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Research Article
Unraveling the Optimal Culture Condition for the Antifungal Activity and IAA Production of Phylloplane *Serratia plymuthica*

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Abstract

Background and Objective: Assessment of the plant growth promoting (PGP) properties of a bacteria *in vitro* required the adjustment of appropriate culture condition. This study was aimed to optimize the culture condition required to stimulate two PGP traits (antifungal activity and indole-3-acetic acid (IAA) production) of phylloplane *Serratia plymuthica* strain UBCF_13. **Materials and Methods:** Evaluation of UBCF_13 antifungal activity against *F. oxysporum f.* sp. *glycine* was conducted under various modifications (pH and addition of exogenous carbons, Nitrogens as well as metals). This strain IAA production was optimized under various culture durations and L-tryptophan (Trp) concentrations. The resulted IAA were then inoculated to several plant's seed to evaluate its effect on plant root and shoot growth. Data were analyzed statistically and the significance was further assessed using Duncan's new multiple range test with a $p<0.05$. **Results:** Application of 50 µL cells-free supernatants (CFS) exhibited the highest fungal suppression under pH 5 (23.02%), $(NH_4)_2 SO_4$ addition (28.25%) and $CaCl_2$ addition (27.14%). The highest IAA concentration (116.09 µg mL^{-1}) was obtained from the 48 h culture containing 0.2% L-Trp. The IAA-containing CFS of UBCF_13 stimulated longer growth in root and shoot of *Solanaceous* plants (chili, tomato and eggplant). **Conclusion:** Antifungal activity of UBCF_13 was predicted to be dependent on acidic condition and the availability of inorganic nitrogen as well as calcium, while its IAA could be produced in short time with the low amount of Trp.

Key words: Culture supernatants, environmental modification, *Fusarium oxysporum*, growth hormone, *Serratia plymuthica* strain UBCF_13

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biocontrol activity of *Serratia plymuthica* gained increasing attention for the last two decades as it showed a broad spectrum of antagonistic against various pathogenic fungi[1-5]. However, most of these reported studies utilized *S. plymuthica* inhabiting plant rhizosphere. The report regarding the bio-control trait of phyllosphere-originating *S. plymuthica* was less documented compared to the rhizosphere one. The previous study found that the cell culture and culture supernatants of phylloplane *S. plymuthica* strain UBCF_13 showed antagonistic activity against *Colletotrichum gloeosporioides*[6]. Nonetheless, the antifungal activity of this strain was required to be further upgraded through culture optimization to achieve better fungal suppression efficiency.

Bacterial metabolites had been utilized for various purposes, including food industry, agriculture application, medical use and pharmaceutical industry. Regarding its utilization for commercial scale, the production of these desirable compounds often required a substantial effort to put in as each compound might demand different condition to produce its optimum yield[7]. Naturally, biosynthesis of most bacterial metabolites was triggered by the unfavorable and stressful environmental conditions threatening the bacterial growth itself[8]. This natural condition was built up from the presence and/or the absence of certain environmental factors resulting in a disturbance for the bacteria itself, thus forcing the cell to release certain metabolite as its survival response[9]. It then became the basis of the culture condition engineering to imitate this natural stimulus required to trigger the production of the targeted metabolites *in vitro*.

The engineering of bacterial culture condition for metabolite production was generally emphasized on the aspect of the nutritional and physical environment. As it was greatly influenced by the environmental conditions, the determination of each environmental factor was the first crucial step to do to identify which factor contributing to significant impact on the production of the targeted metabolites[10,11]. This initial optimization was suggested to be performed using "one variable at a time" approach to determine the medium component to be designed before conducting the subsequent optimization. Once the environmental factors had been completely identified, the metabolite production would be evaluated under the designated medium consisting of previously tested parameters. This approach had been considered as an economical approach as the interaction between each component could be observed[7].

Referred to the previous study[6] the antifungal activity of *S. plymuthica* UBCF_13 was required to be further optimized. In addition, this phylloplane *Serratia* species might also confer other promising plant growth promoting properties, such as IAA producer. Therefore, this study was aimed to identify the environmental factors required to trigger antifungal activity and IAA production of this strain.

MATERIALS AND METHODS

This study was carried out in the Biotechnology Laboratory of Andalas University, Padang West Sumatera, Indonesia for approximately 8 months started from February-October, 2018.

