Environmental Factors Modulating Indole-3-Acetic Acid Biosynthesis by Four Nitrogen Fixing Bacteria in a Liquid Culture Medium

Le Thi Xa[1], Nguyen Khoi Nghia[2*], and Hüseyin Barış Tecimen[3]

[1]School of Education, Soc Trang Community College, Soc Trang Province, Vietnam
[2]Department of Soil Science, College of Agriculture, Can Tho University, Can Tho City, Vietnam
[3]Soil Science and Ecology Department, Faculty of Forestry, Istanbul University-Cerrahpaşa, Istanbul, Turkey

ARTICLE INFO

Received: 29 Nov 2021
Received in revised: 30 Jan 2022
Accepted: 3 Feb 2022
Published online: 28 Feb 2022
DOI: 10.32526/ennrj/20/202100233

Keywords:
Biological nitrogen fixing bacteria/ Indole-3-acetic acid biosynthesis/ Plant growth promotion/ pH/ Sodium chloride/ Tryptophan

*** Corresponding author:**
E-mail: nknghia@ctu.edu.vn

ABSTRACT

This study evaluated the effects of some environmental conditions on IAA biosynthesizing capacity of four nitrogen fixing bacteria, namely *Paenibacillus cineris* TP-1.4, *Bacillus megaterium* MQ-2.5, *Klebsiella pneumoniae* OM-17.2, and *Pseudomonas boreopolis* CP-18.2. Carbon source, pH, NaCl, and tryptophan supplement treatments were set to investigate the effects of those environmental factors on IAA synthesis. The IAA synthesizing capacity of bacterial strains in liquid medium was measured spectroscopically following incubation by Salkowski's reagent method. The results showed that, under the sucrose amendment, the IAA concentrations produced by all four bacterial strains were significantly higher than those of the other four carbon source added treatments. Two of the four bacterial strains produced the highest yield of IAA in liquid medium at pH 7 (TP-1.4 and OM-17.2), whereas pH 8 was optimum for the other two strains (MQ-2.5 and CP-18.2). The MQ-2.5 strain could synthesize IAA fairly well in up to 5% NaCl and produced the highest amount of IAA with 1% NaCl. Furthermore, IAA synthesizing capability of tested bacterial strains increased sharply along with increasing tryptophan content in culture medium except for the TP-1.4 strain. From the current study, these isolates emerged as possible alternatives for future IAA production for plant growth and yield enhancement. Hence, they have a great potential to be used as bio-inoculants for plant growth promotion in eco-friendly and sustainable agriculture.

1. INTRODUCTION

The agriculture industry has applied numerous measures for intensive crop production, such as using chemical fertilizers, pesticides, and plant growth stimulants, which have become widespread to increase productivity and ensure global food security. However, the long term impacts of applying these cultivation techniques made a dramatic decrease in the productivity of some crops (Sirivastava and Singh, 2017), degradation of the soil, and water pollution (Stinner, 2007). Therefore, implementation of sustainable, eco-friendly agriculture and climate change adaptive methods is of utmost concern and has a priority.

Nowadays, chemical fertilizer costs have risen all over the world and this fact has become a big disadvantage for farmers as they suffer from higher input costs and get low profits accordingly. Therefore, the use of plant growth promoting microorganisms in crop cultivation is preferred and encouraged worldwide in order to reduce or partly replace applying chemical fertilizers, or plant growth stimulants which would reduce the input costs and protect the agri-ecosystems, environment, and human health. In particular, the isolation, selection and application of multi-functional microorganisms has shown a significantly higher stimulation on crop growth and yields compared to those of single-function ones (Tewari and Arora, 2014). Among them, the growth functions stimulating microorganisms, including biological nitrogen fixation and synthesis of indole-3-acetic acid, have attracted remarkable attention (Shokri and Emtiazi, 2010; Defez et al., 2017), because nitrogen and indole-3-acetic acid are the two most essential factors that have a strong impact on growth and yield of plants.

Citation: Xa LT, Nghia NK, Tecimen HB. Environmental factors modulating indole-3-acetic acid biosynthesis by four nitrogen fixing bacteria in a liquid culture medium. Environ. Nat. Resour. J. 2022;20(3):279-287. (https://doi.org/10.32526/ennrj/20/202100233)

In a previous study, Xa and Nghia (2019) isolated and selected strains of bacteria that possess significant nitrogen fixation and IAA synthesis functions to be tools for agricultural production. However, it was suggested that environmental factors greatly influence their ability to activate IAA synthesizing functions (Scarcella et al., 2017; Bhutani et al., 2018). Therefore, to ensure the efficacy of these strains when they are inoculated in the greenhouse and field conditions, the environmental and cultural conditions of these strains should be well known. Therefore, within this study, we examined the IAA production capacities of four different selected bacteria against various amended culture mediums in the laboratory. Our findings are expected to broaden the knowledge over the natural and environmentally friendly solutions for sustainable agricultural development under environmental stresses.

2. METHODOLOGY

2.1 Microorganism source

Four nitrogen fixing bacteria, namely *Paenibacillus cineris* TP-1.4, *Bacillus megaterium* MQ-2.5, *Klebsiella pneumoniae* OM-17.2, and *Pseudomonas boreopolic* CP-18.2 were isolated and selected from indigenous microorganisms of different cropping systems in Soc Trang Province, Vietnam (Xa and Nghia, 2019). Four bacterial isolates were maintained in N-free Burks liquid medium under laboratory conditions. N-free Burks liquid medium contained sucrose 10 g, $K_2HPO_4 \cdot 4H_2O$ 0.41 g, KH_2PO_4 1.05 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.015 g, H_3BO_3 0.0025 g, and Mo 0.0025 g per liter (Mehta and Nautiyal, 2001).

