

**IDENTIFICATION AND EVALUATION OF ENZYMES GELATINASE, AMYLASE, AND CATALASE PRODUCED BY RICE ROOT ENDOPHYTIC BACTERIA ISOLATED FROM HAI DUONG WITH ANTIMICROBIAL PROPERTIES AGAINST *Xanthomonas oryzae* pv. *oryzae***

**Nguyen Van Phuong<sup>1,2,\*</sup>, Nguyen Thuy Linh<sup>2</sup>, Trinh Dinh Duy<sup>2</sup>, Tran Le Nam Khanh<sup>2</sup>, Do Doan Thu Giang<sup>2</sup>, Ta Thi Thuy Linh<sup>2</sup>, Mai Thi Phuong Nga<sup>2</sup>, To Thi Mai Huong<sup>2</sup>, Mai Duc Chung<sup>3</sup>, Chu Hoang Ha<sup>4</sup>, Le Tran Binh<sup>4</sup>**

<sup>1</sup>Graduate University of Science and Technology, VAST, Vietnam

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

<sup>3</sup>Agricultural Genetics Institute, Hanoi, Vietnam

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

Received 3 March 2021, accepted 16 June 2021

**ABSTRACT**

Endophytic bacteria (EB) possess different beneficial traits. Endophytic microbes are often functional in that they may carry nutrients from the soil into plants, modulate plant development, increase stress tolerance of plants, suppress virulence in pathogens, increase disease resistance in plants, and suppress development of competitor plant species. They may enhance plant development by carrying nutrients from the soil into plants and protect plants against phytopathogens by synthesizing extra-/intracellular proteolytic enzymes, as well as releasing antimicrobial metabolites and competing with pathogens for habitation and nutrients. In this study, we investigated the ability to produce exo/endo-enzymes such as gelatinase, amylase, and catalase, and antagonistic activity of 77 EB strains isolated from lowland rice roots grown in Doan Ket commune, Thanh Mien district, Hai Duong province, Vietnam. Out of 77 isolates, 76 (98.71%) showed the ability to liquefy gelatin after 7 days with different rates of hydrolysis. The test of starch hydrolysis revealed 58 (75.33%) isolates that were able to hydrolyze starch. Fifty-one out of 77 isolates (66.24%) were able to produce catalase. The antagonistic activity of rice root endophytic bacteria was determined against bacterial leaf blight disease-causing pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), strains X19.2 and VX41. We found that three isolates (TP5, TP7, TP11) showed the ability to inhibit the growth of strain VX41 and twelve isolates (TP3, TP7, TP8, TP9, TP10, TP11, TP12, TP13, TP15, TP17, TP21, TP23) were able to inhibit the growth of strain X19.2. These results are served as a venue for further investigation *in planta* under the conditions of net house and field trials in order to confirm the potential strains for the development of bioinoculant toward controlling the disease caused by *Xoo*.

**Keywords:** Antagonistic activity, catalase, gelatin hydrolysis, endophytic bacteria, starch hydrolysis, rice, *Xanthomonas oryzae* pv. *oryzae*.

*Citation:* Nguyen Van Phuong, Nguyen Thuy Linh, Trinh Dinh Duy, Tran Le Nam Khanh, Do Doan Thu Giang, Ta Thi Thuy Linh, Mai Thi Phuong Nga, To Thi Mai Huong, Mai Duc Chung, Chu Hoang Ha, Le Tran Binh, 2021. Identification and evaluation of enzymes gelatinase, amylase, and catalase produced by rice root endophytic bacteria isolated from Hai Duong with antimicrobial properties against *Xanthomonas oryzae* pv. *oryzae*. *Academia Journal of Biology*, 43(2): 107–117. <https://doi.org/10.15625/15906>

\*Corresponding author email: [nguyen-van.phuong@usth.edu.vn](mailto:nguyen-van.phuong@usth.edu.vn)

©2021 Vietnam Academy of Science and Technology (VAST)