Preparation of bacteria and fungi strains: Bacteria and fungi strains used in this study were obtained from the internal collection of the Biotechnology Laboratory (Andalas University). Phylloplane *S. plymuthica* strain UBCF_13 was cultured in King's agar medium (pH 7.0) for 16 h at room temperature in darkness as well. Evaluation of antifungal activity was conducted using *Fusarium oxysporum* as a pathogen target. Mycelial disk (5×5 mm) was placed onto potato dextrose agar (PDA) (pH 7) and grown for 7 days at room temperature in darkness.

Growth curve of *S. plymuthica* UBCF_13: Analysis of growth curve was conducted by culturing UBCF_13 in King's broth medium for 24 h in darkness at room temperature and 160 rpm. Bacterial cell density was measured every 2 h using spectrophotometer at a wavelength of 600 nm. Optical density values were subsequently converted to obtain the predicted cell density in the unit of cells mL^{-1}. This measurement was performed in three replicates.

Culture modification of *S. plymuthica* UBCF_13: Several modifications applied to the culture of UBCF_13 consisted of pH levels, culture durations and the addition of exogenous trace elements, nitrogen as well as carbon sources. Modification of medium pH was performed using five levels of pH ranging from pH 5-9. The modification was also carried out by adding 1 mM of trace elements ($CaCl_2$, $FeSO_4$, $MgSO_4$, $MnSO_4$ and $ZnSO_4$) and 2% of exogenous carbon (glucose, sucrose, glycerol and ethanol) and nitrogen (tryptone, peptone, yeast extract and ammonium sulfate) sources. UBCF_13 was cultured in King's broth medium modified with each parameter (pH and exogenous metals, carbon as well as nitrogen sources) for 24 h at room temperature and 160 rpm

in darkness. Culture cell density from each modification was determined in duplicates. Afterward, culture supernatants (CSN) were collected by centrifugation (14.000 rpm at 4°C for 15 min) and sterilized using 0.22 µm syringe filter.

Antifungal activity assay: Antifungal effect of UBCF_13 against *F. oxysporum f.* sp. *glycines* was evaluated through antagonistic assay performed using the agar well diffusion method as described by Bauer *et al.*[12]. Fungal mycelial was grown on PDA medium for 2 days at room temperature. Cell-free bacterial CSN (50 µL) resulted from each modification was applied onto four wells located 3 cm away from the position of 2 days-aged fungal mycelia. The fungus was subsequently grown at room temperature for 8 days and the resulting fungal growth inhibition after 8 days was measured using this following formula[13]:

$$\text{Growth inhibition}(\%) = \frac{d_{Uf} - d_{Tf}}{d_{Uf}} \times 100$$

where, d_{Uf} is the diameter of untreated *F. oxysporum* and d_{Tf} is the diameter of bacterial CSN treated fungi. This assay was carried out using five replicates.

Culture optimization of IAA production: The IAA production of this strain was determined using a colorimetric assay based on Mayer[14]. The UBCF_13 was grown in Luria Bertani (LB) medium supplemented with various concentrations of L-tryptophan (Trp) (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5%) under agitated condition (160 rpm) at room temperature and in darkness for 24, 48, 72 and 96 h. Bacterial CSN from each treatment was collected and mixed with 2 mL Salkowski reagent. The solution was homogenized evenly and incubated at room temperature for 25 min. The absorbance of each treatment was measured at 530 nm and followed with the measurement of uninoculated broth as a control. The IAA produced by UBCF_13 cultured in medium without Trp under the same duration was also measured. The IAA concentration was determined according to the standard curve performed using 5-100 µg mL^{-1} IAA (Phyto Technology Laboratories®). The quantification of bacterial IAA was performed in three replicates.

Germination assay of bacterial IAA-inoculated seeds: The IAA produced by UBCF_13 was subsequently applied for the seed inoculation of several plants, such as Chinese mustard, cucumber, chili, tomato and eggplant. Bacterial IAA used for this inoculation was obtained from LB medium supplemented with 100 and 200 µg mL^{-1} (0.1 and 0.2%) L-Trp. Before being inoculated, seeds were surface sterilized using the protocol described by Sindhu *et al.*[15]. The seed inoculation was performed using protocol adopted from Malik and Sindhu[16] with slight modification as this study used the bacterial culture supernatant containing IAA for the inoculation.

