

## ISOLATION AND IDENTIFICATION OF INDOLE ACETIC ACID PRODUCING BACTERIA FROM THE COASTS OF BEN TRE AND TRA VINH PROVINCES

Nguyen Ngoc Lan<sup>1,2</sup>, Vu Van Dung<sup>1,2,3</sup>, Nguyen Thi Kim Lien<sup>1</sup>,  
Nguyen Kim Thoa<sup>4</sup>, Do Huu Nghi<sup>5</sup>, Nguyen Huy Hoang<sup>1,\*</sup>

<sup>1</sup>Institute of Genome Research, VAST, Vietnam

<sup>2</sup>Graduate of Science and Technology, VAST, Vietnam

<sup>3</sup>Institute of Chemistry and Materials, Academy of Military Science and Technology, Vietnam

<sup>4</sup>Institute of Biotechnology, VAST, Vietnam

<sup>5</sup>Institute of Natural Products Chemistry, VAST, Vietnam

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### ABSTRACT

Beneficial plant-growth-promoting bacteria (PGPB) have been reasonably applied to rescue crucial issue for agriculture by salinity soil. Observed most of PGPB was found in endophyte, rhizosphere and soil. Indole acetic acid (IAA)-producing bacteria could naturally stimulate and facilitate plant growth. The knowledge of IAA production and content of bacteria resident in the marine environment has been typically insufficient and limited to date. In recent years, unwarrantable intrusions of sea water have been enlarged in the Mekong River Delta of Vietnam, threatening productive rice fields, local fruits, and cash crops. Therefore, finding PGPB in the coastal regions in the Mekong River Delta as a creative resource for sustainable agriculture is necessary and is a prompt challenge. In this study, IAA-producing bacteria from coastal regions of Ben Tre and Tra Vinh Provinces were isolated and adequately identified. Out of 202 bacterial isolates, 10 isolates showed the possible ability to produce IAA from L-tryptophan. These 10 isolates were objectively evaluated the capacity to produce IAA under 5% (w/v) NaCl in King B and marine broths. The results revealed that IAA production decreased in 5% NaCl, even though bacterial growth increased. These 10 IAA-producing bacteria were classified at the species level, *Marinobacter hydrocarbonoclasticus*, *M. pelagius*, *M. daeipoensis*, and *Mameliella phaeodactyli* by 16S rRNA gene analysis. The most IAA producer in King's B broth, the isolate C7, was investigated in more detail. The isolate C7 produced the maximum IAA amount ( $192.2 \pm 1.14$   $\mu\text{g/ml}$ ) under the presence of 20 g/l yeast extract, 2 g/l of L-tryptophan and 1% NaCl. The isolate C7 was able to grow at 1–17% (w/v) NaCl (optimum, 4%), but not in the absence of NaCl, indicating it is a moderate halophilic bacterium. This study highlighted the considerable ability to produce IAA of marine bacteria, which could be thoughtfully considered to use naturally as biofertilizers to promote plant growth in saline intrusion lands.

**Keywords:** IAA producing bacteria, marine, *Marinobacter*, *Mameliella*, C7, halophile.

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\*Corresponding author email: [nhhoang@igr.ac.vn](mailto:nhhoang@igr.ac.vn)/[hoangibt@yahoo.com](mailto:hoangibt@yahoo.com)

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## INTRODUCTION

Indole acetic acid (IAA) represents the member of the auxin group that have the ability to improve plant growth by stimulating cell elongation and division, tissue differentiation, seed germination and seedling growth (Prinsen et al., 1993). Indole acetic acid is synthesized in many species of many bacteria, fungi, algae, and plants (Tsavkelova et al., 2006; Fahad et al., 2015). The synthesis of IAA in bacteria has been known for a long time that ordinarily occurs via multiple pathways as has been typically observed in plants. Tryptophan carries out a crucial role as the primary precursor for the synthesis of IAA so that the addition of tryptophan to culture media results in higher IAA production (Glickmann & Dessaux, 1995). Bacteria can synthesize IAA via tryptophan-dependent or -independent pathways. There are at least five distinct pathways for the synthesis of IAA from tryptophan (Spaepen et al., 2007). These possible pathways were identified based on intermediates. The indole-3-acetamide (IAM) pathway is the most popular pathway in bacteria. Tryptophan is firstly converted to IAM by enzyme tryptophan-2-monooxygenase. Subsequently, IAM is efficiently converted to IAA by IAM hydrolase. This specific pathway has been illustrated in some bacteria, like *Agrobacterium tumefaciens* (Mashiguchi et al., 2018), *Arthrobacter pascens* (Li et al., 2018) *Pseudomonas chlororaphis* (Dimkpa et al., 2012), and *Bradyrhizobium* spp. (Sekine et al., 1988). The indole-3-pyruvate (IPyA) pathway is observed in plant growth promoter bacteria, like *Pseudomonas putida* (Patten & Glick, 2002) and *Rhizobium tropici* (Imada et al., 2017). Initially, the intermediate IPyA is transferred from tryptophan by aminotransferase. Then, IPyA is converted to indole -3- acetaldehyde (IAAld) by indole-3-pyruvate decarboxylase and finally IAAld is oxidized to IAA. Three remaining pathways consisting of tryptamine, tryptophan side-chain oxidase and indole-3-acetonitrile have been identified in some bacteria, such as *Azospirillum brasiliense* (Bar & Okon, 1993), *Pseudomonas fluorescens* (Oberhänsli et al.,

1991), and *Rhizobium* spp. (Kobayashi et al., 1995), respectively.