## INTRODUCTION

Rice (*Oryza sativa*) is one of the most economically important in many countries including Vietnam. However, production of rice in Vietnam and many rice-growing countries is threatened by pests and diseases causing huge losses. Bacterial leaf blight (BLB) on rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a common and destructive disease which affects millions of hectares of rice field throughout Asia (Jeung et al., 2006). Yield reduction is ranged from 20 to 80% depending on the development stages when the infection occurs. Infection at an earlier stage often causes high yield loss, while infection at the late booting stage mainly affects the grain quality and head rice recovery (Mew et al., 1993). The application of agrochemicals to paddy fields is considered as an easy solution to control the diseases and maintain high yield, however, the overuse of these toxic chemicals causes several problems to the environment and human health due to the high amount of toxic residue. At the same time, the prolonged exposure of agrochemicals to the field also reduce their effectiveness and may lead to the development of insecticide resistant strains (Fitri et al., 2020). This raises up a question about finding alternative solutions which are non-harmful for both human and the environment to stop overusing these chemical products. Endophytic bacteria have been studied as an alternative measure to improve crop yield, grain quality and prevent pathogens; at the same time, the use of endophytes does not cause any serious effects to the plants and the environment (Moronta - Barrios et al., 2018; Fitri et al., 2020).

Endophytic bacteria (EB) are those bacteria that colonize the internal plant tissue without external sign of infection or harmful effect on their host. EB are found on many plant crops including rice (Sen & Chandrasekar, 2014; Van Thi Phuong Nhu & Cao Ngoc Diep, 2014; White et al., 2019). Their interactions with host plants have been extensively studied (Reinhold - Hurek & Hurek, 2011). Many of these interactions have

been shown to benefit the hosts, namely growth promoting via production of phytohormones, nitrogen fixation, nutrient solubilization, and suppression of phytopathogens via production of antibiotics and/or cell wall degrading enzymes such as matrix metalloproteinases (Inoue et al., 2007; Olanrewaju et al., 2017). The beneficial properties of these endophytes make them microbes very promising candidates for biocontrol agents (Ahemad & Kibret, 2014).

Gelatin is a protein derived from the connective tissues of vertebrates, that is, collagen (Leboffe & Pierce, 2010). It is produced when collagen is boiled in water. Gelatin hydrolysis detects the presence of gelatinases. Gelatinases are extracellular proteases secreted by some bacteria. Gelatinases hydrolyze or digest gelatin firstly into polypeptides which then are converted into amino acids benefited to the bacterial cells in their metabolic processes. It has also been reported that the gelatinase B - a matrix metalloprotease can rapidly digest extracellular matrix produced by *Magnaporthe oryzae*, the fungus causing blight disease of wheat. This activity results in the detachment of most infection structures from membrane surfaces (Inoue et al., 2007).

The capacity of producing gelatinase, or other matrix metalloproteinases, by endophytes is a great aid to the plant host in resisting pathogenic fungi. Gelatin hydrolysis catalyzed by bacterial gelatinase leads to the degradation of the fungal extracellular matrix, removal the direct adhesion of fungi to the host, thereby, inhibiting the development and spread of the disease (Inoue et al., 2007). Several studies have focused on their interactions with host plants, especially on a large number of plant crops including rice (Reinhold - Hurek & Hurek, 2011). They have proved some endophytic bacteria isolates that are able to accelerate the growth of rice plants by observation of the root length and canopy height parameters (Fitri et al., 2020). Another study found an endophytic bacteria strain could assist plant nutrient uptake and prevent

plant diseases on the basis of its antagonistic activity, siderophore production, and nitrogen fixation,... (Zhao et al., 2018).

The ability to hydrolyze starch into simpler sugars, by the production of amylase, provides endophytic bacteria one important source of energy and carbon, giving them an advantage in the competition against other microorganisms on the same host. Catalase is an oxygen - scavenging enzyme that protects cells from the toxic effects of hydrogen peroxide and, in some cases, an extremely toxic superoxide during development by converting it to water and oxygen (Cappuccino & Welsh, 2017). It has been reported that catalase plays a major role in combating the toxic effect of reactive oxygen species in plant cells under abiotic and biotic stresses (Choodamani et al., 2009; Hameed & Iqbal, 2014; Sofo et al., 2015).