Seeds were germinated based on supplier recommendation as the germination rate of each plant's seed varied. Seeds of Chinese mustard, cucumber and tomato were germinated for 6 days, while the remaining plant seeds were germinated for 10 days. Percentage of final germination was determined at the last day of each seed germination. Root and shoot length were measured at 6th day for Chinese mustard, cucumber and tomato, while the measurement on chili and eggplant was performed at 5th and 10th day. The effect of this bacterial IAA inoculation in each plant's seed was compared with non-inoculated (soaked in sterile aquadest) and synthetic IAA (10 µM) inoculated seeds.

Statistical analysis: One-way analysis of variance (ANOVA) was performed using SPSS version 23.0[17] and significances among treatments were further analyzed using Duncan's New Multiple Range Test[18] (DNMRT) with a $p<0.05$. Data were presented as a graph representing mean values and complemented with standard deviations and statistical notations (if any).

RESULTS

Difference of bacterial growth patterns under modified culture condition: The *S. plymuthica* UBCF_13 displayed a specific growth pattern under normal condition as visualized in Fig. 1. The application of various modifications to the culture of UBCF_13 affected its growth in different ways. Several parameters, such as glucose and peptone addition, stimulated higher growth rate marked by higher cell density compared to normal culture (Fig. 2a and b). Modification on medium pH showed no particular effect towards UBCF_13 growth, except in pH 5 showing the growth inhibition (Fig. 2c). Significant growth constraint was clearly observed from the bacterial culture supplemented with the trace elements (Fig. 2d) suggesting this strain is sensitive towards metals.

Difference of UBCF_13 antifungal activity under modified culture condition: Addition of carbon sources seemed to suppress the antifungal activity of UBCF_13 (Fig. 3a) suggesting that the biosynthesis of its antifungal compounds occurred under the absence of carbon sources. A similar trend

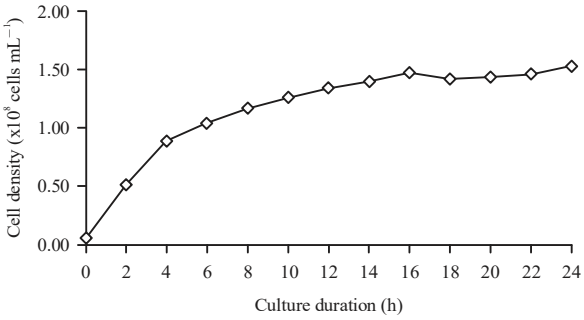

Fig. 1: Growth dynamics of *S. plymuthica* UBCF_13 during 24 h culture
Values were mean±SD (n = 3)