Sea water has been intruded into many coastal areas in the Mekong River Delta of Vietnam, threatening productive rice fields, fruit, cash crops, and aquaculture (CGIAR Research Centers in Southeast Asia, 2016). Estimating, 25,900 ha of 400,000 ha of cropland was left fallow. Rice areas affected by drought and salinity intrusion rapidly increased from 139,000 ha in mid-March 2016 to 224,552 ha by mid-April 2016. Salinity intrusion have also affected 13,000 ha of cash crops, 25,500 ha of fruit trees and 14,400 ha of aquaculture. Therefore, finding the plant growth promoting bacteria in the coastal regions in the Mekong River Delta of Vietnam as a creative resource for agriculture is necessary and challenge. The marine environments contain a remarkably diverse microorganisms, and they retain unique properties to adapt to harsh condition like high salt concentration with limited nutrition (De Carvalho & Femandes, 2010). Marine microorganisms hold many applications in biotechnology, such as production of enzymes (Mohapatra et al., 2003; Trincone, 2011) and pharmaceutical substances (Blunt et al., 2018; Calado et al., 2018). Up to date, only a few published studies have inspected plant growth promoting activities of marine bacteria. Bacteria *Pseudomonas* spp. olive green (OG) isolated from marine water of the Gulf of Khambhat showed an ability to produce 29 µg/ml IAA (Goswami et al., 2013). *Pseudomonas aeruginosa* strain BS8 produced 19 µg/ml IAA in the medium supplemented with 500 µg/ml L-tryptophan (Goswami et al., 2015). Nayomi & Thangavel (2015) found 14 isolates which are able to produce IAA in the necessary presence of L-tryptophan. Therefore, isolation of IAA-producing salt-tolerant marine bacteria may be one of the creative methods by which to alleviate the environmental problem of salinity and promoting the plant growth under salinity. The aim of this study was to investigate the IAA producing bacteria from the coastal regions of Ben Tre and Tra Vinh Provinces, Vietnam.

## **MATERIALS AND METHODS**

### **Isolation and screening of IAA producing bacteria**

Six soil samples and six water samples were carefully collected from the coastal regions in Ben Tre Province (9.8334 N, 106.6597 E) and Tra Vinh Province (9.6199 N, 106.5592 E), Vietnam on April 27-28, 2017. Ten grams of sand or 10 ml of water were mixed with 90 ml sterile distilled water in 250 ml flask and shaken at 160 rpm for 10 min. The suspensions were serially diluted to  $10^{-5}$  using sterile saline solution. An aliquot of 100  $\mu$ l diluted samples was spread onto the marine agar medium (Gellix, South of Korea) supplemented with 5% (w/v) NaCl. The plates were incubated at 30°C for up to 3 days. Single colonies were selected and streaked on sterile marine agar plates to properly obtain a pure culture. Bacterial isolates were stored at -80°C in marine broth supplemented with 25% glycerol or slant agar at 4°C.

Ability to produce IAA of isolates was determined qualitatively following the methods described by Glickmann & Dessaux (1995), using King's B broth [Per liter: peptone 20 g; glycerol 10 ml;  $K_2HPO_4 \cdot 3H_2O$  1.5 g;  $MgSO_4 \cdot 7H_2O$  1.5 g] supplemented with 1 g/ml L-tryptophan and 5% (w/v) NaCl. After incubation at 30°C for 5 days, the supernatant was collected by centrifugation at 8,000 rpm for 5 min. One ml of the supernatant was mixed with 2 ml of Salkowski's reagent [ $FeCl_3$  12 g/l in 7.9 M sulfuric acid solution] and kept in the dark for 30 min to develop pink colour, indicating IAA production.

