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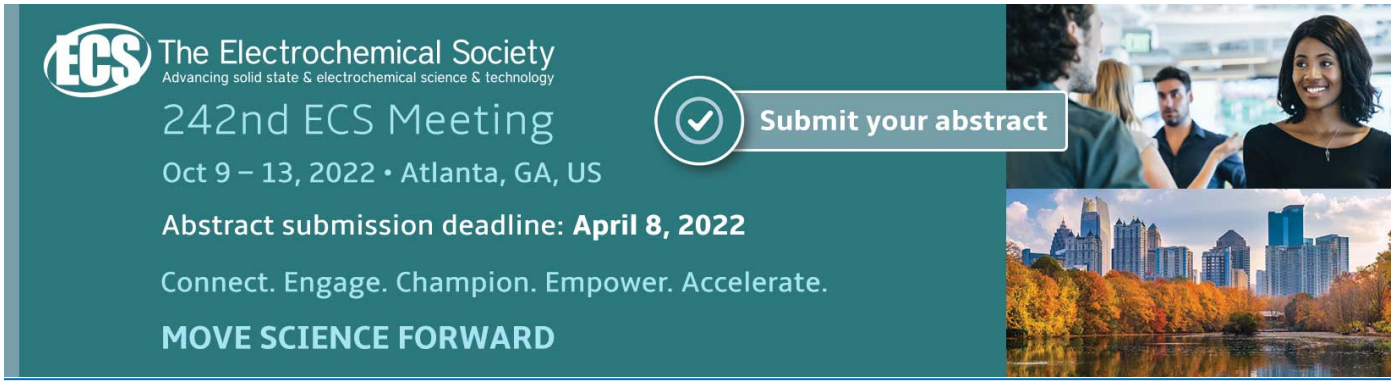

Different pH levels medium effects in IAA production of phylloplane bacterium *Serratia plymuthica* strain UBCF_13

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Abstract. *Indole-3-acetic acid* [IAA] is the main source of auxin in plants that plays a major role in controlling various physiological processes. The ability of bacterium in IAA biosynthesis depends on several environmental factors, usually triggered by acidic stresses. This study was aimed to optimize the IAA production by phylloplane bacterium *Serratiaplymuthica* strain UBCF_13 with various pH levels. Optimization was carried out by using 0.2% L-Tryptophan in Luria Bertani[LB] media with various pH levels namely pH 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0 and 9.0. UBCF_13 showed the best IAA production at pH 6.0 and 0.2% L-Tryptophan exhibited IAA concentration in the amount of 24.13 ppm. The UBCF_13 extracellular protein profile analysis showed that at pH 6.0 induced L-Tryptophan 0.2% produced protein bands of 60.8 kDa and 45 kDa in size. These protein bands are assumed to be *ipdC* and *nitrilase* proteins which are involved in the IAA biosynthesis. The application of IAA was carried out using chili peppers [*Capsicum annuum* L.] germination of the Lotanbar genotype. The application was using supernatant produced from media of pH 6.0 and 0.2% L-Tryptophan. The results show that root and hypocotyl lengths were 4.10 cm and 2.90 cm, respectively. Meanwhile, root and hypocotyl lengths in the control sample only 2.78 cm and 1.88 cm. The results indicated that the IAA production of UBCF_13 can promote the growth of chili seedling. A further detailed study on the visualization of protein profiles at the optimum treatment needs to be done. Two-DimensionalElectrophoresis is recommended to be applied so that protein bands can be accurately separated to identify detailed proteins involved in the IAA biosynthesis.

Keywords: IAA, L-Tryptophan, pH, *Serratiaplymuthica*, UBCF_13

1. Introduction

Indole-3-aceticacid [IAA] is the main source of auxin in plants. IAA plays a role in controlling various physiological processes, such as cell division and enlargement, tissue differentiation and plant responses to light and gravity [22] [23]. Several factors that influence IAA biosyntheses in the bacteria are genetic and environmental. Genetic factors that influence IAA biosyntheses are the location of auxin biosynthetic regulatory genes in the bacteria genome, gene expression, and the activity of gene regulatory transcription factors when responding to stress conditions [*RpoS* and *GacS*/ *GacA*][6]. Meanwhile, environmental factors that influence IAA biosyntheses by the bacteria are environmental stresses such as acidic pH, osmotic pressure, carbon limitations and decreased bacteria growth rate [7] [19].