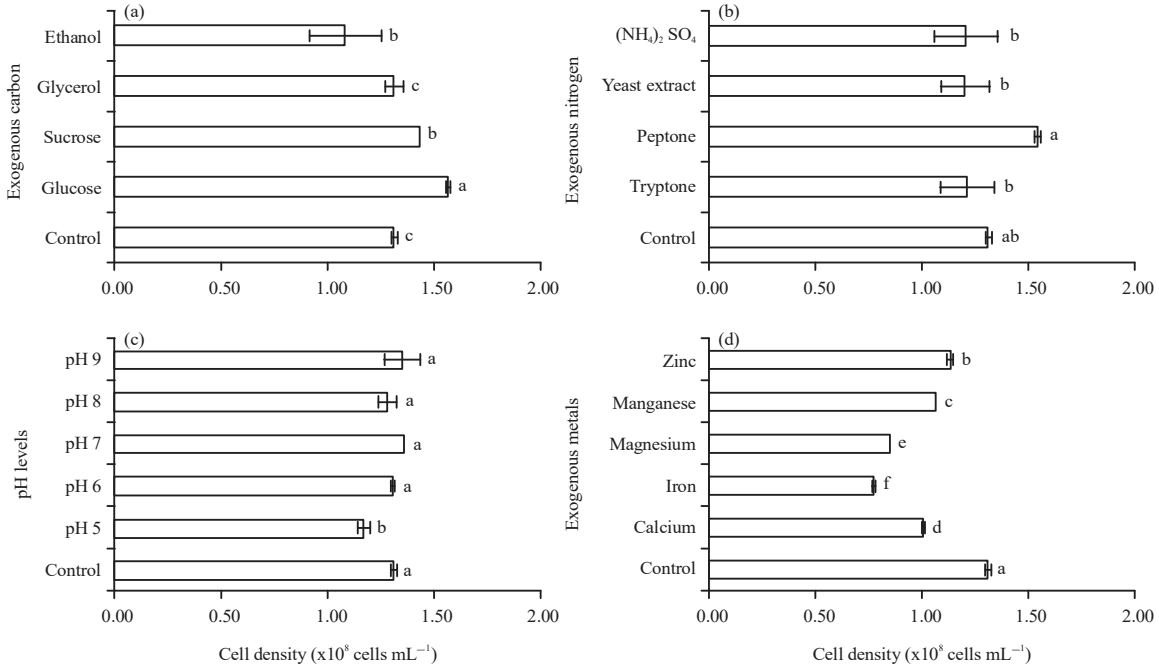

Fig. 2(a-d): Growth of *S. plymuthica* UBCF_13 in a culture condition modified with various exogenous (a) Carbon, (b) Nitrogen sources, (c) pH levels and (d) Exogenous metals
Values were mean±SD (n = 2), Bars followed by the same lowercases were insignificantly different based on DNMRT with a $p<0.05$

was also found in peptone and yeast extract supplemented culture. However, tryptone and ammonium sulfate addition resulted in higher antifungal effect up to 26.87 and 28.25%, respectively (Fig. 3b). From pH modification, the acidic culture (pH 5 and 6) triggered better fungal suppression of this strain, while the neutral and alkaline condition diminished its antifungal effect (Fig. 3c). Additionally, the antifungal activity of UBCF_13 also elevated significantly under the presence of calcium only (Fig. 3d).

Difference of UBCF_13 IAA production under various culture durations and L-Trp concentrations: Culture duration and Trp concentration showed a significant contribution in affecting the IAA production of this strain. The resulted IAA from all Trp concentrations exhibited optimum production after more than 24 h culture. Each Trp concentration revealed a specific pattern regarding the dynamic of its IAA production during the culture. The lowest concentration of Trp resulted in the highest IAA concentration (63.6 μg mL^{-1}) after 96 h culture (Fig. 4). Both 0.1 and 0.2% L-Trp showed a remarkable increase in IAA production after 48 h but 0.2% L-Trp resulted in the highest IAA concentration about 116.09 μg mL^{-1} (Fig. 4). However, IAA production triggered by this 0.2% L-Trp dropped drastically when the culture duration was prolonged. In contrast, IAA production resulted from 0.1% L-Trp constantly increased along with the longer culture

