower to higher levels, it might be beneficial to compare to those used for synthesizing IAA process. Therefore, the bacteria could use the precursor to synthesize IAA abundantly together with increasing biomass during incubating time. Moreover, when

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different inoculum concentrations as various amounts of IAA producer were added into culture media, the growth and IAA production depended on the number of inoculated cells (Figure 2). It also means that, at the higher amount of IAA producer, the growth and IAA production might be greater than at lower amounts. The increase of growth is inversely linked to the increase of IAA production. This tendency indicated a positive correlation.

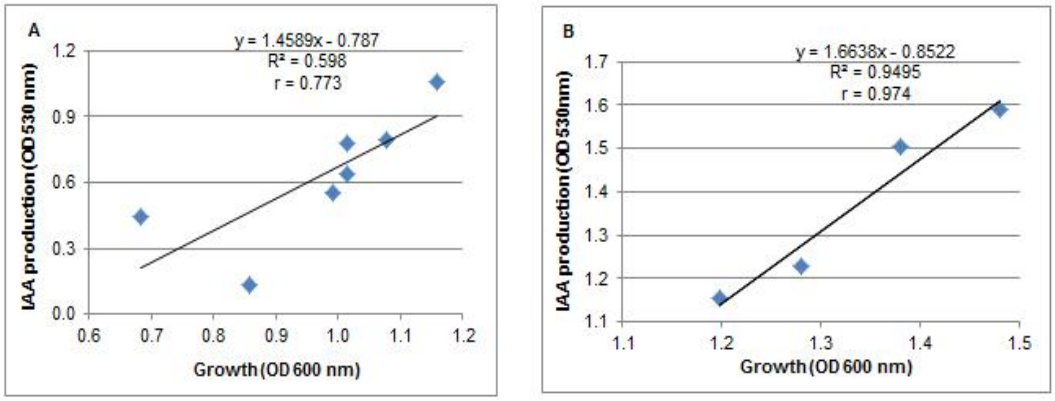

Fig. 3 . The correlation between growth and indole acetic acid production in various concentrations of tryptophan (A) and different cell number (B) experiments of *L. fusiformis* UD270

TABLE 6
THE CORRELATION BETWEEN GROWTH AND INDOLE ACETIC ACID PRODUCTION IN VARIOUS CONCENTRATIONS OF TRYPTOPHAN EXPERIMENTS OF *L. FUSIFORMIS* UD270

		Growth	IAA
Growth	Pearson correlation	1	0.773*
	Sig. (2-tailed)		0.041
	N	7	7
IAA	Pearson correlation	0.773*	1
	Sig. (2-tailed)	0.041	
	N	7	7

*Correlation is significant at the 0.05 level (2-tailed)

TABLE 7
THE CORRELATION BETWEEN GROWTH AND INDOLE ACETIC ACID PRODUCTION IN DIFFERENT CELL NUMBER EXPERIMENTS OF *L. FUSIFORMIS* UD270

		Growth	IAA
Growth	Pearson correlation	1	0.974*
	Sig. (2-tailed)		0.026
	N	4	4
IAA	Pearson correlation	0.974*	1
	Sig. (2-tailed)	0.026	
	N	4	4

*Correlation is significant at the 0.05 level (2-tailed)

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V. CONCLUSION

The endophyte *L. fusiformis* strain UD270 has shown the ability to synthesize IAA in the medium supplemented with L-tryptophan. Moreover, the different sizes of inoculum cell concentration lead to variable growth and IAA production for each concentration. In terms of effective application, the best growth and IAA production were determined by the suitable concentration of L-tryptophan and the amount of inoculum for IAA production which were 5mg mL^{-1} and 10^9 CFU mL^{-1}, respectively, pointing to a positive correlation between these two parameters. This IAA producing bacteria should be utilized to improve its potential capacity involving plant-bacteria interaction in further research.