IAA biosyntheses can go through several pathways including the *indole-3-acetamide*[IAM] pathway, the *indole-3-pyruvate* pathway [IPyA], the *tryptamin*e pathway, and the *indole-3-acetonitrile* pathway. Some enzymes also play a role in IAA biosyntheses such as *tryptophan-2-monooxygenase*, *nitrilase*, IAM *hydrolase*, *aminotransferase*, and *indole-3-pyruvate decarboxylase*[20] [21]. The protein produced during the IAA biosyntheses process can be identified by a proteomic-based approach. Proteomic-based approaches examine several proteins to be expressed and a complete understanding of the genomic aspects. Through this approach, the expression of the amount of protein that appears during certain biological processes can be studied simultaneously [10].

Serratiaplymuthica can produce *indole-3-aceticacid*[IAA]auxohormonephytohormone[12]. *S. plymuthica* strain UBCF_13[accession no: KX394779] was isolated from the phylloplane mustard [*Brassica juncea* L.] plant. In one study Aisyah*et al*. [2] reported that this bacterium was able to produce IAA optimally [116.09 ppm] at a modification of L-Tryptophan 0.2% with a culture duration of 48 hours [3]. However, the potential of UBCF_13in producing IAA needs to be studied in more depth. This study was aimed to optimize the IAA production by phylloplane bacterium *S. plymuthica* strain UBCF_13 with various pH levels. Optimization was carried out using the 0.2% L-Tryptophan inducer in the Luria Bertani[LB] medium at various pH levels, namely pH 4.0; 4,5; 5.0; 5.5; 6.0; 6.5; 7.0 and 9.0.

2. Methods

The bacterium was isolated from phylloplane mustard [*Brassica juncea* L.]. *S. plymuthica* strain UBCF_13 no. accessions: KX394779 [internal collection of FP-UA Biotechnology Laboratory], LB [Luria Bertani] media, L-Tryptophan, Salkowski reagent, *trichloroacetic acid*[TCA], sample buffer, water agar media, and chili seeds [*Capsicum annuum* L.] genotype Lotanbar.

2.1 L-Tryptophaninductionculture of 0.2% at a variouspH level

LB media was adjusted pH [pH 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 9.0] and induced L-Tryptophan 0.2%. 1 mL of bacterium cell culture was cultured on the LB media for 48 hours, 160 rpm, dark conditions, and at room temperature.

2.2 Measurement of IAA

The bacterium density of OD 600 nm was measured using a spectrophotometer. IAA generated by UBCF_13 is checked using the colorimetric method. This method was adopted from the Gordon and Weber protocol [11]. UBCF_13supernatant was immersed in the Salkowski reagent at a ratio of 1: 2 for 30 minutes. Next, IAA measurements were checked with a spectrophotometer with a wavelength of 530 nm.

2.3 Analysis of extracellular proteomic profiles

Isolation of bacterium extracellular proteins based on the Nouwens*et al*. [18]protocol. Visualization of protein profiles with SDS-PAGE. Several protein bands are identified to be up or down. This identification is also accompanied by information [size and level of expression difference].

2.4 Chili germination with the IAA supernatant application UBCF_13
The seeds are soaked with a bacterium IAA supernatant that has been sterile for 30 minutes. Seeds are germinated in agar water media [10 seeds/bottle] and stored at room temperature [28 ^{o}C]. As a control, seeds were not immersed in UBCF_13 supernatant and directly germinated in the media [14].

3. Results and Discussion

3.1 Bacterium culture density after L-Tryptophan induction of 0.2%
The average absorbent value of starter culture was 1.57 while the density of bacterium culture after L-Tryptophan induction was 0.2% and without induction at various pH levels had different values [Figure 1]. The *S. plymuthica* strain UBCF_13 was unable to grow at pH 4.0. This is because pH 4.0 has a high acidity so it is not suitable for bacterium growth. Marlisa[15] states that the optimum pH for *S. plymuthica* growth of UBCF_13is at pH 7.0 with the absorbance value of bacterium at OD 600 nm being 1.7.