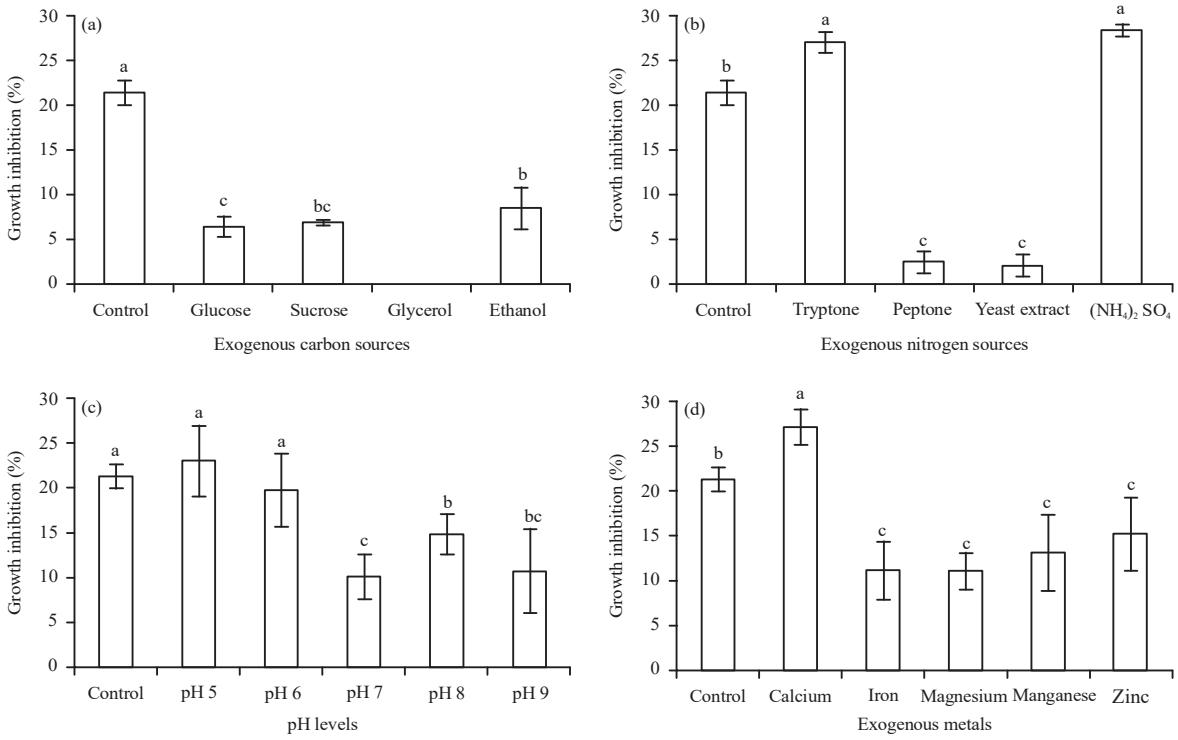

Fig. 3(a-d): Antifungal activity of UBCF_13 CSN against *F. oxysporum* f. sp. *glycines* after 8 days under culture condition modified with different exogenous (a) Carbon, (b) Nitrogen sources, (c) pH levels, as well as and (d) Exogenous metals

Values were mean±SD (n = 5), Bars followed by the same lowercases were insignificantly different based on DNMRT with a p<0.05

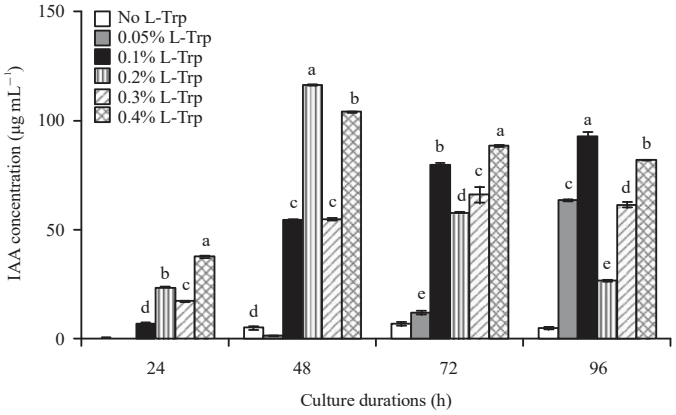

Fig. 4: IAA production of *S. plymuthica* UBCF_13 under different culture durations and L-tryptophan concentrations

Values were mean±SD (n = 3), Bars followed by the same lowercase at the same duration level were insignificantly different based on DNMRT with a p<0.05

duration (Fig. 4). Higher Trp concentrations induced a rapid increase in IAA secretion after a certain period of culture but tended to be highly unstable over the longer duration.

Effect of bacterial IAA inoculation on the germination of various plant seeds: Seed response toward the inoculation of UBCF_13 IAA varied according to the plant type. Growth promoting effect due to this bacterial IAA application was clearly observed in *Solanaceous* plant seeds (chili, tomato and eggplant) based on its germination percentage, root and shoot growth. No significant effect exhibited from IAA-inoculated Chinese mustard and cucumber seeds. The

Table 1: Seed germination percentage of several plants after the application of bacterial IAA

	Germination of each plant's seeds at 6th or 10th day after inoculation (%)				
	6th			10th	
Treatments	Chinese mustard	Cucumber	Tomato	Chili	Eggplant
Control	70	60	80	90	100
10 µM IAA	60	40	95	90	100
$_{UBCF\_13}$ IAA (0.1% L-Trp)	50	55	100	100	100
$_{UBCF\_13}$ IAA (0.2% L-Trp)	50	55	75	100	100

Table 2: Effect of UBCF_13 IAA inoculation on root length of several plants

	Root length (cm) of each plant seeds				
Treatments	Chinese mustard	Cucumber	Tomato	Chili	Eggplant
Control	5.86±1.49^{ab}	7.20±0.84^{a}	8.54±1.28^{a}	1.54±0.23^{b}	1.98±0.39^{b}
10 µM IAA	6.74±1.43^{a}	0.60±0.52^{c}	7.74±0.49^{ab}	1.84±0.43^{b}	2.08±0.33^{b}
$_{UBCF\_13}$ IAA (0.1% L-Trp)	3.88±2.41^{bc}	2.30±1.04^{b}	7.78±0.41^{ab}	2.70±0.54^{a}	2.72±0.70^{b}
$_{UBCF\_13}$ IAA (0.2% L-Trp)	1.42±2.34^{c}	1.90±1.18^{b}	7.34±0.59^{b}	1.46±0.09^{b}	4.44±0.76^{a}