### **Assessment of IAA production in King's B and marine broths**

To assess the effect of saline on the ability of IAA production, the isolates were inoculated in King's B broth supplemented with 5% (w/v) NaCl and marine broth [Per liter: peptone 5 g; yeast extract 1 g;  $C_6H_5FeO_7$  0.1 g; NaCl 19.45 g;  $MgCl_2$  5.9 g;  $MgSO_4 \cdot 7H_2O$  3.24 g;  $CaCl_2$  1.8 g; KCl 0.55g;  $NaHCO_3$  0.16 g; KBr 0.08 g;  $SrCl_2$  34 mg;

$H_3BO_3$  22 mg;  $Na_2SiO_3$  4 mg;  $NH_4NO_3$  1.6 mg;  $Na_2PO_4$  8 mg] with 3% (w/v) NaCl to achieve a final concentration of 5% (w/v) NaCl. King's B broth and marine broth without supplementary NaCl were used for comparison. L-tryptophan was added to all media to make up final concentration of 1.0 g/L. Each experiment was carried out with three replicates and the inoculums were incubated at 180 rpm in a shaker incubator at 30°C in the dark for 5 days. After centrifugation, 1 ml of the supernatant was mixed with 2 ml of Salkowski's reagent and kept in the dark for 30 min. IAA production was vigilantly measured by optical densitometry at the wavelength of 530 nm. Uninoculated broth served as a control. A standard curve was prepared with 5–100  $\mu$ g/ml IAA (Sigma) for quantification. Growth of bacterial strains was monitored by measuring optical density at the wavelength of 600 nm.

### **Sequencing and analysis of 16S rRNA gene**

The IAA producing isolates were identified in a traditional manner based on the analysis of 16S rRNA gene sequencing as described previously (Tran Bao Tram et al. 2018).

### **Effects of nitrogen sources, L-tryptophan and NaCl on growth and production of IAA by the isolate C7**

The growth and production of IAA by the isolate C7, in the presence of yeast extract, tryptone, meat extract, peptone, L-proline,  $NaNO_3$ , glycine, and urea were tested. Ingredients of the media were as follows (per litre): nitrogen, 20 g (yeast extract, tryptone, meat extract, peptone) or 5 g (L-proline,  $NaNO_3$ , glycine, and urea); 1.5 g  $K_2HPO_4 \cdot 3H_2O$ ; 1.5 g  $MgSO_4 \cdot 7H_2O$ ; 10 g NaCl and 1 g L-tryptophan.

The effect of L-tryptophan was checked employing the above medium with yeast extract as nitrogen and supplemented with 1% (w/v) NaCl. The concentration of L-tryptophan varied from 0 to 3 g/l (at intervals of 0.5 g/l).

The effect of NaCl was examined at the concentrations of 0, 1, 2, 4, 5, 6, 8, 9, 10, 12, 14, 15, 17 and 20% (w/v) NaCl in the medium containing (per litre) 20 g yeast extract, 1.5 g K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 1.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, and 2 g L-tryptophan.

Starter culture was prepared in 20 ml of marine broth and incubated overnight at 30°C with continuous shaking at 160 rpm. Each test was carried out with three replicates. Measurements of IAA and growth were carried out as above. Optical densities higher than 2.99, were measured after an appropriate dilution and the readings were multiplied with the dilution factor.

## RESULTS

### Isolation of bacteria and screening of IAA producing bacteria

A total of 202 bacterial colonies was isolated from 12 samples collected from coastal regions of Ben Tre and Tra Vinh Provinces. All isolates were evaluated for the ability to produce IAA by the Salkowski method. Ten out of 202 colonies were able to produce IAA and were designated BDS 1.2.2, BDW 1.1.1, BDW 1.1.2, BDW 1.1.3, B9, B7, CBW 1.1.1, CBW 1.1.3, CBW 2.2.3 and C7 (table 1). All of them were identified as gram-negative and rod-shaped.

Table 1. Information of IAA-producing bacterial strains isolated in the coastal regions of Ben Tre and Tra Vinh Provinces

No.	Isolates	Isolation source	Colony
1	BDS 1.2.2	Sands, Ba Dong coast, Tra Vinh	White, circular, entire, elevated, opaque, mucoid
2	BDW 1.1.1	Seawater, Ba Dong coast, Tra Vinh	White, circular, entire, elevated, mucoid
6	BDW 1.1.2	Seawater, Ba Dong coast, Tra Vinh	White, circular, entire, elevated, mucoid
7	BDW 1.1.3	Seawater Ba Dong coast, Tra Vinh	White, circular, entire, elevated, mucoid
9	B9	Seawater, Ba Dong coast, Tra Vinh	Opalescent, circular, entire, elevated, mucoid
10	B7	Seawater, Ba Dong coast, Tra Vinh	Opalescent, circular, entire, elevated, mucoid
3	CBW 1.1.1	Seawater, Con Bung coast, Ben Tre	White, circular, entire, elevated, mucoid
4	CBW 1.1.3	Seawater, Con Bung coast, Ben Tre	White, circular, entire, elevated, mucoid
5	CBW 2.2.3	Seawater, Con Bung coast, Ben Tre	White, circular, entire, elevated, mucoid
8	C7	Seawater, Con Bung coast, Ben Tre	Opalescent, circular, entire, elevated, mucoid

### Assessment of IAA production in King's B and marine broths

As King'B is a basic medium to test IAA producing activity and marine broth is a native habitat of marine bacteria, we used these two media for the assessment of IAA production. Fig. 1 illustrates the growth and

IAA production of the ten bacterial isolates. Isolates BDW 1.1.1, CBW 2.2.3, and BDW 1.1.3 could grow well in all conditions, in contrast the other strains were able to grow better in moderate halophilic conditions (Fig. 1a). The best growths of isolate BDW 1.1.1; isolates CBW 2.2.3 and BDW 1.1.3 were observed in King'B broth and King'B

broth supplemented with 5% (w/v) NaCl, respectively. Isolates BDS 1.2.2, CBW 1.1.1, CBW 1.1.3, C7, B7 and B9 displayed the best

growth in marine broth supplemented with 3% (w/v) NaCl.