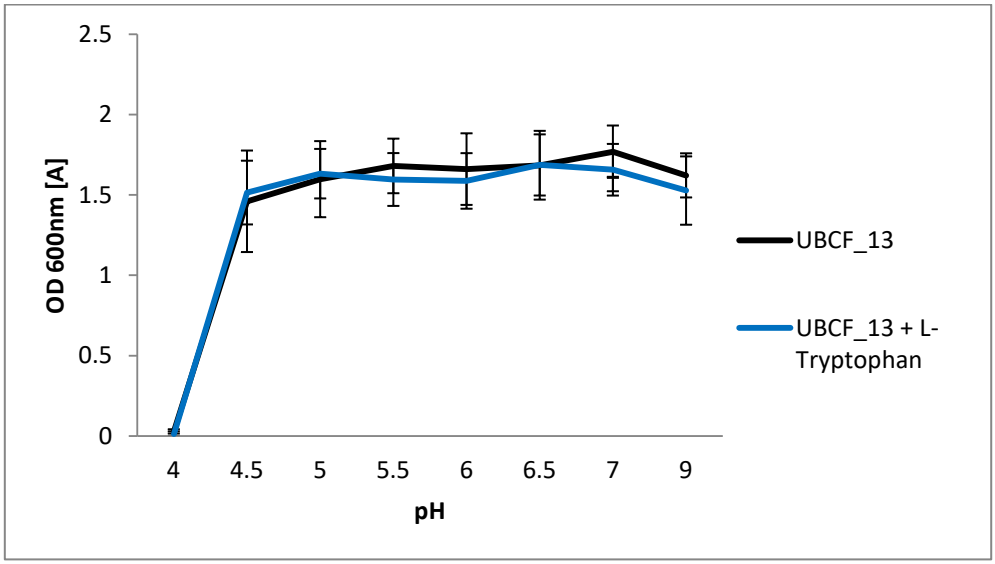

Figure 1. Bacteriumdensity of *S. Plymuthica* strain UBCF_13 with and without L-Tryptophan induction of 0.2 % at various pH levels. Data is the average value of four groups with standard deviations displayed in the form of error bars.

Based on the measurement of bacterium density at OD 600 nm, optimum UBCF_13 grows on pH 7.0 media without inducing L-Tryptophan with an absorbance value of 1.77. Whereas at the same pH as the media-induced by L-Tryptophan 0.2%, the absorbance value decreased to 1.66. Likewise, at pH 6.0, the density of bacterium culture induced by 0.2% L-Tryptophan decreased from an absorbance value of 1.66 to 1.59. This decrease indicates that the administration of 0.2% L-Tryptophan to bacterium can suppress bacterium growth. Following the statement of Zhang *et al*. [24] that environmental stresses such as acid pH affect IAA biosyntheses. The bacterium that experiences stress will produce secondary metabolites and synthesize Tryptophan into IAA. This occurs in the stationary phase and results in a decrease in the rate of bacterium growth.

3.2 IAA production at various pH levels

The interaction between pH level factor and L-Tryptophan induction factor was not significant in IAA production by UBCF_13bacterium. The graph of the interaction of pH level interactions with the induction of 0.2% L-Tryptophan does not intersect from pH 4.0 to pH 9.0 as shown in Figure 2.

In general, the highest IAA production concentration was found at a single factor of pH 6 level and a single factor of 0.2% L-Tryptophan induction of 24.13 ppm. Based on a single pH factor, the average IAA production of the bacterium UBCF_13at a pH level of 6.0 ie 17.36 ppm was not significantly different from the media pH of 7.0 which was 14.50 ppm. At the pH level of 4.0, the bacterium does not produce IAA. This was not significantly different from IAA production at pH 4.5, 5.0 and pH 9.0 [Table 1].