2.2 Environmental factors on IAA production

2.2.1 Effect of different carbon sources on IAA synthesis by four bacterial strains in Burks liquid medium

Five different carbon sources composed of fructose, glucose, glycerol, mannose and sucrose at the concentration of 1% were considered as different treatments and the control treatment was without bacteria. Each treatment had three replicates, corresponding to three different incubation flasks. Bacterial strains were grown in 100 mL Erlenmeyer flasks containing 50 mL fresh N-free Burks liquid medium for four days. Then, an aliquot of 300 µL bacterial suspension was transferred to 50 mL Erlenmeyer flasks containing 30 mL fresh N-free Burks liquid medium, pH 7, 100 mg/L tryptophan and different carbon sources as different treatments to achieve bacterial numbers of 10^6 CFU/mL. The flask samples were put on an orbital shaker at 100 rpm in the dark and under laboratory conditions for ten days. The synthesized IAA concentrations were determined after 0, 2, 4, 6, 8, and 10 days of incubation by Salkowski's reagent method at a wavelength of 530 nm followed by the modified method described by Bric et al. (1991).

2.2.2 Effect of different pH values on IAA synthesis

To determine the effect of different pH values of N-free Burk's medium on IAA production, an experiment was conducted at pH values of 3, 5, 7, 8, and 9. Each pH level had their own corresponding control treatment without bacterial inoculation. The carbon form providing the highest IAA production from the results of the test in section 2.2.1 was added as an energy source to the bacteria. Concentration of IAA production was measured at 0, 2, 4, 6, 8, and 10 days after inoculation according to Bric et al. (1991).

2.2.3 Effect of different NaCl concentrations

The experiment was established to evaluate the effect of different concentrations of NaCl on IAA production of four bacterial strains. The 0%, 1%, 2%, 3%, 4%, and 5% NaCl concentration series was used as individual treatments. Each NaCl concentration treatment accordingly had a corresponding control treatment without microbial inoculation. The best pH value and carbon source from the results of the tests in section 2.2.1 and 2.2.2 were applied in the liquid culture medium of this experiment. The synthesized IAA concentrations were determined after 0, 2, 4, 6, 8, and 10 days of incubation by the method of Bric et al. (1991).

2.2.4 Effect of different concentrations of tryptophan

This experiment was conducted to find out the best concentration of tryptophan added in N-free Burks liquid medium for optimizing IAA production using tryptophan concentrations of 100, 200, 300, 400, and 500 mg/L. The control treatment was without tryptophan with pH 7. Each IAA concentration treatment, accordingly, had a corresponding control treatment without microbial inoculation. The optimal pH, carbon source, and NaCl concentration achieved from section 2.2.1, 2.2.2, and 2.2.3 were adjusted in the cultured medium of this experiment.

2.3 Data analysis

The data were analyzed by ANOVA with MINITAB software with 16.2 versions.

3. RESULTS AND DISCUSSION

3.1 Effects of different carbon sources on IAA production synthesized by four bacterial isolates

The results presented in Figure 1 reveal that the different carbon sources caused variation in the production of IAA by four bacterial isolates. During the experiment period, sucrose was observed to be the best carbon source at supporting the four bacterial strains to boost the IAA synthesis, and OM-17.2 was the highest IAA producing strain compared to the other three isolates. The OM-17.2 strain reached its maximum IAA production amount after four days of incubation at 93.9 mg IAA/L in the sucrose amended treatment, followed by the treatments with glycerol, glucose, mannose, and fructose. Two other strains, MQ-2.5, and CP-18.2 synthesized up to 32.8 mg/L and 43.0 mg/L IAA in sucrose added medium after 6 and 4 days of incubation, respectively, slightly higher than glucose, glycerol, mannose, and fructose added treatments. The TP-1.4 strain reached its highest IAA production rate of 26.3 mg/L in the sucrose added treatment after the 10 days incubation. To sum up, from this result, sucrose can be considered as the best carbon source for the four tested bacteria to synthesize at a worthy amount of IAA in liquid culture.