Values were Mean±SD (n = 5). Numbers followed by the same lower case at the same column were insignificantly different based on DNMRT with a $p<0.05$

Table 3: Effect of UBCF_13 IAA inoculation on shoot length of several plants

	Shoot length (cm) of each plant seeds				
Treatments	Chinese mustard	Cucumber	Tomato	Chili	Eggplant
Control	1.72±0.40^{a}	1.18±0.24^{a}	2.28±0.59	0.64±0.05^{b}	0.66±0.05^{b}
10 µM IAA	1.40±0.42^{a}	0.36±0.34^{c}	2.70±0.45	0.76±0.15^{b}	1.26±0.63^{b}
$_{UBCF\_13}$ IAA (0.1% L-Trp)	1.52±0.67^{a}	0.82±0.36^{ab}	2.68±0.44	1.04±0.26^{a}	1.50±0.83^{ab}
$_{UBCF\_13}$ IAA (0.2% L-Trp)	0.24±0.54^{b}	0.70±0.12^{bc}	2.30±0.27	0.76±0.09^{b}	2.32±0.82^{a}

Values were Mean±SD (n = 5). Numbers followed by the same lower case at the same column were insignificantly different based on DNMRT with a $p<0.05$

presence of bacterial IAA improved the germination percentage of chili and tomato seeds (Table 1) but showed no effect on eggplant seeds germination (Table 2). Table 3 revealed that the chili and eggplant seeds showed different trend in utilizing the bacterial IAA to stimulate its root growth. The longest root of chili seeds was achieved from the inoculation of 0.1% Trp-induced IAA (Table 2), however, application of higher Trp-induced IAA suppressed its root growth. Meanwhile, eggplant seed revealed different trends showing the increase of root length along with the increase of bacterial IAA concentration applied. Longer shoots were also recorded from the bacterial IAA-inoculated *Solanaceous* seeds compared to uninoculated seeds. The shoot of tomato and chili revealed the longest growth after the inoculation of 0.1% L-Trp induced IAA, while the eggplant shoot conferred the longest size due to a higher concentration of Trp-induced IAA (Table 3).

DISCUSSION

Modification of various culture parameters exhibited an opposite trend towards UBCF_13 growth and fungal suppression efficacy. It also revealed that the culture parameter inhibiting the bacterial growth might enhance its antifungal activity. It then implied that production or secretion of antifungal metabolites from this strain preferred the growth and carbon-limited conditions. As proposed by Sanchez *et al.*[8], most of the bacterial secondary metabolites favor a stimulus produced by the stressful conditions leading to growth repression and nutrient starvation. Moreover, Gram-negative bacteria, such as *Serratia* were known to synthesize its secondary metabolites under carbon repression state. The maximum metabolite production of this species could be achieved when the cells were exposed to the nutrient deprivation state[19].

The presence of specific nitrogen sources determined the resulted metabolites and its production[20,21]. Out of all culture parameter tested, this strain showed the highest fungal suppression activity from the culture supplemented with ammonium sulfate (Fig. 3b). This result implied that the antifungal-related compounds of this strain were induced by inorganic nitrogen source. Ammonium is considered an easily assimilated nitrogen source preferred by most microorganisms[22]. A certain amount of ammonium could trigger the depletion of medium pH as the consequence of its rapid assimilation, leading to a decrease of growth rate and the increase of metabolite biosynthesis[7]. This statement was also in line with the result of this study revealing the best

antifungal activity under pH modification was achieved from the acidic condition (pH 5) (Fig. 3c). Previous studies mentioned that certain pH levels might affect the availability of certain ions and membrane permeability required to support the growth and production of particular enzymes[23,24].