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REFERENCES

[1] B. R. Glick, "Plant growth-promoting bacteria: Mechanisms and applications," *Scientifica*, vol. 20, no. 1, pp. 1–15, 2012. **DOI:** 10.6064/2012/963401

[2] P. F. O'Connell, "Sustainable agriculture-a valid alternative," *Outlook on Agriculture*, vol. 21, no. 1, pp. 5-12, 1992. **DOI:** 10.1177/003072709202100103

[3] A. Esitken, H. E. Yildiz, S. Ercisli, M. Figen Donmez, M. Turan and A. Gunes, "Effects of Plant Growth Promoting Bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry," *Scientia Horticulturae*, vol. 124, no. 1, pp. 62–66, 2010. **DOI:** 10.1016/j.scienta.2009.12.012

[4] C. R. Sari, J. Taylor, Y. Hong and S. Jackson, "Development of a transient viral CRISPR expression system to manipulate flowering time in plants," *International Journal of Applied and Physical Sciences*, vol. 2, no. 3, pp. 65-70, 2016. **DOI:** 10.20469/ijaps.2.50002-3

[5] D. Egamberdiyeva, "Plant-growth-promoting rhizobacteria isolated from a calcisol in a semi-arid region of Uzbekistan: Biochemical characterization and effectiveness," *Journal of Plant Nutrition and Soil Science*, vol. 168, no. 1, pp. 94–99, 2005. **DOI:** 10.1002/jpln.200321283

[6] M. Miransari and D. L. Smith, "Plant hormones and seed germination," *Environmental and Experimental Botany*, vol. 99, pp. 110-121, 2014. **DOI:** 10.1016/j.envexpbot.2013.11.005

[7] J. Vessey, "Plant growth promoting rhizobacteria as biofertilizers," *Plant and Soil*, vol. 255, no. 2, pp. 571–586, 2003. **DOI:** 10.1023/A:1026037216893

[8] F. Ahmad, I. Ahmad and M. S. Khan, "Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan," *Turkish Journal of Biology*, vol. 29, no. 1, pp. 29-34, 2005.

[9] U. Bharucha, K. Patel and U. B. Trivedi, "Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (Brassica nigra)," *Agricultural Research*, vol. 2, no. 3, pp. 215-221, 2013. **DOI:** 10.1007/s40003-013-0065-7

[10] B. Mohite, "Isolation and characterization of Indole Acetic Acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth," *Journal of Soil Science and Plant Nutrition*, vol. 13, no. 3, pp. 638–649, 2013. **DOI:** 10.4067/S0718-95162013005000051

[11] P. Tallapragada, R. Dikshit and S. Seshagiri, "Isolation and optimization of IAA produce *Burkholderia* seminalis and its effect on seedlings of tomato," *Songklanakarin Journal of Science & Technology*, vol. 37, no. 5, pp. 553-559, 2015.

[12] R. T. Vendan, Y. J. Yu, S. H. Lee and Y. H. Rhee, "Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion," *Journal of Microbiology*, vol. 48, no. 5, pp. 559–565, 2010. **DOI:** 10.1007/s12275-010-0082-1

[13] W. J. Kim and H. G. Song, "Interactions between biosynthetic pathway and productivity of IAA in some rhizobacteria," *The Korean Journal of Microbiology*, vol. 48, no. 1, pp. 1-7, 2012. **DOI:** 10.7845/kjm.2012.48.1.001

[14] V. Sgroy, F. Cassán, O. Masciarelli, M. F. Del Papa, A. Lagares and V. Luna, "Isolation and characterization of endophytic Plant Growth-Promoting (PGPB) or Stress Homeostasis-Regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera," *Applied Microbiology and Biotechnology*, vol.85, no. 2, pp. 371–381, 2009. **DOI:** 10.1007/s00253-009-2116-3

[15] J. M. Lynch and J. M. Whipps, "Substrate flow in the rhizosphere," *Plant and Soil*, vol. 129, no. 1, pp. 1-10, 1990. **DOI:** 10.1007/bf00011685

[16] V. Chernenok, A. Kurishbayev, A. Kudashev and Y. Nurmanov, "Diagnostics and optimization of crops' nitrogen nutrition in rainfed conditions of the Northern Kazakhstan," *Journal of Applied and Physical Sciences*, vol. 1, no. 1. pp. 1-8, 2015. **DOI:** 10.20474/-japs1.1.1