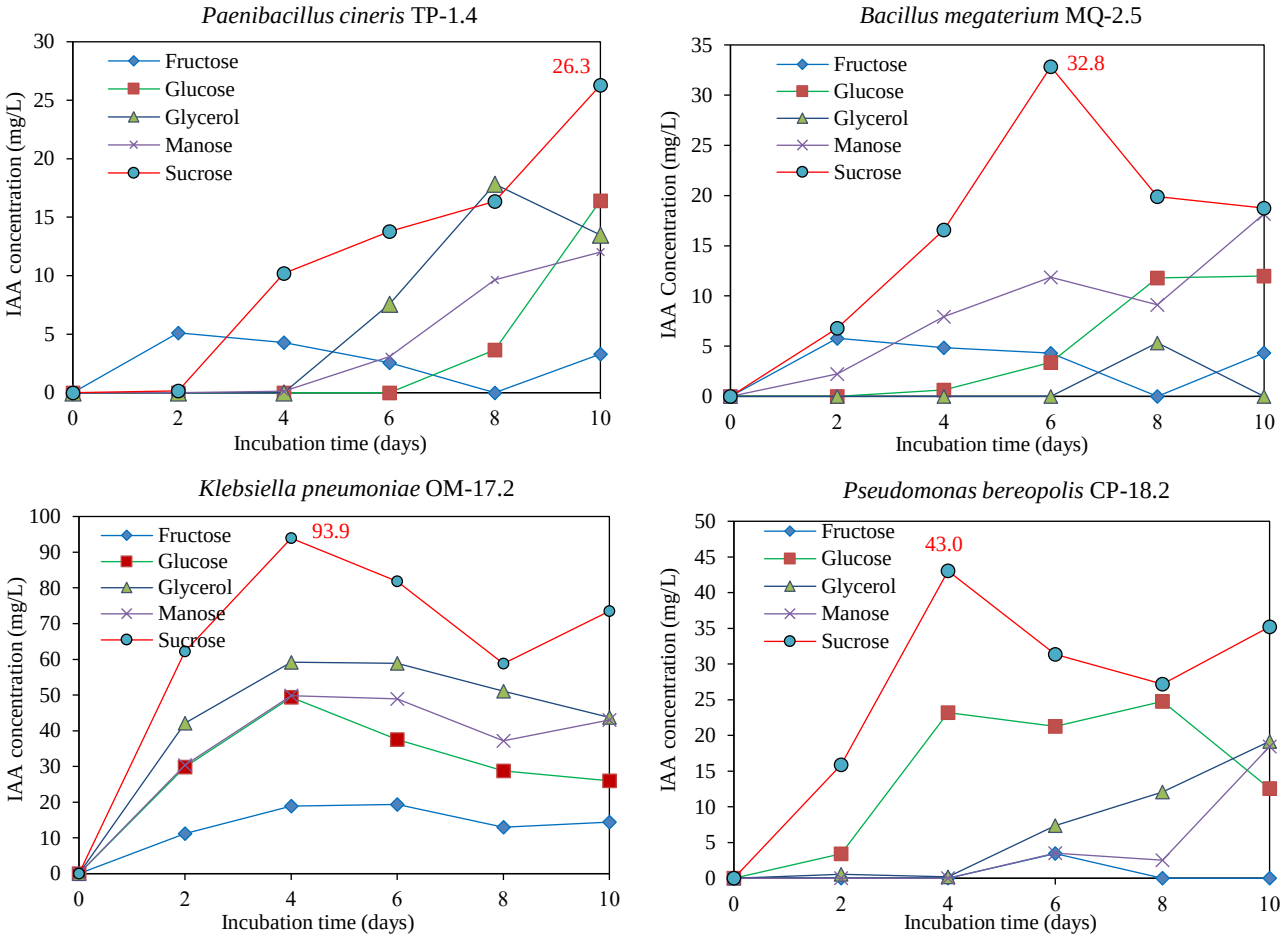

Figure 1. Effects of different carbon sources on the IAA production by four bacterial strains in Burks liquid medium during 10 days of incubation

This result is consistent with the study of Nutaratat et al. (2015) who recorded that the strain of *Rhodosporidium paludigenum* synthesized the highest amount of IAA in liquid culture medium containing 1% sucrose compared to arabinose, dextrose, fructose, galactose, glycerol, lactose, maltose, mannitol, mannose, my-inositol, raffinose, sorbitol, sorbose, starch, xylitol, and xylose. Similarly, Kucuk and Cevheri (2016) also detected that among glucose, fructose, mannitol, and sucrose, the highest IAA synthesis by the strain of *Rhizobium* sp. P2 was found to be in sucrose-amended culture medium. In this case,

it is clear that sucrose is the best carbon source for IAA synthesis. However, in the study of Scarcella et al. (2017) on *Trichosporon asahii*, a yeast strain showed that IAA production in the sucrose added treatment was superior at pH 6.0, but the pH of 4.5 in this study gave the highest IAA production in the glucose added treatment. These results indicate an association between the carbon source and medium pH. For *Rhodotorula mucilaginosa*, the highest IAA production was observed at pH 6.0 with glucose. In a research study of Bhutani et al. (2018) on *Bacillus aryabhattai*, the MBN3, MJHN1, and MJHN10 strains, the most suitable carbon sources were found to be mannitol, sucrose, and glucose, respectively. This result is consistent with the results of Wagi and Ahmed (2019) for *Bacillus cereus* (So3II) and *B. subtilis*. Alfonso et al. (2021) also noted that although the highest growth rate was achieved when glucose was the carbon source, the lowest IAA concentration was found in the glucose added medium. Upon the findings from reviewed studies, we can postulate that different types of sugar sources in a media have basal differences due to varied utilization of sugars by bacteria during their growth (Shanti et al., 2007; Sridevi et al., 2008).

3.2 Effects of different pH levels

The IAA production performances by four bacterial strains in different pH levels are presented in Figure 2. It was clear that, for *Paenibacillus cineris* TP-1.4 and *Klebsiella pneumoniae* OM-17.2 strains, the best pH value for IAA synthesis was pH 7, while for the two others was pH 8. The TP-1.4 and OM-17.2 strains showed their maximum IAA production after eight days of incubation with a value of 33.8 and 76.6 mg/L, respectively. The MQ-2.5, and CP-18.2 strains performed the maximum IAA production at rates 40.0, and 25.0 mg/L after eight and 10 days of incubation, respectively. The IAA production by four bacterial strains showed the same trend as following the order as pH 8>pH 9>pH 5>pH 3. It was clear to see that under the acidic environment of liquid culture (pH=3, and pH=5) the IAA production of bacterial strains decreased dramatically.