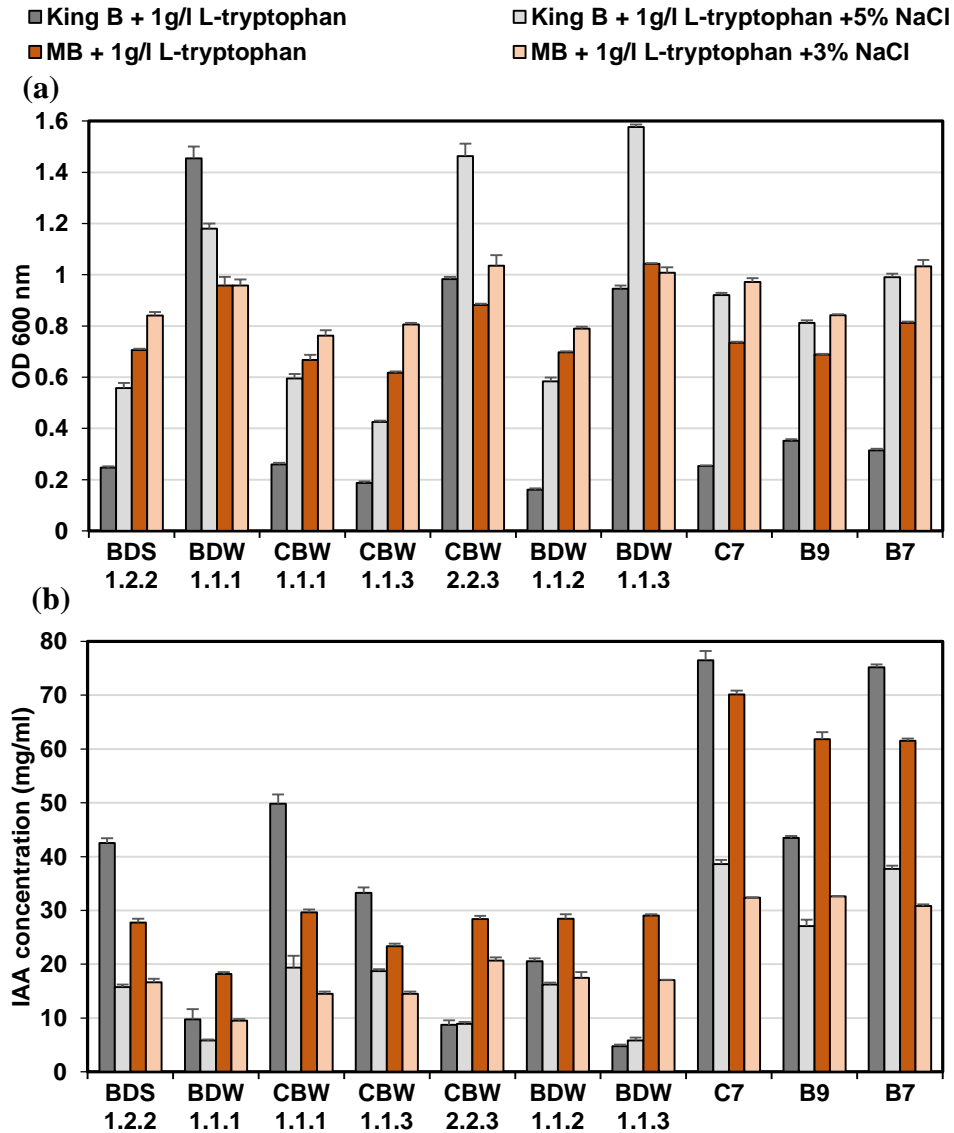


Figure 1. Growth (a) and IAA production (b) in saline and non-saline conditions by ten bacterial isolates

The isolate C7 was identified to be the most efficient IAA producing isolate in all conditions, compared to the other strains (Fig 1b). In details, the isolate C7 produced 76.49 mg/ml IAA in King' B broth and 33.05 mg/ml IAA in marine broth supplemented 3% (w/v) NaCl. Isolates B7 and B9 also produced the

high amounts of IAA in marine broth with 61.82 mg/ml and 61.54 mg/ml, respectively. Isolates BDW1.1.1, CBW 2.2.3, BDW 1.1.2, BDW 1.1.3, and B9 produced greater amount of IAA in marine broth than in King'B broth. In contrast, isolates BDS 1.2.2, CBW 1.1.1, CBW 1.1.3, C7, and B7 showed the most

effective IAA production at the most limited growth in King'B broth, compared to the rest of the other conditions (Fig. 2). Under the presence of higher NaCl, IAA productions

were significantly decreased, showing that higher NaCl is unfavorable to IAA production of bacterial isolates.