[17] M. B. Yusuf, B. M. Firuza and O. S. Khairulmaini, "Variation in soil physiochemical properties at different land use sites in Northeastern Nigeria," *International Journal of Applied and Physical Sciences*, vol. 3, no. 1, pp. 26-34, 2017. **DOI:** 10.20469/ijaps.3.50004-1

[18] J. Hallmann, A. Quadt-Hallmann, W. F. Mahaffee and J. W. Kloepper, "Bacterial endophytes in agricultural crops," *Canadian Journal of Microbiology*, vol. 43, no. 10, pp. 895-914, 1997. **DOI:** 10.1139/m97-131

[19] A. V. Sturz, B. R. Christie and J. Nowak, "Bacterial endophytes: potential role in developing sustainable systems of crop production," *Critical Reviews in Plant Sciences*, vol. 19, no. 1, pp. 1-30, 2000. **DOI:** 10.1080/07352680091139169

[20] J. Davison, "Plant beneficial bacteria," *Nature Biotechnology*, vol. 6, no. 3, pp. 282-286, 1988. **DOI:** 10.1038/nbt0388-282

[21] J. W. Kloepper, R. Lifshitz and R. M. Zablotowicz, "Free-living bacterial inocula for enhancing crop productivity," *Trends in Biotechnology*, vol. 7, no. 2, pp. 39-44, 1989. **DOI:** 10.1016/0167-7799(89)90057-7

[22] S. N. Jones and E. A. Bergey, "Habitat segregation in stream crayfishes: Implications for conservation," *Journal of the North American Benthological Society*, vol. 26, no. 1, no. 134-144, 2007. **DOI:** 10.1899/0887-3593(2007)26[134:hsisci]2.0.co;2

[23] P. Trivedi, T. Spann and N. Wang, "Isolation and characterization of beneficial bacteria associated with citrus roots in Florida," *Microbial Ecology*, vol. 62, no. 2, pp. 324–336, 2011. **DOI:** 10.1007/s00248-011-9822-y

[24] R. L. Melnick, C. Suárez, B. A. Bailey and P. A. Backman, "Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases," *Biological Control*, vol. 57, no. 3, pp. 236-245, 2011. **DOI:** 10.1016/j.biocontrol.2011.03.005

[25] W. Bootkotr and W. Mongkolthanaruk, "Properties of bacteria beneficial to the promotion of plant growth, "

in *Proceedings of Ponencia presentada en: International Conference on Life Science & Biological Engineering*, Singapur, 2012.

[26] Fisher Scientific, "*L-5-Hydroxytryptophan, 99%, ACROS organics*," n.a. [Online]. Available from: goo.gl/JpGt2o

[27] S. Spaepen and J. Vanderleyden, "Auxin and plant-microbe interactions," *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 4, pp. 1–13, 2011. **DOI:** 10.1101/cshperspect.a001438

[28] M. Park, C. Kim, J. Yang, H. Lee, W. Shin, S. Kim and T. Sa, "Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea," *Microbiological Research*, vol. 160, no. 2, pp. 127–133, 2005. **DOI:** 10.1016/j.micres.2004.10.003

[29] A. Kumar, A. Srivastava, I. Y. Galaev and B. Mattiasson, "Smart polymers: Physical forms and bioengineering applications," *Progress in Polymer Science*, vol. 32, no. 10, pp. 1205-1237, 2007. **DOI:** 10.1016/j.progpolymsci.2007.05.003

[30] V. Jeyanthi and P. Ganesh, "Production , optimization and characterization of phytohormone indole acetic acid by *pseudomonas* fluorescence," *International Journal of Pharmaceutical & Biological Archives*, vol. 4, no. 2, pp. 514–520, 2013.

[31] S. Spaepen, J. Vanderleyden and R. Remans, "Indole-3-acetic acid in microbial and microorganism-plant signaling," *FEMS Microbiology Reviews*, vol. 31, no. 4, pp. 425–448, 2007. **DOI:** 10.1111/j.1574-6976.2007.00072.x

— This article does not have any appendix. —