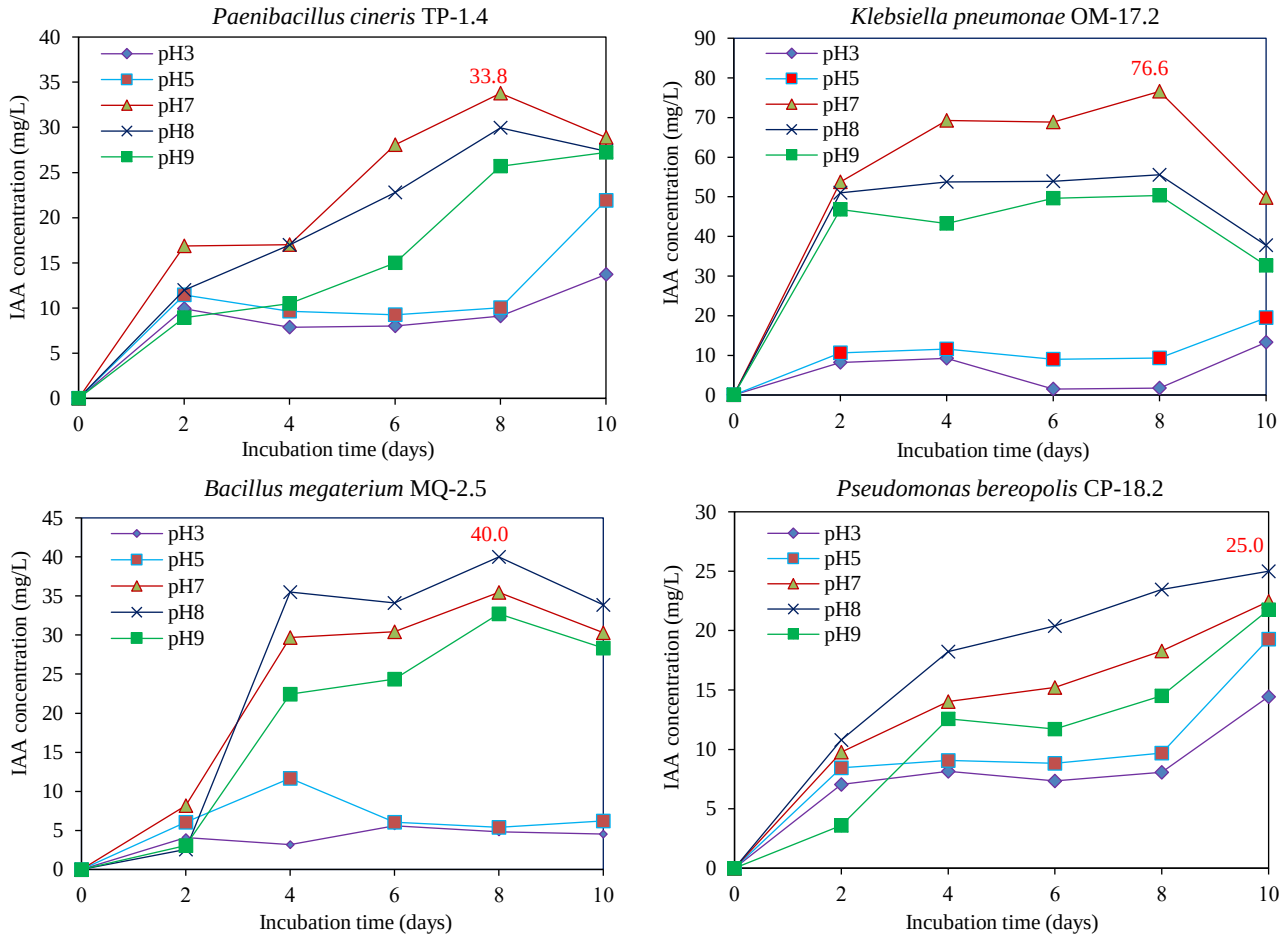

Figure 2. Effects of different pH levels of liquid medium on the IAA production by four bacterial strains in Burks liquid medium during 10 days of incubation

Our results showed similarity with findings of Nutaratat et al. (2015) who suggested that pH 7 was suitable for IAA synthesizing performance of *Rhodosporidium paludigenum*. In addition, Khamna et al. (2010) reported that, pH 7.0 was suitable for maximum IAA production by *Streptomyces* sp. or *Bacillus subtilis* (Kumari et al., 2018), pH 7.2 was the optimum range for IAA production of *Rhizobium* strain VMA 301 (Mandal et al., 2007), pH 7.5 was best for *Pseudomonas putida* UB-1 (Bharucha et al., 2013) and pH 7.2 was the optimum IAA production range (Shanti et al., 2007). However the best IAA production was found at pH 8 (Sachdev et al., 2009). In the study by Chandra et al. (2018), three different isolates were tested and the isolate CA2003 showed maximum IAA production at pH 5 and decreased towards the pH range of 6-9; while CA2001 was opposite. The maximum IAA production of this strain was observed at pH 9 and CA2004 produced maximum IAA at pH 6, but lower IAA production was recorded at pH 5, 7, 8, and 9. Overall, the acid medium is not suitable for IAA synthesis of bacteria (Ona et al., 2005; Mohite, 2013).