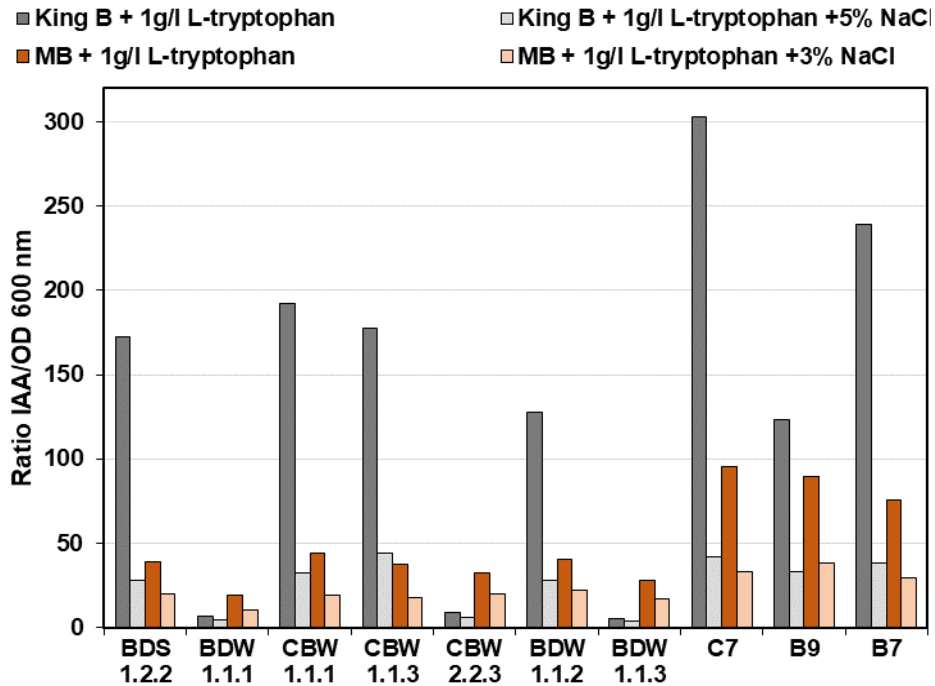


Figure 2. Ratio of optical density of bacterial growth to IAA production (IAA/OD 600 nm) in saline and non-saline conditions by ten bacterial isolates

### Species identification of bacteria isolates using 16S rRNA gene sequence

The 16S rRNA gene of the ten IAA producing isolates was sequenced and compared with those available from the GenBank database (table 2). Based on 16S rRNA gene sequence similarity, the isolate BDS 1.2.2 was identified as *Mameliella phaeodactyli* (99.85%); the isolate BDW 1.1.1 was classified as *Marinobacter daepoensis* (99.93%); the isolates CBW 1.1.1, CBW 1.1.3, CBW 2.2.3, BDW 1.1.2, and BDW 1.1.3 were identified to be *Marinobacter hydrocarbonoclasticus* (99.86–100%); and isolates C7, B9, and B7 were the members of *Marinobacter pelagius* (99.09%). Interestingly, bacterial isolates producing IAA were the representatives of the genus *Marinobacter*. The sequences data has been submitted to GenBank database

under the accession numbers given in table 2.

Maximum-likelihood phylogenetic tree clearly illustrated the taxonomic positions of the ten IAA-producing bacterial strains (Fig. 3). The isolate BDS 1.2.2 together with the type strain *Mameliella phaeodactyli* KD53<sup>T</sup> represented a monophyletic separate genus-level lineage. The genus *Marinobacter* is divided into three groups. The group I was formed by the isolates CBW 1.1.1, CBW 1.1.3, CBW 2.2.3, BDW 1.1.2, and BDW 1.1.3 and the type strain *Marinobacter hydrocarbonoclasticus* ATCC 49840<sup>T</sup>. In group II, the isolate BDW 1.1.1 clustered with the type strain *Marinobacter daepoensis* SW-156<sup>T</sup> with 100% bootstrap support. Three isolates C7, B7, and B9 grouped with the type strain *Marinobacter pelagius* HS 255<sup>T</sup> in group III.