Regarding the effect of exogenous metals modification, it was found that the antifungal activity of UBCF_13 reached the highest value from the calcium-supplemented culture (Fig. 3d). It suggested the possible existence of a calcium-dependent metabolite associated with this strain antifungal activity. Previous studies reported that Gram-positive bacteria, *Streptomyces coelicolor* produced a calcium-dependent antibiotic, categorized as non-ribosomal peptide synthase (NRPS), conferring a promising antimicrobial activity[25,26]. However, the presence of this metabolite type produced by Gram-negative bacteria was still questioned.

This present study revealed that this phylloplane bacteria produced maximum IAA concentration after 48 h culture (Fig. 4) suggesting its IAA biosynthesis might occur during the late growth phase. Longer culture duration could maintain the high production of IAA at certain levels of Trp concentration (Fig. 4) as the excess amount of Trp would be toxic for the bacteria. Unlike the biosynthesis of most secondary metabolites in Gram-negative bacteria, IAA biosynthesis in *S. plymuthica* was reported to be quorum sensing (QS)-independent, thus it did not depend on the presence of N-acyl-homoserine lactone (AHL) and high population density[1,27].

The use of cell-free supernatants (CFS) to inoculate the plant seeds with the bacterial IAA revealed that plants could utilize the IAA directly without the help of the producing bacteria. The use of this medium had not been widely reported, yet. It was successfully displayed a significant growth improvement, particularly in *Solanaceous* plants, based on its root and shoot growth (Table 2, 3). However, these results were in contradiction with Malik and Sindhu[16], where the inoculation of IAA-producing *Pseudomonas* isolates on chickpea seeds resulted in stunting root and shoot growth. Tabatabaei *et al.*[28] also reported the decrease of durum wheat germination rate and percentage due to the elevated IAA concentration produced by the inoculated *Pseudomonas*. Both studies emphasized that low concentration of IAA was quite powerful to stimulate the plant growth, while higher IAA amount would lead to growth suppression. In addition, the application of this IAA-containing CFS might minimize the risk of IAA accumulation in plant cells, commonly occurred in the inoculation of IAA-producing bacteria, thus leading to better plant growth.

CONCLUSION

The modification on several culture parameters performed in this study had contributed some hints regarding the favorable environmental parameters required to stimulate its antifungal activity and IAA production. However, further studies were needed to perform the metabolites production in a culture designated using the combination of multiple parameters recommended by this present study. This information would provide a reference for the development of large-scale IAA and antifungal metabolites production culture of this strain.

SIGNIFICANCE STATEMENT

This study discovered some preferred culture parameters triggering higher antifungal activity and IAA production of phylloplane *S. plymuthica* UBCF_13. It would serve as a reference for the design of a recommended production medium where all the contributing parameters are combined proportionally. Therefore, mass production of antifungal compounds and IAA from this strain would be the next issue to be investigated thoroughly. This present study also proved that the inoculation of IAA-containing CFS offered a promising yet efficient method to deliver beneficial secreted metabolites to plant cells with a lower risk of uncontrolled metabolites accumulation.

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REFERENCES

1. Liu, X., J. Jia, R. Popat, C.A. Ortori and J. Li *et al*., 2011. Characterisation of two quorum sensing systems in the endophytic *Serratia plymuthicastrain* G3: Differential control of motility and biofilm formation according to life-style. BMC Microbiol., Vol. 11. 10.1186/1471-2180-11-26.
2. Neupane, S., 2013. Genomics and Transcriptomics of Plant Beneficial *Serratia* spp. Swedish University of Agricultural Sciences, Uppsala, Pages: 50.
3. Jankiewicz, U. and M.S. Brzezinska, 2015. Purification, characteristics and identification of chitinases synthesized by the bacterium *Serratia plymuthica* MP44 antagonistic against phytopathogenic fungi. Applied Biochem. Microbiol., 51: 560-565.