3.3 Effects of different sodium chloride concentrations

The results of the effects of different NaCl concentration on the IAA production are presented in Figure 3. It is obvious that the highest IAA production by strains MQ-2.5 and OM-17.2 was found in the 1% NaCl added treatments, while the highest IAA production by strains TP-1.4 and CP-18.2 was found in the no NaCl added treatment. For both with and without NaCl treatments, the most efficient IAA synthesis was done by OM-17.2. The highest amount of IAA production was recorded in the 1% NaCl added and no NaCl added treatments after the fourth day of incubation at rates 97.2 mg/L and 82.7 mg/L, respectively. However, with increasing NaCl concentration, the synthesized IAA amount by the strain OM-17.2 decreased accordingly from 97.2 mg/L to 80.1, 57.8, 32.8, and 10.1 mg/L in treatments supplied with 2%, 3%, 4%, and 5% NaCl, respectively.

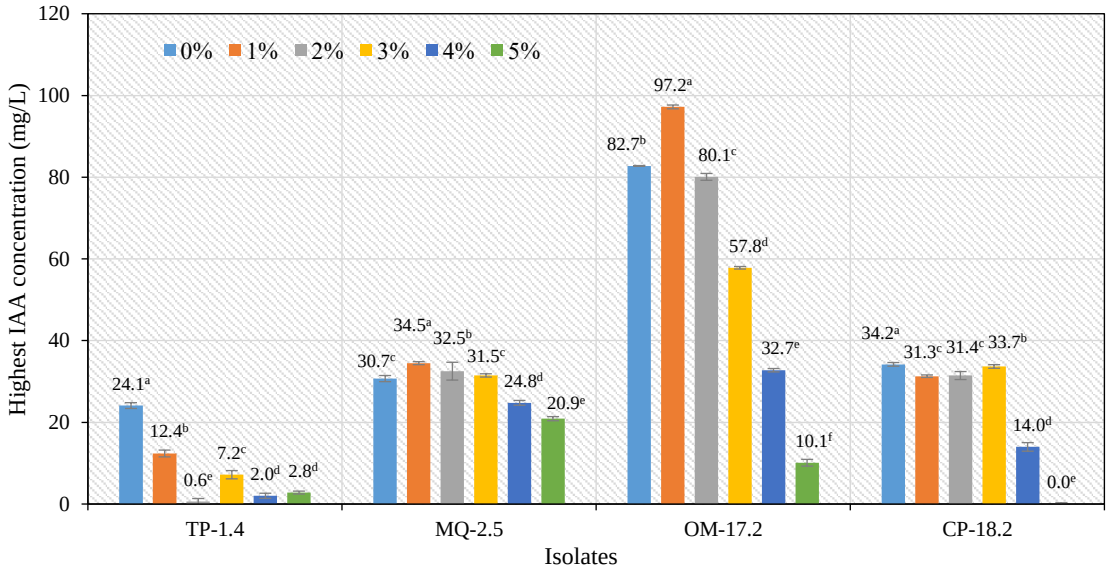

Figure 3. The highest concentration of IAA in Burks liquid medium synthesized by four bacterial strains in the different NaCl concentrations

Although the MQ-2.5 strain did not produce IAA as high as the strain OM-17.2, the amount of IAA produced by MQ-2.5 varied between 20.9 and 34.5 mg/L. The highest IAA amount synthesized by MQ-2.5 was in the treatment of 1% NaCl, however with gradually increased NaCl content to 2, 3, 4, and 5%, the synthesis of IAA decreased very slightly and still remained at a high level (over 20 mg/L IAA in the treatment of 5% NaCl). In short, under the NaCl stress, the MQ-2.5 strain showed the optimum IAA synthesis at 1% NaCl treatment and it tolerated NaCl concentration up to 5% which allowed MQ-2.5 to be assessed as a halotolerant bacteria (Willey et al., 2009). Turning to the strain CP-18.2, the results showed its best capacity of IAA synthesis in the treatment of 3% NaCl supplementation. However, under 5% NaCl conditions, this bacterial strain was completely suppressed in producing IAA. Unfortunately, with the

presence of NaCl in liquid culture, the IAA synthesizing process by strain TP-1.4 was strongly inhibited while the content of IAA produced by this strain was found to be highest at the treatment without NaCl addition (24.1 mg/L). However, when NaCl concentration in the liquid culture increased to 1%, 2%, 3%, 4%, and 5%, the IAA content synthesized by strain TP-1.4 sharply decreased.