Table 2. BLASTN analysis of 10 IAA-producing isolates showing the closest species in GenBank database

No.	Isolate	Length (bp)	NCBI Accession	Top-hit taxon	Identity (%)
1	BDS 1.2.2	1390	MK850321	<i>Mameliella phaeodactyli</i>	99.85
2	BDW 1.1.1	1480	MK850322	<i>Marinobacter daepoensis</i>	99.93
3	CBW 1.1.1	1480	MK850323	<i>Marinobacter hydrocarbonoclasticus</i>	99.86
4	CBW 1.1.3	1482	MK850324	<i>Marinobacter hydrocarbonoclasticus</i>	99.93
5	CBW 2.2.3	1430	MK850325	<i>Marinobacter hydrocarbonoclasticus</i>	99.86
6	BDW 1.1.2	1481	MK850326	<i>Marinobacter hydrocarbonoclasticus</i>	100
7	BDW 1.1.3	1428	MK850327	<i>Marinobacter hydrocarbonoclasticus</i>	100
8	C7	1480	MK850328	<i>Marinobacter pelagius</i>	99.09
9	B9	1484	MK850329	<i>Marinobacter pelagius</i>	99.09
10	B7	1484	MK850330	<i>Marinobacter pelagius</i>	99.09

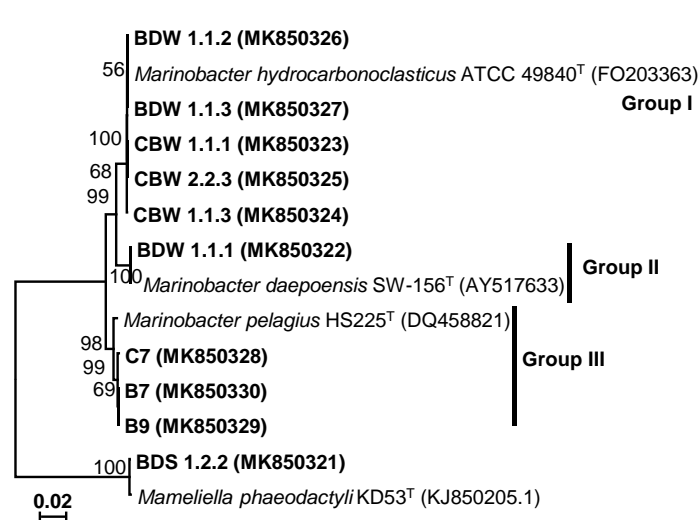


Figure 3. The maximum-likelihood phylogenetic relationship of IAA producing marine bacteria based on 16S rRNA sequence analysis. Marine bacteria isolates are shown in bold with their nucleotide sequence accession numbers indicated in brackets. The significance of each branch is indicated by a bootstrap value  $\Rightarrow$  50 are indicated for each node (1,000 replicates). Bar, 0.01 substitution per nucleotide position

#### Effect of nitrogen sources, L-tryptophan and NaCl on growth and production of IAA by the isolate C7

The effects of different nitrogen sources on the IAA production and the growth of the

isolate C7 were shown in Figure 4a. No growth was observed in glycine (Fig. 4a). The isolate C7 was able to grow slowly under the presence of urea and NaNO<sub>3</sub> but grow well in yeast extract, meat extract, peptone and tryptone (Fig. 4a). Among them, yeast extract

was found to serve as the most suitable source for IAA production ( $148.16 \pm 0.31 \mu\text{g/ml}$ ). Meat extract, peptone, and tryptone also resulted in the intense production of IAA ( $124.38 \pm 0.37$ ;  $112.24 \pm 0.46$ ; và  $93.46 \pm 0.71 \mu\text{g/ml}$ , respectively). Under the presence of L-proline, a moderate amount of IAA ( $54.40 \pm 0.05 \mu\text{g/ml}$ ) was produced (Fig. 4a). The most poor IAA production was observed under the presence of urea and  $\text{NaNO}_3$  (IAA concentration  $< 20 \mu\text{g/ml}$ ). Although the isolate C7 showed the steadiest growth in peptone, the highest IAA production was achieved in yeast extract. Growth ability as well as IAA production of the isolate C7 was low in  $\text{NaNO}_3$  and urea.

To identify the optimum concentration of L-tryptophan on IAA production by the isolate C7, the isolate C7 was cultured under the presence of various concentrations of L-

tryptophan (0 to 3 g/l) (Fig. 4b). IAA production was gradually increased starting at 0.5 g/l, reached its peak ( $191 \pm 1.52 \mu\text{g/ml}$ ) at 2.0 g/l, and then decreased along with the increase of L-tryptophan concentration up to 3 g/l. The growth of the isolate C7 gradually decreased in proportional with the increase of the L-tryptophan concentration (Fig. 4b).

The isolate C7 was able to grow and produce IAA over a broad range (1–17%) of NaCl with the optimum growth at 4% (Fig. 4c), but unable to grow without NaCl. No growth was observed at 20% (w/v) NaCl. The highest IAA production by the isolate C7 ( $192.2 \pm 1.14 \mu\text{g/ml}$ ) was obtained at 1% NaCl. The IAA production by the isolate C7 gradually decreased when the NaCl concentration increased (Fig. 4c). IAA was not produced at the NaCl concentration of 15%.