Currently, there have been numerous studies in Vietnam on rice rhizospheric microflora, either free-living or endophytic microorganisms. However, the vast majority of these studies were conducted in the South of Vietnam (Kennedy et al., 2008; Van Thi Phuong Nhu & Cao Ngoc Diep, 2014; Ly Ngoc Thanh Xuan et al., 2016a, b; Hoang Minh Tam & Cao Ngoc Diep, 2017; Nguyen Ngoc Lan et al., 2019) and no such research has been done in the North of Vietnam, except for a production program of biofertilizer from bacterial strains isolated in Hanoi (Kennedy et al., 2008; Phan Thi Cong et al., 2009). Therefore, looking at the rice rhizospheres from the Red River Delta for beneficial endophytes capable of potential biofertilizer and/or biocontrol agents for rice is a promising way forward. In this study, a set of seventy-seven endophytic bacteria isolated from Hai Duong province was *in vitro* screened for exo/endo-enzyme production including gelatinase, amylase, and catalase. We also screened the antagonistic activity against the bacterial strains *Xoo* causing bacterial leaf blight disease on rice. Findings in this study provide preliminary results for further investigation *in planta* in order to identify potential strains for development of

bioinoculant toward controlling the disease caused by *Xoo*. Furthermore, the study could also contribute to the protection of environment by reducing the use of insecticides, and sustainable development of agriculture in Vietnam.

## MATERIALS AND METHODS

### Bacterial strains

A set of seventy-seven rice root endophytic bacteria isolated from submerged paddy field in Hai Duong province as described in the previous article (Nguyen Van Phuong et al., 2020) was used for all experiments in this study. Two *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains X19.2 and VX41 provided by Agricultural Genetics Institute, Hanoi were used for determining the antibacterial activity of endophytic bacteria.

### *In vitro* qualitative assay for exoenzyme gelatinase production

The gelatinase production assay was carried out according to the nutrient gelatin stab method as described in Cappuccino & Welsh (2017) with some modifications. Briefly, the procedure was described as follows: an inoculum of 24 hours - old EB was heavily stab-inoculated into cylinder glass tubes containing 4 mL of nutrient gelatin medium (peptone, 5 g/L; beef extract, 3 g/L; gelatin, 120 g/L). This high gelatin concentration resulted in a solid medium and also served as the substrate for the activity of gelatinase. The inoculated tubes and an uninoculated control tube were incubated at  $28 \pm 2$  °C and were checked for gelatin liquefaction at 2, 4 and 7 days post incubation (dpi). Gelatin starts to liquefy at temperature of 28 °C. In order to determine the liquefaction in relation to gelatinase activity, the tubes were submerged in an ice bath for 30 minutes. Afterwards, the tubes are kept in inclined position to check the amount of degraded gelatin. Liquefied gelatin remained in liquid form post incubation in ice, while the control tube was still in solid - state. The rate of gelatin hydrolysis was estimated as follow: the cultures that remain liquefied demonstrate rapid, moderate and slow gelatin hydrolysis at

2, 4 and 7 dpi, respectively. The gelatin hydrolysis levels were qualitatively identified at 7 dpi based on the amount of gelatin medium that remain liquefied: no gelatin hydrolysis - the medium was in complete solid state as the control tube; low - < 1/3; moderate - 1/3-1/2; high - 1/2-2/3; very high - complete liquefied.

#### ***In vitro* qualitative assay for exoenzyme amylase production**

The assay for amylase production was adapted from Cappuccino & Welsh (2017). The experimental procedure was performed as follows: the EB isolates were streaked on the medium plates containing Tryptic Soy Broth (TSB) (5 g/L), starch (20 g/L), and agar (15 g/L). After 24 hours of incubation at  $28 \pm 2$  °C, the test plates were completely flooded for 10 minutes with Lugol solution containing KI 1% (w/v) and I<sub>2</sub> 0.5% (w/v) before the Lugol solution was discarded. The test plates were air-dried and illuminated to determine the presence of starch in the medium. Starch in the presence of iodine will develop a dark-blue color on the surface of the medium, indicating the absence of amylase and representing a negative result. If the starch has been hydrolyzed, a clear zone of hydrolysis will be observed around the colonies indicating a positive result. The strain *Xoo* VX41 was used as the negative control.

#### ***In vitro* qualitative assay for endoenzyme catalase production**

The ability to produce catalase was screened following the plate method as described by Cappuccino & Welsh (2017). The test bacterial strains were streaked on the plates of 1/6 Tryptic Soy Agar (TSA) medium. After 24 hours of incubation at  $28 \pm 2$  °C, one drop of hydrogen peroxide solution 3% was dropped on the bacterial colonies. The absence or presence of bubbles indicated negative or positive catalase test, respectively.