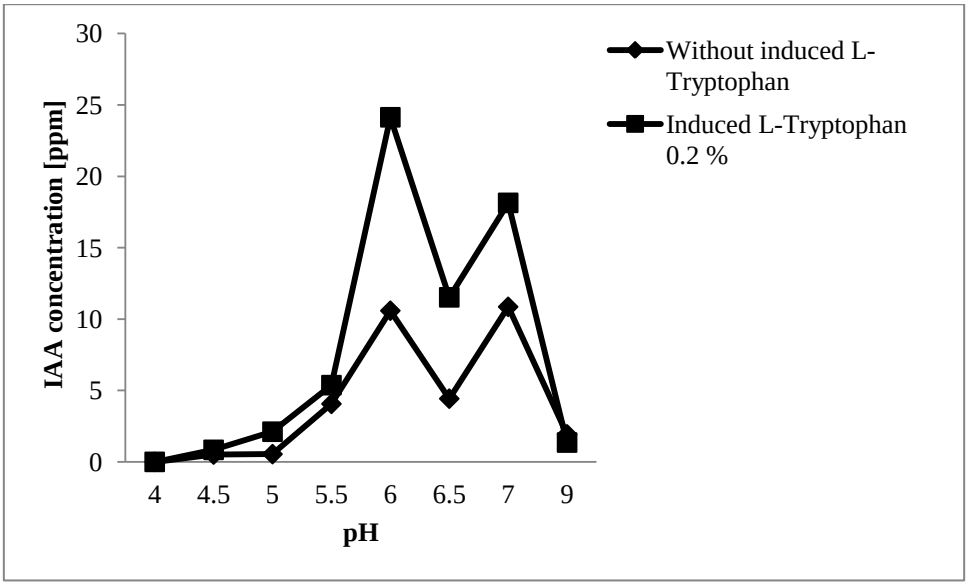

Figure 2. RelationshipbetweenpH Level withandwithoutL-Tryptophaninduction on IAA production by UBCF_13.

Table 1. Effect of pHandInduction of L-Tryptophan 0.2% on IAA Production UBCF_13

pH	IAA production UBCF_13[ppm]	IAA production UBCF_13+ L-Tryptophan[ppm]	Average[ppm]
pH 4.0	0.00	0.00	0.00 a
pH 4.05	0.51	0.84	0.68 a
pH 5.0	0.54	2.11	1.33 a
pH 5.5	4.06	5.37	4.72 b
pH 6.0	10.59	24.13	17.36 c
pH 6.55	4.43	11.52	7.98 b
pH 7.0	10.86	18.14	14.50 c
pH 9.0	1.93	1.36	1.64 a
Average[ppm]	4.11 A	7.93 B	

The *S. plymuthica* UBCF_13 has the same optimal media pH with *Bacillus* spp. MQH-19 also produced the highest IAA at pH 6.0 and decreased 62% at pH 5.0 [1]. The value followed by the same lower case notation is not significantly different in the single factor of pH and the value followed by the same capital letter, not significantly different in the single factor of induction of L-Tryptophan 0.2% according to DNMRT at the level of 5%.

Based on the single factor L-Tryptophan induction, IAA production by UBCF_13induced L-Tryptophan 0.2 % was significantly different from without the induction of L-Tryptophan [Table 1]. In

a single factor without L-Tryptophan induced, the average IAA produced by the bacterium was only 4.11 ppm while those induced by L-Tryptophan were 0.2%, the average IAA production increased by 51.85% to 7.93 ppm. This is consistent with the statement of Block *et al*. [5] that the production of compounds [IAA] is stimulated by the amino acid L-Tryptophan as its precursor.

3.3 Analysis of UBCF_13 extracellular protein profile with SDS-PAGE

UBCF_13 extracellular protein profile can be seen in Figure 3. Based on the results of the visualization of protein profiles, proteome profiles at 45 kDa and 60.8 kDa up-regulated at pH 6 treatment induced with L-Tryptophan 0,2%. The 45 kDa protein band is assumed to be *nitrilase*. In previous studies, Gong [9] reported that nitrilase is an enzyme that plays a role in the biosynthesis of L-Tryptophan with a size of 45 kDa. The activity of this enzyme is greatly influenced by the formation of biomass and culture conditions, such as carbon sources, nitrogen sources, inducers, temperature, pH, enzyme modifiers, and organic solvents. Meanwhile, the 60.8 kDa protein band is assumed to be the *ipdC* gene. Based on GenBank with accession number HQ910435.1 the *ipdC* gene has a size of 1676 bp with a protein band size of 60.8 kDa. Ona *et al*. [19] state that the expression of the *ipdC* gene involved in the IAA biosyntheses process in *Azospirillumbrasilense* increases when the bacterium is in acidic pH conditions.