The halophilic bacteria identified as *Bacillus megaterium* ST2-9 synthesized the highest amount of IAA at the treatment supplied with 3% NaCl (Nghia et al., 2017). Similarly, Sarkar et al. (2018) observed a sharp decline in IAA production produced by *Enterobacter* sp. P23 strain with gradually increasing NaCl concentration (160 µg/mL at 150 mM NaCl and <20 µg/mL at 600 mM NaCl). Egamberdiyeva (2009) reported that IAA-producing bacteria supported plant growth under salt stress with a significantly increased plant growth. Additionally, Nakbanpote et al. (2014) proved that *Pseudomonas* sp. PDMZnCd2003 isolated from a Zn/Cd contaminated soil was classified as a salt-tolerant bacteria. This strain also indicated a good capacity of IAA synthesis, biological nitrogen fixation, and phosphate solubilization in liquid medium containing 8% (w/v) NaCl. Smith (2000) inferred that, IAA was an auxin hormone required by most plant cells for proliferation and root initiation. Badawy et al. (2021) showed that *Aspergillus ochraceus* produced 146 and 176 µg/mL IAA in a medium provided with 15 and 30% seawater, respectively. Therefore, this fungus was considered as a plant growth promoting and salt tolerant agent under seawater irrigation conditions. From the result of this experiment we can conclude that three strains, MQ-2.5, OM-17.2, and CP-18.2 can be considered as salt tolerant plant growth promoting bacteria. This had implications for agriculture in the Mekong Delta region of Vietnam where it is currently affected by salinity and drought.

3.4 Effects of different L-tryptophan concentrations

The test results to evaluate the effects of different L-tryptophan concentrations on IAA synthesizing ability of four bacterial strains presented in Figure 4 manifested that all four isolates preferred tryptophan for IAA production. Maximum IAA production was found in the treatments amended with 500 mg/L tryptophan for MQ-2.5, and CP-18.2, 400 mg/L tryptophan for TP-1.4, and 300 mg/L tryptophan for OM-17.2. Among four bacterial strains, CP-18.2 was found to be the most sensitive with tryptophan precursor. In the 100 mg/L tryptophan added treatment, the synthesized IAA was 32 mg/L, with a dramatic increase in the 500 mg/L tryptophan added treatment (128 mg/L IAA). A similar trend was observed for the MQ-2.5 strain with the highest IAA concentration of 84.6 mg/L. In particular, the OM-17.2 strain showed its highest concentration of IAA (123.4 mg/L) in the 300 mg/L tryptophan treatment, however, the IAA content dropped sharply when tryptophan content increased to 400 or 500 mg/L. The IAA synthesis of TP-1.4 was less affected by the tryptophan content than the other three isolates and TP-1.4 did not synthesize IAA as good as the other three bacteria under the same tryptophan supplementation conditions.

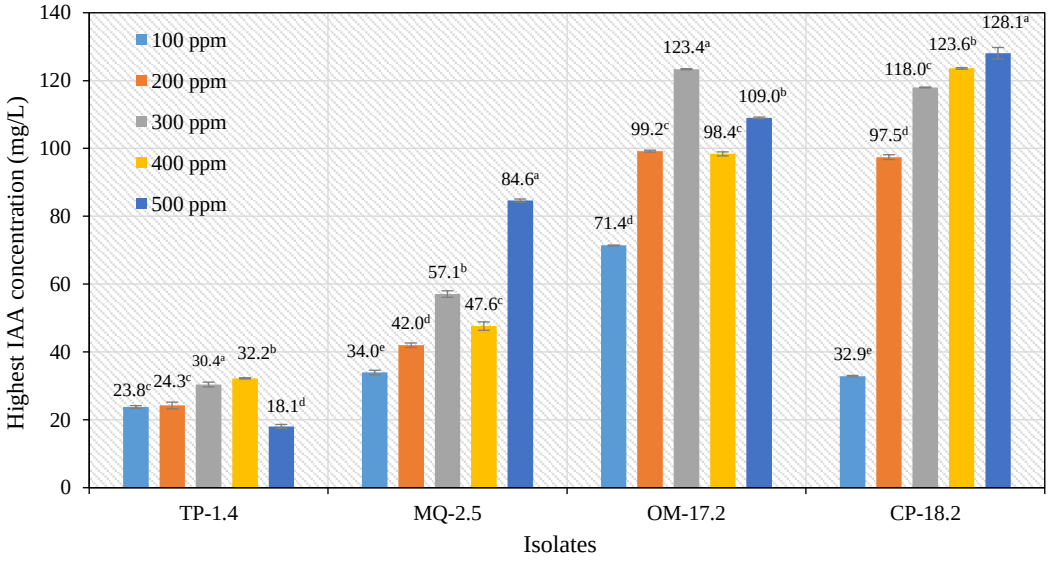

Figure 4. The highest concentrations of IAA synthesized by four different bacterial strains in Burks liquid medium containing different tryptophan concentrations

The IAA synthesis behaviors of four isolates when cultivated in Burks media supplied with or without tryptophan were varied. No IAA production at tryptophan-free medium for all four bacterial strains proved the dependency of those isolates on the tryptophan pathway for IAA synthesis.