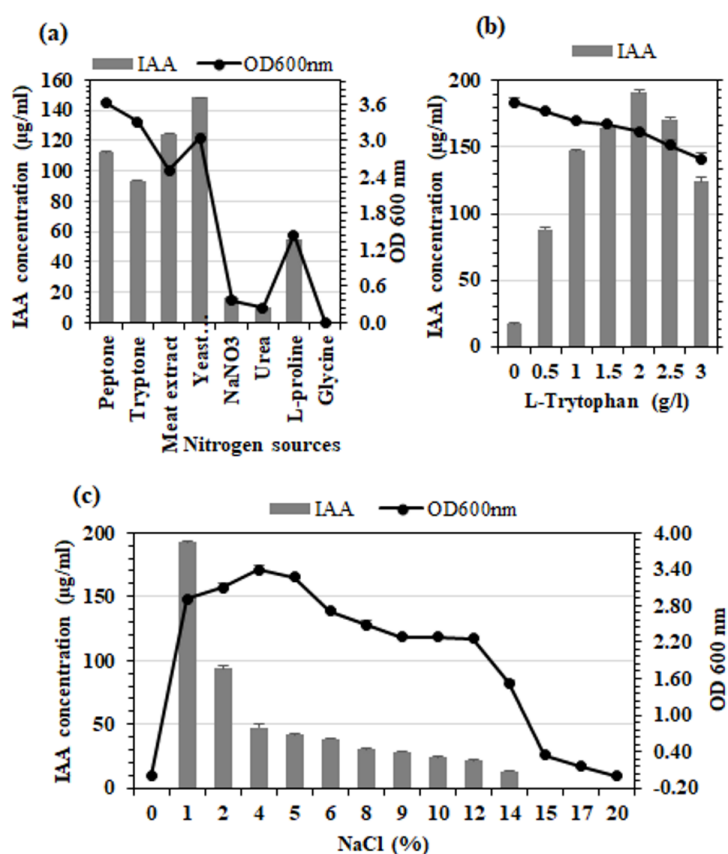


Figure 4. Effect of nitrogen sources, L-tryptophan and salt on growth and IAA production of isolate C7: a) Nitrogen sources; b) L-tryptophan; c) NaCl



## DISCUSSION

Recent assessments have documented salt intrusions in Mekong River Delta. Numerous studies have reported IAA-producing salt tolerant bacteria from rice plants (Nguyen Van Minh, 2017), and from the soil of rice-shrimp farming systems in the Mekong Delta, Vietnam (Nguyen Khoi Nghia et al., 2017). Almost all the studies on marine bacteria have typically focused on their antibiotic (Wiese & Imhoff, 2019); degradation capability of plastic (Urbanek et al., 2018), oil (Farag et al., 2018), and petroleum hydrocarbon (Mahjoubi et al., 2018). Research on plant growth promoting activity of marine bacteria is limited. The study of Uchgaonkar et al. (2018) screened siderophore producing bacteria in sea water in India. In case of IAA production, the study of Nayomi & Thangavel (2015) screened IAA producing bacteria from sea water and sediment in India. As the development of new sources for IAA producing bacteria from marine environments is promising, in this study, we investigated the ability to produce IAA of bacteria isolated from the marine environment in Vietnam. The number of IAA-producing isolates from coastal regions of Ben Tre and Tra Vinh Provinces was considerably low compared with that of Thiruvananthapuram, India (Nayomi & Thangavel, 2015). This difference may relate to the different sampling. In details, in the study of Nayomi & Thangavel (2015), they isolated bacteria from marine water and sediments, but in this study we isolated from marine water and sands. Sediment may have more diverse bacteria than sands. The number of IAA-producing bacterial isolates from the marine environment was similar with that found in rhizosphere soils from banana, cotton, maize and wheat (Mohite, 2013), but lower than those found in the rhizosphere of halophyte or soils (Siddikee et al., 2010).

In contrast to the studies of Goswami et al. (2013, 2015) and Nayomi & Thangavel (2015), in which bacterial strains of the genera *Pseudomonas* and *Acinetobacter* produced IAA, in this study no such genera were

obtained. On the other hand, this study is the first on the ability of IAA production from *Marinobacter* spp. and *Mameliella* spp. Therefore, our study established the potential for ubiquitous IAA-producing bacteria in the marine environment. This suggests marine bacteria could be a creative source for the use of these bacteria as biofertilizers to improve the growth and crop productivity in saline intrusion lands.

In this study, we also investigated several factors affecting the growth and IAA production of the isolate C7. Due to the optimal growth at 4% NaCl and no growth at 0% NaCl, the isolate C7 was characterized as a moderate halophilic strain as classified previously by Kushner (1978). The salt tolerance of the isolate C7 was similar with the type strain of *Marinobacter pelagius* (Xu et al., 2008). Based on 16S rRNA gene sequence, we identified the isolate C7 as *Marinobacter Pelagius*. Hereafter, we designate the isolate C7 as *Marinobacter pelagius* strain C7.