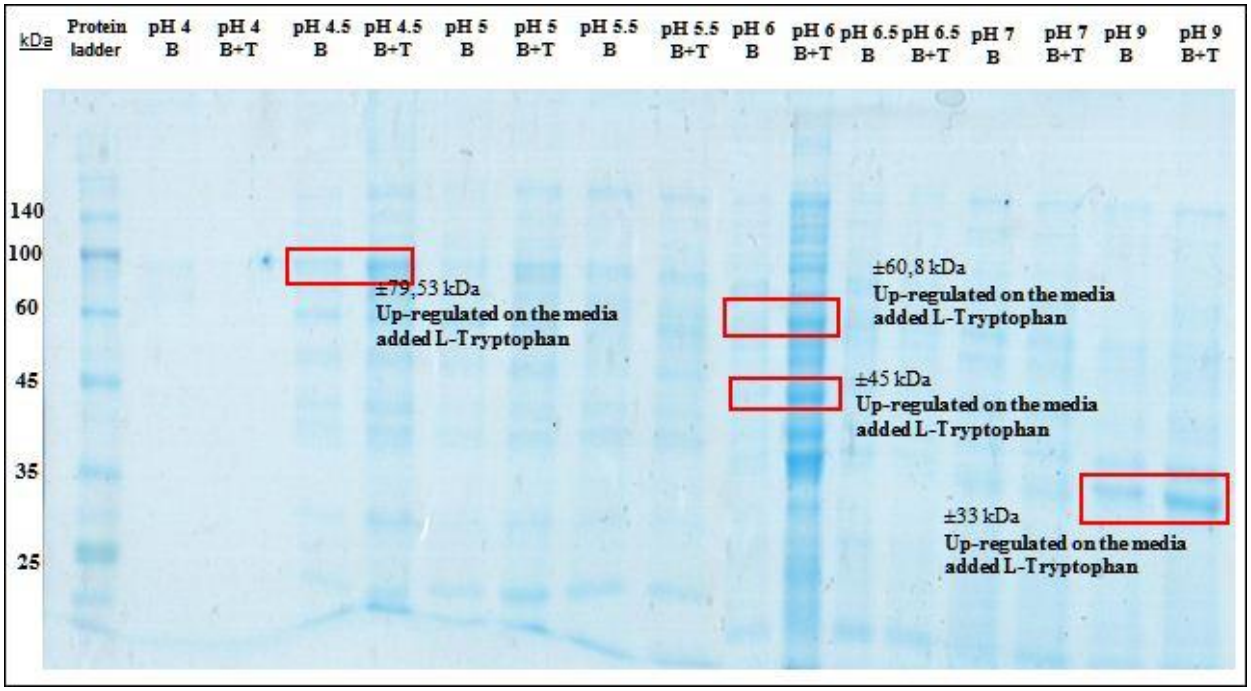

Figure 3. Extracellular protein profile of *S. Plymuthica* strain UBCF_13 bacterium was cultured on media with and without induced 0.2% L-TryptophaninvariouspH level

Proteome profiles with pH measures 4.5 and pH 9.0 induced by L-Tryptophan were up-regulated at 79.53 kDa and 33 kDa. It is assumed that this band is an enzyme that can reduce the activity of enzymes to produce IAA. Based on data on NCBI with Gene ID: 829389 the *YUC1* gene is found on chromosome number 4 with a size of 2171 bp. This gene has a protein band size of around 79.53 kDa. The *YUC1* gene acts as a catalyst for the *5-[4-chlorophenyl]-4H-1,2,4-triazole-3-thiol [yucasin]* enzymes that inhibit IAA biosyntheses via the *IPyA* pathway competitively [17]. To confirm this, further purification and analysis related to the type of protein in a certain band size are needed.

3.4 The temptation of the Lotanbar genotype chili seeds with the supernatant applicationUBCF_13
Based on observations made for 10 days, it is known that root and hypocotyl growth in IAA-given seeds was higher than in controls, which were 4.10 ± 0.75 cm and 2.90 ± 0.33 cm compared to controls [Figure 4-5]. Increased growth compared with control occurred in the roots of 1.32 cm and 1.02 cm in the hypocotyl. Previous studies suggest that IAA stimulates cell division, cell elongation and tissue differentiation and lateral root formation [4].