Our results showed consistency with Ahmad et al. (2005) who tested IAA synthesizing capacity of 10 *Azotobacter* spp. strains and 11 strains of *Pseudomonas* sp. in liquid media containing 0, 1, 2, and 5 mg/mL tryptophan and observed lower IAA (2.68-10.80 mg/mL) yield in the liquid media without tryptophan addition. Seven *Azotobacter* strains produced IAA at ranges between 7.3 to 32.8 mg/mL and *Pseudomonas* sp. strains produced 41.0 to 53.2 mg/mL with 5 mg/mL tryptophan. In addition, Ahmad et al. (2008) showed that *Azotobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. strains could not synthesize IAA properly without tryptophan, and they showed their highest IAA production ranged from 7.03 µg/mL to 22.02 µg/mL when the culture medium included 500 µg/mL tryptophan. Bhutani et al. (2018) indicated a gradual increase in the IAA production with an increase in L-tryptophan concentration. *Bacillus aryabhat* MBN3 and *Bacillus aryabhat* MJHN1 had a maximum IAA production in the treatment provided with 500 µg/mL tryptophan while *Bacillus aryabhat* MJHN10 produced the highest IAA in the treatment added with 300 µg/mL tryptophan. The study of Suliasih and Widawati (2020) illustrated that maximum IAA production by *Bacillus siamensis* was achieved after 96 h of incubation in a medium supplemented with 250 µg/mL of tryptophan, pH 8 and sucrose as carbon. In addition, Mohite (2013) suggested that IAA was not produced in negligible quantities of L-tryptophan. There were significantly different demand levels of L-tryptophan for varying microorganisms. For many bacteria, the conversion of tryptophan into IAA is of uppermost importance (Costacurta and Venderleyden, 1995). Manulis et al. (1994) reported that various *Streptomyces* spp. could secrete IAA when fed with tryptophan, while according to the results of Swain et al. (2007), IAA producing *Bacillus* spp. were tryptophan dependent and several other bacteria strains as well (Patten and Glick, 2002).

The results of four experiments in this study allowed us to conclude that different strains of bacteria also have different abilities in synthesizing IAA, and sucrose is the best carbon source for IAA synthesis for all four bacterial strains. In particular, the OM-17.2 strain has the highest ability to synthesize IAA in an environment with pH 7, 1% NaCl, and 300 mg/L tryptophan. Strain CP-18.2 showed optimum IAA production at pH 8, and 500 mg/L tryptophan. Notably, the MQ-2.5 strain was a salt-tolerant bacteria and can synthesize IAA in saline conditions up to 5% and this strain produced the highest IAA under pH 8, NaCl 1%, and 500 mg/L tryptophan. Meanwhile, the TP-1.4 strain had a stable IAA production and was least affected by environmental conditions. It produces the highest concentration of IAA in liquid culture medium under pH 7, without and with 400 mg/L tryptophan.

4. CONCLUSION

In summary, the environmental factors of the culture medium such as pH, carbon source, NaCl, and tryptophan concentration strongly influenced the IAA synthesizing capacity of four bacterial isolates. Under the low pH culture medium the IAA synthesizing capacity of the bacterial strains was reduced and increased under high pH culture medium (pH=7 and pH=8). Sucrose was the best carbon source to promote an increase of the IAA synthesis for all four strains. *Bacillus megaterium* MQ-2.5 was considered as the best salt tolerant plant growth promoting bacteria and tryptophan precursors also played an important role in enhancing IAA synthesis by all four strains. All four bacterial strains in this study emerged as potential alternatives for IAA production and they could be applied in the greenhouse and field conditions as bio-inoculants for more sustainable field production. In particular, two strains OM-17.2 and CP-18.2 can be used to produce safe biological IAA products to replace synthetic IAA used widely in agriculture.

ACKNOWLEDGEMENTS

This research was funded by Can Tho University Upgrade Project VN14-P6 with an ODA-A7 loan from the Japanese Government.

REFERENCES

Ahmad F, Ahmad I, Khan MS. Indole acetic acid production by the indigenous isolates of *Azotobacter* and *Pseudomonas fluorescent* in the presence and absence of tryptophan. Turkish Journal of Biology 2005;29(1):29-34.

Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 2008;163(2):173-81.

Alfonso FC, Vigueras-Ramírez G, Rosales-Colunga LM, Monte-Martínez A, Hernández RO. Propionate as the preferred

carbon source to produce 3-indoleacetic acid in *B. subtilis*: Comparative flux analysis using five carbon sources. Molecular Omics 2021;17:554-64.

Badawy AA, Alotaibi MO, Abdelaziz AM, Osman MS, Khalil AMA, Saleh AM, et al. Enhancement of seawater stress tolerance in barley by the endophytic fungus *Aspergillus ochraceus*. Metabolites 2021;11:Article No. 428.

Bharucha U, Trivedi UB, Patel K. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on Mustard (*Brassica nigra*). Agricultural Research 2013;2(3):215-22.

Bhutani N, Maheshwari R, Negi M, Suneja P. Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. Israel Journal Plant Science 2018;65(1-2):83-96.

Bric JM, Bostock RM, Silverstone SE. Rapid insitu assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Applied and Environmental Microbiology 1991;57:535-8.

Chandra S, Askaria S, Kumaria M. Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. Journal of Genetic Engineering and Biotechnology 2018;16:581-6.

Costacurta A, Vanderleyden J. Synthesis of phytohormones by plant associated bacteria. Critical Reviews in Microbiology 1995;21:1-18.

Defez R, Andreozzi A, Bianco C. The overproduction of indole-3-acetic acid (IAA) in endophytes upregulates nitrogen fixation in both bacterial cultures and inoculated rice plants. Microbial Ecology 2017;74(2):441-52.

Egamberdiyeva D. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiologiae Plantarum 2009;31:861-4.

Khamna S, Yokota A, Peberdy JF, Lumyong S. Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsian Journal of BioSciences 2010;4:23-32.

Kucuk C, Cevheri C. Indole acetic acid production by *Rhizobium* sp. isolated from Pea (*Pisum sativum* L. ssp. arvense) Bezelye (*Pisum sativum* L. ssp. arvense)'den İzole edilen *Rhizobium* sp. Tarafından İndol Asetik Asit Üretimi. Turkish Journal of Life Sciences 2016;1(1):43-5.