#### **Antagonistic activity against *Xanthomonas oryzae* pv. *Oryzae***

The antibacterial activity of the EB was evaluated by agar plate diffusion method

following Monteiro et al. (2005) with some modifications. The *Xoo* strains X19.2 and VX41 stocks were recovered on 1/2 TSA medium plates for 48 hours at  $28 \pm 2$  °C. The bacteria were then suspended in PSB solution, pH 6.8 and adjust to OD<sub>600</sub> = 1 (10<sup>9</sup> CFU/mL). Two hundred microliters of the *Xoo* suspension was spread on 1/2 TSA medium. Subsequently, a single colony from 24-hour-old culture of each endophytic bacteria were inoculated at the center of a tested plate. The experiment was conducted with ten replicates per isolate. Inhibition zones formed by the isolates against *Xoo* after 48 hours of inoculation at  $28 \pm 2$  °C were recorded for selection of strong antagonists.

## **RESULTS AND DISCUSSION**

### **Production of enzymes by endophytic bacteria isolated from rice rhizosphere**

In present study, we described the *in vitro* screening for exo/endo-enzyme producers from a set of seventy-seven rice root endophytic bacterial strains isolated from submerged paddy in Hai Duong province. The results of gelatin hydrolysis assay showed that after 2 days of incubation, the gelatin liquefaction was observed for 22 isolates (28.95%) with 17 isolates (22.37%) showing partial gelatin liquefaction and 5 isolates (6.58%) exhibiting complete liquefaction. Meanwhile, the gelatin liquefaction was not observed for remain 54 isolates (71.05%). However, at 7 dpi, the gelatin liquefaction was found in most of the isolates, 76/77 (98.71%) (Table 1). This results indicate that the rate and level of gelatin hydrolysis varies among bacterial strains. Five strains including TP5, TP23, TP59, TP60, and TP61 showed high and very high levels of gelatin liquefaction at 2 dpi (Table 1, Fig. 1), the cultures remained complete liquefied. Previous study reported that 24 out of 72 isolates showed *in vitro* antifungal activity against *Magnaporthe oryzae*, strain 007-6 with the inhibition ratio of more than 50% (Nguyen Van Phuong et al., 2020). The ability to gelatin hydrolysis of EB provides them not

only benefits in terms of amino acid for their living processes but also dedicates them the ability to compete for the place and to inhibit the growth of other pathogens. Therefore, the

reveal of rice root endophytic bacteria which are able to hydrolyze gelatin plays a critical role in finding the potential beneficial strains for the development of biocontrol agents.

Table 1. Enzyme production and antibacterial activities of 77 rice root endophytic bacteria

No.	Name of strain	Gelatin liquefaction <sup>a</sup>			Starch hydrolysis <sup>a</sup>	Catalase production <sup>b</sup>	Inhibition of <i>Xoo</i> <sup>b</sup>	
		2 dpi	7 dpi	Rate of hydrolysis			VX41	X19.2
1	TP1	-	++	Slow	+	+	-	-
2	TP2	++	+++	Moderate	+	+	-	-
3	TP3	++	++++	Moderate	+	+	-	+
4	TP4	++	++++	Moderate	+++	+	-	-
5	TP5	++++	++++	Rapid	++	+	+	-
6	TP6	-	+	Slow	+	+	-	-
7	TP7	+	++++	Slow	++	+	+	+
8	TP8	++	++++	Moderate	+++	+	-	+
9	TP9	+	++	Slow	+	-	-	+
10	TP10	-	+	Slow	+	+	-	+
11	TP11	-	++++	Slow	+	+	+	+
12	TP12	-	++++	Slow	+	+	-	+
13	TP13	-	++++	Slow	+	+	-	+
14	TP14	-	++++	Slow	+	+	-	-
15	TP15	-	++++	Slow	+	-	-	+
16	TP16	-	++++	Slow	+	+	-	-
17	TP17	-	++++	Slow	+	-	-	+
18	TP18	-	++++	Slow	+	+	-	-
19	TP19	-	-	ND	+	+	-	-
20	TP20	-	++	Slow	+	+	-	-
21	TP21	-	++	Slow	+	-	-	+
22	TP22	-	++++	Slow	+	+	-	-
23	TP23	+++	++++	Rapid	+	+	-	+
24	TP24	-	+	Slow	+	+	-	-
25	TP25	-	++	Slow	+	+	-	-
26	TP26	-	++	Slow	+	+	-	-
27	TP27	+	+++	Slow	+	+	-	-
28	TP28	-	++++	Slow	+	+	-	-
29	TP30	-	++	Slow	+	+	-	-
30	TP31	-	++++	Slow	+	+	-	-
31	TP32	+	++++	Slow	-	+	-	-
32	TP33	+	++++	Slow	-	+	-	-
33	TP34	+	++++	Slow	-	+	-	-
34	TP35	-	+	Slow	-	+	-	-
35	TP36	-	+	Slow	-	+	-	-
36	TP37	-	++++	Slow	+	+	-	-
37	TP38	-	++++	Slow	+	+	-	-
38	TP39	-	++	Slow	+	+	-	-
39	TP40	-	++	Slow	+	-	-	-
40	TP41	-	++++	Slow	+	+	-	-
41	TP42	-	++++	Slow	+	-	-	-