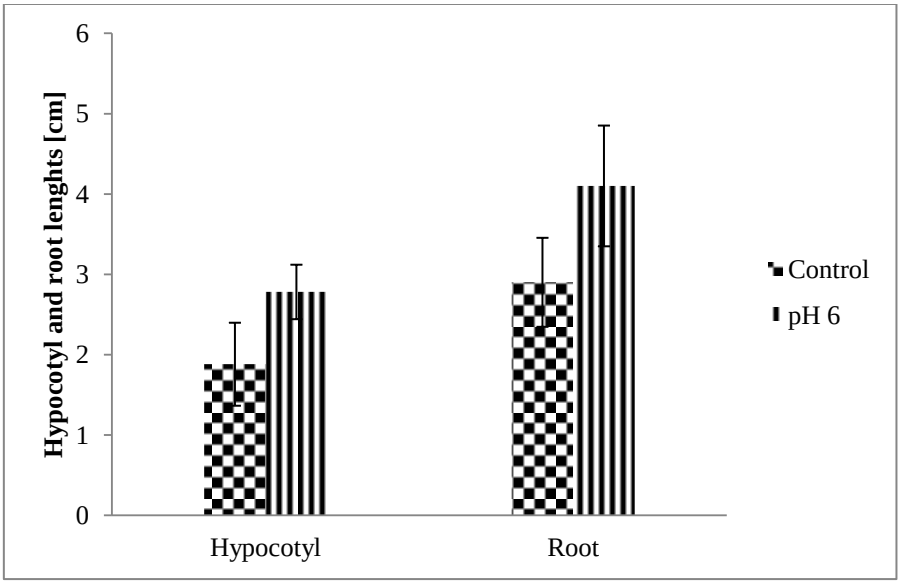

Figure 4. Sprout growth of Lotanbar genotype day 10th with root and hypocotyl variables.

The highest IAA concentration obtained from this study [19.14 ppm] is still lower than the Maldoni study [13] which reached IAA 116.09 ppm. This is presumably because the concentration of starter culture given is lower, so the IAA produced is also lower. This study used 1 mL of starter culture for 100 mL of LB media with L-Tryptophan 0.2%, while the Maldoni study [13] used 1 mL of starter culture for 20 mL LB with L-Tryptophan 0.2%. The difference in the volume of LB used affects the density of bacterium culture so that bacterium productivity produces different secondary metabolites. Following the statement of Masurekar[16] that the source of nutrition influences the growth of bacterium and the production of secondary metabolites. The availability of nutrients at 20 mL LB is less than the nutrition at 100 mL LB. This resulted in a bacterium struggle for nutrients in the 20 mL LB medium so that the IAA produced at 20 mL LB was higher than in 100 mL LB.

Maldoni was study [13] of chili peppers added to the Petri dish had a root length of 1.32 cm and hypocotyl of 0.62 cm. The use of a Petri dish as a germination container is less effective than a bottle. This is because the Petri dish size is lower than the bottle so it gives the effect of the growth of chili sprouts. The growth of controls in the Maldoni study [13] was lower than the controls in this study. Increased root growth in the treatment applied supernatant by 0.13 cm from root growth in the control. Meanwhile, hypocotyl growth in the application of the supernatant decreased by 0.02 cm from the control[13]. The decrease in hypocotyl growth obtained by Maldoni[13] is presumably due to the concentration of IAA given has reached a maximum so that it inhibits growth. Following the statement of Dhungana [8] that the application of IAA biosynthetic inhibitors results in a reduction in endogenous IAA followed by suppressing the extension and growth of tomato seedlings.