4. Schmidt, R., V. de Jager, D. Zuhlke, C. Wolff and J. Bernhardt *et al*., 2017. Fungal volatile compounds induce production of the secondary metabolite Sodorifen in *Serratia plymuthica* PRI-2C. Scient. Rep., Vol. 7. 10.1038/s41598-017-00893-3.
5. Aisyah, S.N., H. Harnas, S. Sulastri, R. Retmi and H. Fuaddi *et al*., 2016. Enhancement of a novel isolate of *Serratia plymuthica* as potential candidate for an antianthracnose. Pak. J. Biol. Sci., 19: 250-258.
6. Aisyah, S.N., S. Sulastri, R. Retmi, R.H. Yani and E. Syafriani *et al*., 2017. Suppression of *Colletotrichum gloeosporioides* by indigenous phyllobacterium and its compatibility with rhizobacteria. Asian J. Plant Pathol., 11: 139-147.
7. Masurekar, P.S., 2008. Nutritional and Engineering Aspects of Microbial Process Development. In: Natural Compounds as Drugs, Petersen, F. and R. Amstutz (Eds.)., Springer, Germany, pp: 291-328.
8. Sanchez, S., A. Chavez, A. Forero, Y. Garcia-Huante and A. Romero *et al*., 2010. Carbon source regulation of antibiotic production. J. Antibiot., 63: 442-459.
9. Gokulan, K., S. Khare and C. Cerniglia, 2014. Metabolic Pathways: Production of Secondary Metabolites of Bacteria. In: Encyclopedia of Food Microbiology, Batt, C. and M. Tortorello (Eds.)., 2nd Edn., Academic Press, San Diego, C.A. pp: 561-569.
10. Mazzucotelli, C.A., I. Durruty, C.E. Kotlar, M.R. Moreira, A.G. Ponce and S.I. Roura, 2014. Development of a microbial consortium for dairy wastewater treatment. Biotechnol. Bioprocess Eng., 19: 221-230.
11. Srividya, S. and M. Mala, 2011. Influence of process parameters on the production of detergent compatible alkaline protease by a newly isolated *Bacillus* sp. Y. Turkish J. Biol., 35: 177-182.
12. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
13. Islam, M.R., Y.T. Jeong, Y.S. Lee and C.H. Song, 2012. Isolation and identification of anti-fungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. Mycobiology, 40: 59-66.
14. Mayer, A.M., 1958. Determination of indole acetic acid by the salkowsky reaction. Nature, 182: 1670-1671.
15. Sindhu, S.S., S.K. Gupta and K.R. Dadarwal, 1999. Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of growth of green gram (*Vigna radiata*). Biol. Fertil. Soils, 29: 62-68.
16. Malik, D.K. and S.S. Sindhu, 2011. Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol.. Mol. Biol. Plants, 17: 25-32.
17. Gaur, A.S. and S.S. Gaur, 2006. Statistical Methods for Practice and Research: A Guide to Data Analysis Using. SPSS, Inc., SAGE Publications, India.
18. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
19. Williamson, N.R., P.C. Fineran, F.J. Leeper and G.P. Salmond, 2006. The biosynthesis and regulation of bacterial prodiginines. Nat. Rev. Microbiol., 4: 887-899.
20. Gesheva, V., V. Ivanova and R. Gesheva, 2005. Effects of nutrients on the production of AK-111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. Microbiol. Res., 160: 243-248.
21. Rokem, J.S., A.E. Lantz and J. Nielsen, 2007. Systems biology of antibiotic production by microorganisms. Natural Prod. Rep., 24: 1262-1287.
22. Sanchez, S. and A.L. Demain, 2002. Metabolic regulation of fermentation processes. Enzyme Microb. Technol., 31: 895-906.
23. Guimaraes, L.M., R.L. de Araujo Furlan, L.M. Garrido, A. Ventura, G. Padilla and M.C.R. Facciotti, 2004. Effect of pH on the production of the antitumor antibiotic retamycin by *Streptomyces olindensis*. Biotechnol. Applied Biochem., 40: 107-111.
24. Gomaa, E.Z., 2012. Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: Their potential in anti-fungal biocontrol. J. Microbiol., 50: 103-111.
25. Bentley, S.D., K.F. Chater, A.M. Cerdeno-Tarraga, G.L. Challis and N.R. Thomson *et al*., 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature, 417: 141-147.
26. Borodina, I., P. Krabben and J. Nielsen, 2005. Genome-scale analysis of *Streptomyces coelicolor* A3(2) metabolism. Genome Res., 15: 820-829.
27. Muller, H., C. Westendorf, E. Leitner, L. Chernin and K. Riedel *et al*., 2009. Quorum-sensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HRO-C48. FEMS Microbiol. Ecol., 67: 468-478.
28. Tabatabaei, S., P. Ehsanzadeh, H. Etesami, H.A. Alikhani and B.R. Glick, 2016. Indole-3-Acetic Acid (IAA) producing *Pseudomonas* isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.). Span. J. Agric. Res., Vol. 14, No. 1.