42	TP43	-	++++	Slow	+	+	-	-
43	TP44	-	++++	Slow	+	-	-	-
44	TP45	-	++++	Slow	+	-	-	-
45	TP46	-	++++	Slow	+	-	-	-
46	TP47	++	++++	Moderate	+	-	-	-
47	TP48	++	++++	Moderate	+	-	-	-
48	TP49	+	++	Slow	+	-	-	-
49	TP50	+	++++	Slow	+	-	-	-
50	TP51	-	++++	Slow	+	+	-	-
51	TP52	++	++	Moderate	-	-	-	-
52	TP53	-	++	Slow	-	-	-	-
53	TP54	++	++	Moderate	-	-	-	-
54	TP55	++	++++	Moderate	-	-	-	-
55	TP56	+	++	Slow	-	-	-	-
56	TP57	-	++	Slow	-	-	-	-
57	TP58	-	++++	Slow	-	+	-	-
58	TP59	++++	++++	Rapid	-	+	-	-
59	TP60	++++	++++	Rapid	-	+	-	-
60	TP61	+++	++++	Rapid	-	+	-	-
61	TP62	++	++++	Moderate	-	-	-	-
62	TP63	-	++	Slow	-	+	-	-
63	TP64	-	+++	Slow	-	+	-	-
64	TP65	++	++++	Moderate	+	+	-	-
65	TP66	-	++++	Slow	+	-	-	-
66	TP67	-	++	Slow	+	+	-	-
67	TP68	-	++++	Slow	-	-	-	-
68	TP69	-	++	Slow	+	-	-	-
69	TP70	-	++	Slow	+	+	-	-
70	TP71	-	++	Slow	+	-	-	-
71	TP72	-	++++	Slow	+	-	-	-
72	TP73	++	++++	Moderate	+	-	-	-
73	TP74	++	++++	Moderate	+	+	-	-
74	TP75	++	++	Moderate	+	+	-	-
75	TP76	++	++	Moderate	+	+	-	-
76	TP77	++	++++	Moderate	++	+	-	-
77	TP78	++	++++	Moderate	+	+	-	-
	Total Positive (Nos)		76		58	51	3	12
	% Positive		98.71		75.33	66.24	3.9	15.59

Note: <sup>a</sup>The signs -, +, ++, +++ and ++++ indicate no gelatin liquefaction, low, moderate, high, and very high level of gelatin liquefaction (completely hydrolyzed), respectively; <sup>b</sup>the signs - and + indicate negative and positive test, respectively; dpi - days post incubation; Nos - Number of strains; ND - Note Detected.

Many bacteria are able to produce extracellular enzymes which participate in different catalytic chemical reactions outside of the cell. In this manner, nutrient sources, such as starch, that are too large to be absorbed through the cell membrane can be broken down into smaller molecules and transported into the cell via diffusion. In

this starch hydrolysis assay, the EB isolates are grown on agar plates containing starch. The starch hydrolysis was indicated by

transparent clear zones while the non-hydrolyzed starch forms dark blue color with iodine.