Mandal SK, Mondal KC, Dey S, Pati BR. Optimization of cultural and nutritional conditions for indole-3-acetic (IAA) production by a *Rhizobium* sp. isolated from root nodules of *Vigna mungo* (L.) Helpper. Research Journal of Microbiology 2007;2:239-46.

Manulis S, Shafri RH, Epstein E, Lichter A, Barash I. Biosynthesis of Indole 3-acetic acid via the indole 3-acetamide pathway in *Streptomyces* spp. Microbiology 1994;140:1045-50.

Mehta S, Nautiyal CS. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiology 2001;43:51-6.

Mohite B. 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 2013;13(3):638-49.

Nakbanpote AW, Panitlurtumpaia N, Sangdeea A, Sakulponea N, Sirisoma P, Pimthong A. Salt-tolerant and plant growth promoting bacteria isolated from Zn/Cd contaminated soil: Identification and effect on rice under saline conditions. Journal of Plant Interactions 2014;32:37-41.

Nghia NK, Tien TTM, Oanh NTK, Nuong NHK. Isolation and characterization of indole acetic acid producing halophilic bacteria from salt affected soil of rice-shrimp farming system in the Mekong Delta, Vietnam. Agriculture, Forestry and Fisheries 2017;6(3):69-77.

Nutaratat P, Amsri W, Srisuk N, Arunrattiyakorn P, Limtong S. Indole-3-acetic acid production by newly isolated red yeast *Rhodosporidium paludigenum*. Journal of General and Applied Microbiology 2015;61:1-9.

Ona O, Impe JV, Prinsen E, Vanderleyden J. Growth and indole-3-acetic acid biosynthesis of *Azospirillum brasilense* Sp245 is environmentally controlled. FEMS Microbiology Letters 2005;246:125-32.

Patten CL, Glick BR. Role of *Pseudomonas putida* indo lactic acid in development of the host plant root system. Applied and Environmental Microbiology 2002;68:3795-801.

Sachdev DP, Chaudhari HG, Kasture VM, Dhavale DD, Chopade BA. Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumonia* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Indian Journal of Experimental Biology 2009;47:993-1000.

Sarkar A, Ghosh PK, Pramanik K, Mitra S, Sorent, Pandey S, et al. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Research in Microbiology 2018;169:20-32.

Scarcella ASA, Junior RB, Bastos RG, Magri MMR. Temperature, pH and carbon source affect drastically indole acetic acid production of plant growth promoting yeasts. Brazilian Journal of Chemical Engineering 2017;34(02):429-38.

Shanti M, Keshab C, Dey S. Optimization of cultural and nutritional conditions for indole acetic acid production by a *Rhizobium* sp. isolated from root nodules of *Vigna mungo* (L.) Hepper. Research Journal of Microbiology 2007;2:239-46.

Shokri D, Emtiazi G. Indole-3-acetic acid (IAA) production in symbiotic and non-symbiotic nitrogen-fixing bacteria and its optimization by Taguchi design. Current Microbiology 2010;61(3):217-25.

Sirivastava A, Singh A. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. International Journal of Agricultural, Science and Research 2017;7(4):2250-321.

Smith H. Phytochromes and light signal perception by plants: An emerging synthesis. Nature 2000;407:585-91.

Kumari S, Prabha C, Singh A, Kumari S, Kiran S. Optimization of indole-3-acetic acid production by diazotrophic *B. subtilis* DR2 (KP455653), isolated from rhizosphere of *Eragrostis cynosuroides*. International Journal of Pharma Medicine and Biological Sciences 2018;7(2):20-7.

Sridevi M, Yadav NCS, Mallaiah KV. Production of indole acetic acid by *Rhizobium* isolates from *Crolatariaspecies*. Research Journal of Microbiology 2008;3(4):276-81.

Stinner DH. The science of organic farming. In: Lockeretz W, editor. Organic Farming: An International History. Oxfordshire, UK and Cambridge, Massachusetts: CAB International; 2007. p. 978-1000.

Suliasih, Widawati S. Isolation of Indole Acetic Acid (IAA) producing *Bacillus siamensis* from peat and optimization of the culture conditions for maximum IAA production. IOP Conference Series: Earth and Environmental Science (The 9th International Symposium for Sustainable Humanosphereer) 2020;572:12-25.

Swain MR, Naskar SK, Ray RC. Indole 3-acetic acid production and effect on sprouting of yam. (*Dioscorea rotundata* L.)

minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. Polish Journal of Microbiology 2007;56:103-10.

Tewari S, Arora NK. Multifunctional exopolysaccharides from *Pseudomonas aeruginosa* PF23 involved in plant growth stimulation, biocontrol and stress amelioration in sunflowers under saline conditions. Current Microbiology 2014;69(4): 484-94.

Wagi S, Ahmed S. *Bacillus* spp.: potent microfactories of bacterial IAA. Peer Journal 2019;7:e7258.

Willey JM, Sherwood LM, Woolverton CJ. Prescott's Principles of Microbiology. New York, USA: McGraw-Hill; 2009.

Xa LT, Nghia NK. Isolation and selection of biological nitrogen fixing and indole-3-acetic acid synthesizing bacteria from different cropping systems in Soc Trang Province, Vietnam. International Journal of Innovative Studies in Sciences and Engineering Technology 2019;5(11):15-23.