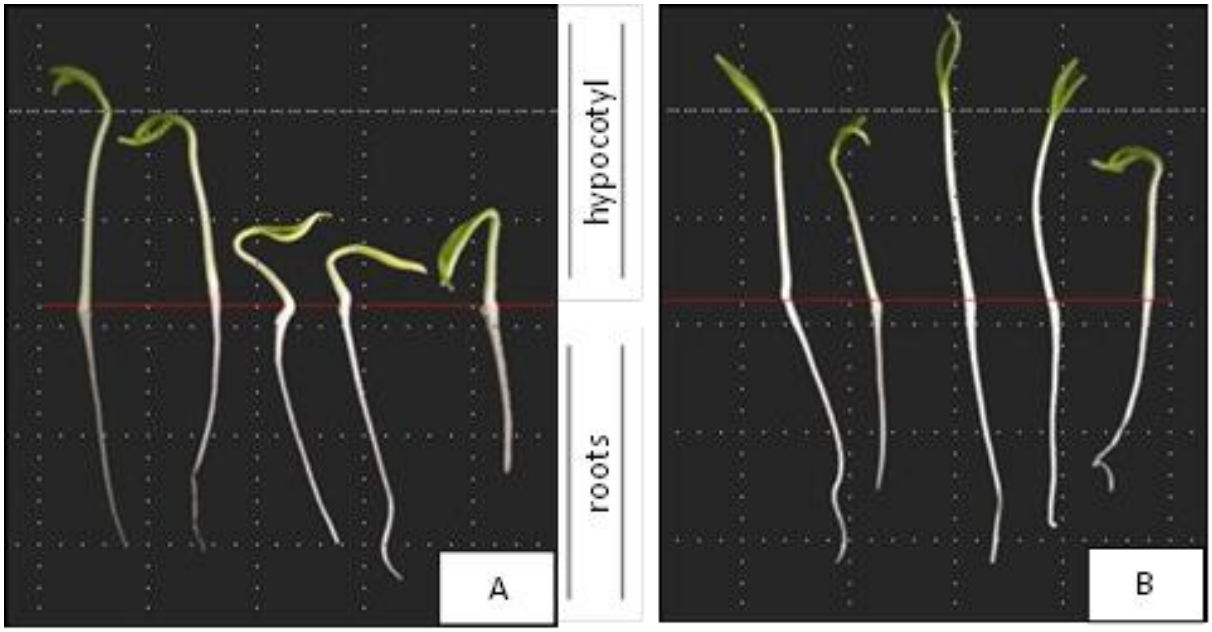

Figure 5. Growth of Lotanbar genotype chili sprouts. [A] Control [without UBCF_13 supernatant application. [B] Sprouts with UBCF_13 supernatant application on pH 6 media-inducedby 0.2% L-Tryptophan.

4. Conclusion

This study concludes that there is no interaction between L-Tryptophan induction factor and pH acidity level in IAA production by *S. plymuthica* strain UBCF_13. The optimum IAA productionby *S. plymuthica* strain UBCF_13was 24.13 ppm obtained in media with a single factor of L-Tryptophan induction of 0.2% and a single factor of pH 6.0.

Acknowledgment
We are grateful to the General Directorate of Higher Education through PMDSU Research Grant fiscal with contract No. T/15/UN.16.17/PT.01.03/PP/2019.

References

[1] Acuna, J.J., M.A. Jorquera1, O.A. Martínez, D. Menezes−Blackburn, M.T. Fernández, P. Marschner, R. Greiner and M.L. Mora1. 2011. *Indole Acetic Acid* and *Phytase* Activity Produced by *Rhizosphere bacilli* as Affected by pH and Metals. *Journal of Soil Science and Plant Nutrition*, **11**: 1-12.

[2] Aisyah, S.N., S. Sulastri, R. Retmi, R.H. Yani, E. Syafriani, L. Syukriani, F. Fatchiyah, A. Bakhtiar and J. Jamsari. 2017. Suppression of *Colletotrichum gloeosporioides* by Indigenous Phyllobacterium and Its Compatibility with Rhizobacteria. *Asian Journal of Plant Pathology*, **11**: 139-147.

[3] Aisyah, S.N., J. Maldoni, I. Sulastri, W. Suryati, Y. Marlisa, L. Herliana and J. Jamsari. 2019. Unraveling The Optimal Culture Condition for The Antifungal Activity and IAA Production of Phylloplane *Serratia plymuthica*. *Plant Pathology Journal*, **18**: 31-38.

[4] Bhutani, N., R. Maheshwari, M. Negi, and P. Suneja. 2018. Optimization of IAA Production by Endophytic *Bacillus* spp. from *Vigna radiata* for Their Potential Use as Plant Growth Promoters. *Israel Journal of Plant Sciences*, **65**: 83-96.

[5] Block, A., E. Schmelz, J.B. Jones and H.J Klee. 2005. Coronatine and Salicylic Acid: The

Battle Between *Arabidopsis* and *Pseudomonas* for Phytohormone Control. *Molecular Plant Pathology*, **6**: 79-83.

[6] Brandl, M., B. Quinones and S. Lindow. 2001. Heterogeneous Transcription of An *Indole Acetic Acid* Biosynthetic Gene in *Erwinia herbicola* on Plant Surfaces. *Proceedings of The National Academy of Sciences*, **98**: 3454-3459.

[7] Broek, A.V., P. Gysegom, O. Ona, N. Hendrickx, E. Prinsen, J. Van Impe and J. Vanderleyden. 2005. Transcriptional Analysis of The *Azospirillum brasilenseIndole-3-pyruvate Decarboxylase* Gene and Identification of A *Cis-acting* Sequence Involved in Auxin Responsive Expression. *Molecular Plant-Microbe Interactions*, **18**: 311-323.