As a moderate halophile, *M. pelagius* strain C7 grew better in marine broth than King'B broth, but produced IAA most effectively in King'B. It can be explained that the high concentration of NaCl in marine broth (1.95%) could affect to IAA production of the strain C7. This is confirmed further by examining the dose-effect of NaCl to IAA production of the strain C7 (Fig. 3c), in which, the IAA production was significantly decreased from 2% NaCl after reaching its peak at 1% NaCl. Synthesis of IAA from L-tryptophan consists of membrane-bound and extracellular enzymes, of which enzyme activity is considerably affected by external saline conditions (Ventosa et al., 1998). These processes are different between bacteria groups. In case of salt-sensitive bacteria *Bradyrhobium* PN13-3, approximate 30% and 100% IAA reduction was observed at 200 mM and 400 mM NaCl, respectively, indicating high salt concentrations inhibited the enzyme activity (Dong et al., 2017). In contrast, salt-tolerant bacteria *Bradyrhobium* RJS9-2, IAA production significantly

increased about 20% and 30% at 200 nM NaCl and 400 mM NaCl, respectively, compared to no NaCl (Dong et al., 2017). It could be suggested that enzyme activity was stimulated by salt concentration up to 400 mM. As another possible explanation, IAA production might raise the salt-tolerant strain RJS9. In the study of Dong et al. (2017) at the concentration of 500 mM, osmoprotective compounds of strain RJS9 decreased, indicating that strain RJS9 started salt sensitivity at this concentration. In our study, the strain C7 is a moderate halophilic bacterium and can grow abundantly at an approximate NaCl concentration of 855 mM, but IAA production reduced nearly 50%, compared to non-saline (Fig. 1). Indeed, concentration of 855 mM NaCl is high that inhibits enzyme activity, including enzymes involved in synthesis of IAA from L-tryptophan.

The strain C7 had limitations in carbon utilization but could use peptone in King's B and marine broths. Therefore, we tested the effect of nitrogen sources to the ability of IAA production of this strain, such as yeast extract, meat extract, peptone, tryptone, L-proline, urea and NaNO<sub>3</sub>. Yeast extract was the best nitrogen source for IAA production for the strain C7. This is consistent with that observed in *Pseudomonas* sp. (Balaji et al., 2012) and *Enterobacter* sp. (Nutarata et al., 2017). The strain C7 could not grow under the presence of glycine, suggesting that cells of the strain C7 were susceptible to glycine. Glycine induced the lysis of various microorganisms such as *Bacillus subtilis*, *Escherichia coli*, *Bacteroides ruminicola*, *Streptomyces* sp., *Pseudomonas* sp. etc. (Hishinuma et al., 1969). Glycine also inhibited the growth of diverse bacterial species by inhibiting cell wall synthesis (Hammes et al., 1973). Glycine has been demonstrated to incorporate into the nucleotide-activated peptidoglycan precursors, resulting accumulation of glycine-containing precursors which lead to a disturbance of the normal balance between peptidoglycan synthesis and controlled

enzymatic hydrolysis during growth. The strain C7 is a gram-negative bacterium, which owns a thin cell wall, and therefore, cell wall may become weaker by no growth at 0.5% glycine, compared to 80% inhibition of growth at 0.55–10% glycine of gram-positive bacteria such as *Lactobacillus* sp. and *Corynebacterium* sp. (Hammes et al., 1973). The presence of urea and NaNO<sub>3</sub> at the concentration of 0.5% (w/v) reduced the IAA production, compared to the other favorable organic nitrogen sources. This negative effect has also been reported by Othman et al. (2013), in which, nitrogen from urea lowered the IAA production of *Stenotrophomonas maltophilia* Sb16.

L-tryptophan is an IAA precursor, and as a result, the supplementation of L-tryptophan in the medium enhanced IAA biosynthesis. The result of IAA production at 2 g/l L-tryptophan of the strain C7 in this study is consistent with the previous studies of Park et al. (2015) and Wagi & Ahmed (2019), who also obtained maximum IAA productions of 2-3 g/l L-tryptophan for *Enterobacter* sp. and *Bacillus* sp. However, other bacteria in the study of Mohite et al. (2013) indicated the optimum L-tryptophan concentrations ranging from 0.5 to 15 g/l. This difference might be attributed the different adaptability of each bacterial strain to L-tryptophan. The optimum L-tryptophan for IAA production may not be the optimum L-tryptophan for growth of bacteria. In this study, the strain C7 showed a gradual decrease in the growth with the increase of L-tryptophan. It can be explained that L-tryptophan could enhance cellular reactive oxygen species generation, causing bacterial death (Li et al., 2019).

## CONCLUSION

This study highlights a diverse IAA-producing bacteria in marine origin belonging to the genera *Marinobacteria* and *Mameliella*. This is also the first report dealing with the IAA-producing ability of these two genera. The strain C7 belonging to *Marinobacter pelagius* has shown promising plant growth promoting activity indicated by IAA

production and can be used as bio-inoculants in agricultural environments.

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