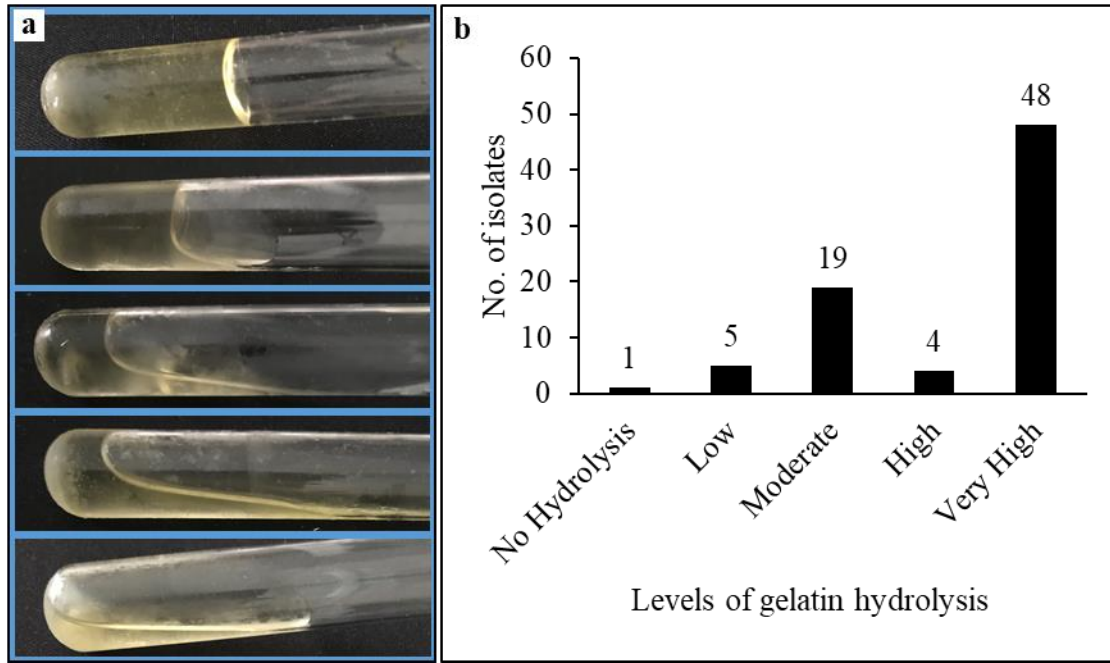


Figure 1. Levels of gelatin liquefaction by rice root EB at 7 dpi. (a) Different liquefied states of gelatin medium in the test tubes after 30 min of incubation in ice. Top down represents the levels of gelatin liquefaction from low to very high, respectively. (b) Distribution of EB isolates by levels of gelatin hydrolysis

A total of 77 EB strains was screened for exoenzyme amylase production. The results showed that 58 EB isolates (75.33%) were able to hydrolyze starch and 19 remain isolates were false to hydrolyze starch on test plates. Based on the intensive levels of the light going through the transparent zones, 58 amylase-producers represented the strains with low level of starch hydrolysis. The moderate and high levels of starch hydrolysis were observed in five isolates (TP5, TP7 and TP77, and TP4, and TP8, respectively) (Table 1, Fig. 2).

Similarly, the qualitative assay for endoenzyme catalase production of rice root EB isolates was conducted. During aerobic respiration, many bacteria produce hydrogen peroxide. Increasing level of this substance

will cause the death of the cells unless they can be enzymatically degraded. Hydrogen peroxide is produced when aerobes, facultative anaerobes, and microaerophiles use the aerobic respiratory pathway, in which oxygen is the final electron acceptor, during degradation of carbohydrates for energy production (Cappuccino & Welsh, 2017). Bacteria capable of producing catalase rapidly degrade hydrogen peroxide into water and oxygen. By adding one drop of hydrogen peroxide solution 3% on the 24-hour-old bacterial colonies, the catalase - producers were determined by appearance of free oxygen bubbles. The results showed that 51 out of 77 EB isolates (66.24%) were positive with catalase production. For the other 26 EB isolates (33.76%), the free oxygen bubbles did not appear (Table 1).