[8] Dhungana, S.A. and K. Itoh. 2019. Effects of Co-Inoculation of *Indole-3-Acetic Acid*-Producing and-Degrading Bacterial Endophytes on Plant Growth. *Horticulturae*, **5**: 1-9.

[9] Gong, J.S., Z. Lu, H. Li, J.S. Shi, Z.M. Zhou and Z.H. Xu. 2012. Nitrilases in *Nitrile* Biocatalysis: Recent Progress and Forthcoming Research. *Microbial Cell Factories*, **11**: 142-160.

[10] Gonzalez-Fernandez, R. and J.V. Jorrin-Novo. 2011. Contribution of Proteomics to The Study of Plant Pathogenic Fungi. *Journal of Proteome Research*, **11**: 3-16.

[11] Gordon, S.A. and R.P. Weber. 1951. Colorimetric Estimation of *Indole Acetic Acid*. *Plant Physiology*, **26**: 192-195.

[12] Liu, X., J. Jia, R. Popat, C.A. Ortori, J. Li, S.P. Diggle, K. Gao and M. Cámara. 2011. Characterisation of Two Quorum Sensing Systems in The Endophytic *Serratia plymuthica* Strain G3: Differential Control of Motility and Biofilm Formation According to Life-style. *BioMed Central Microbiology*, **11**: 26-37.

[13] Maldoni, J. 2019. Optimasi Produksi IAA *Serratia plymuthica* Strain UBCF_13 melalui Modifikasi Konsentrasi Induser dan Durasi Kultur Induksi. [Skripsi]. Padang: Universitas Andalas.

[14] 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*]. *Physiology and Molecular Biology of Plants*, **17**: 25-32.

[15] Marlisa, Y. 2019. Pengaruh Modifikasi pH Media Kultur Pertumbuhan Terhadap Aktivitas Antijamur dari Supernatan Kultur Bakteri *Serratia plymuthica* Strain UBCF_13. [Skripsi]. Padang: Universitas Andalas.

[16] Masurekar, P.S. 2008. Nutritional and Engineering Aspects of Microbial Process Development. *Natural Compounds as Drugs*, **1**: 91-328.

[17] Nishimura, T., K.I. Hayashi, H. Suzuki, A. Gyohda, C. Takaoka, Y. Sakaguchi, and Y. Kamiya. 2014. *Yucasin* is A Potent Inhibitor of *YUCCA*, A Key Enzyme in Auxin Biosynthesis. *The Plant Journal*, **77**: 352-366.

[18] Nouwens, A.S., M.D. Willcox, B.J. Walsh and S.J. Cordwell. 2002. Proteomic Comparison of Membrane and Extracellular Proteins from Invasive [*PAO1*] and Cytotoxic [6206] Strains of *Pseudomonas aeruginosa*. *International Edition*, **2**: 1325-1346.

[19] Ona, O., I. Smets, P. Gysegom, K. Bernaerts, J. Van Impe, E. Prinsen and J. Vanderleyden. 2003. The Effect of pH On Indole-3-Acetic Acid [IAA] Biosynthesis of *Azospirillumbrasilense* Sp7. *INT Science Service*, **35**: 199-208.

[20] Saleh, S.S. and B.R. Glick. 2001. Involvement of *GacS* and *RpoS* in Enhancement of The Plant Growth-promoting Capabilities of *Enterobacter cloacae* CAL2 and UW4. *Canadian Journal of Microbiology*, **47**: 698-705.

[21] Spaepen, S., J. Vanderleyden and R. Remans. 2007. Indole-3-Acetic Acid in Microbial and Microorganism-plant Signaling. *Federation of European Microbiological Societies*, **31**: 425-448.

[22] Teale, W.D., I.A. Paponov and K. Palme. 2006. Auxin in Action: Signalling, Transport and The

Control of Plant Growth and Development. *Nature Reviews Molecular Cell Biology*, **7**: 847-859.

[23] Woodward, A.W. and B. Bartel. 2005. Auxin: Regulation, Action, and Interaction. *Annals ofBotany*, **95**: 707-735.

[24] Zhang, C., X. Zhang and S. Shuen. 2014. Proteome Analysis for Antifungal Effects of *Bacillus subtilis* KB-1122 on *Magnaporthe grisea* P131. *Journal of Microbial Biotechnology*, **30**: 176