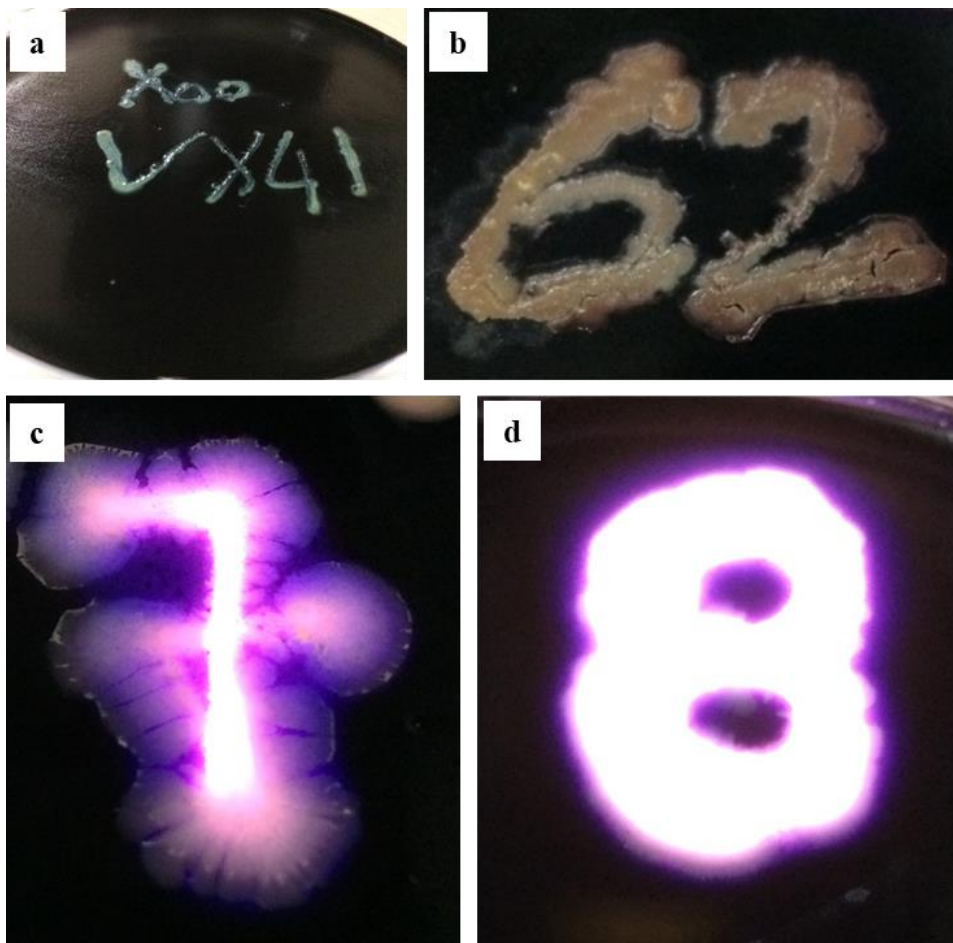


Figure 2. Starch hydrolysis by rice root EB strains on the starch nutrient plates. (a) The *Xoo* strain VX41 - negative control; (b) Strain TP62 was unable to hydrolyze starch in the medium, no transparent areas around colonies were observed; (c and d) Different levels of starch hydrolysis by the TP7 and TP8 isolates, respectively were demonstrated by transparent areas and intensive levels of light going through

**Antibacterial activity of endophytic bacteria against *Xanthomonas oryzae* pv. *Oryzae***

A total of 77 EB isolates were screened to identify the antagonistic activity against two strains X19.2 and VX41 of *Xoo*. The isolates with positive antagonistic effect were determined by producing a halo zone of inhibition around the bacterial colony on culture medium (Fig. 3). Therefore, these isolates were considered as potential antagonists. The results showed that three isolates TP5, TP7 and TP11 (3.9%) were able to inhibit the growth of the strain VX41,

whereas 12 isolates (15.59%) demonstrated positive effect on the strain X19.2. Specifically, the isolate TP17 exhibited the highest antagonistic activity on the growth of strain X19.2 indicating by the inhibition halo zone of more than 30 mm in diameter, accounted for 58.98% of inhibition (Fig. 3; data not showed). Interestingly, the isolates TP7 and TP11 were able to inhibit the growth of both strains VX41 and X19.2 on the tested plates (Table 1).

Based on these primary results, further investigations *in planta* under conditions of net house and field trial will be necessary to



draw clear conclusion on the antibacterial activity of the rice root endophytic bacteria. For instance, under greenhouse conditions, El-shakh and colleagues have found that the *Bacillus* strain D29 showed the highest level of antibacterial activity (57.86%) against *Xoo* followed by A15, H8 and A13 respectively (El-shakh et al., 2015). Moreover, many studies demonstrated all

*Bacillus* strains tested reduced the leaf blight, lesion length and wilting in rice plants compared to control plants (El-shakh et al., 2015; Trinh Thanh Trung et al., 2017; Nguyen Huynh Nha Uyen et al., 2018). Another study of Niño-Liu and colleagues had the same conclusion that the inhibition rate of tested *Bacillus* strains was highest against *Xoo* (Niño-Liu et al., 2006).

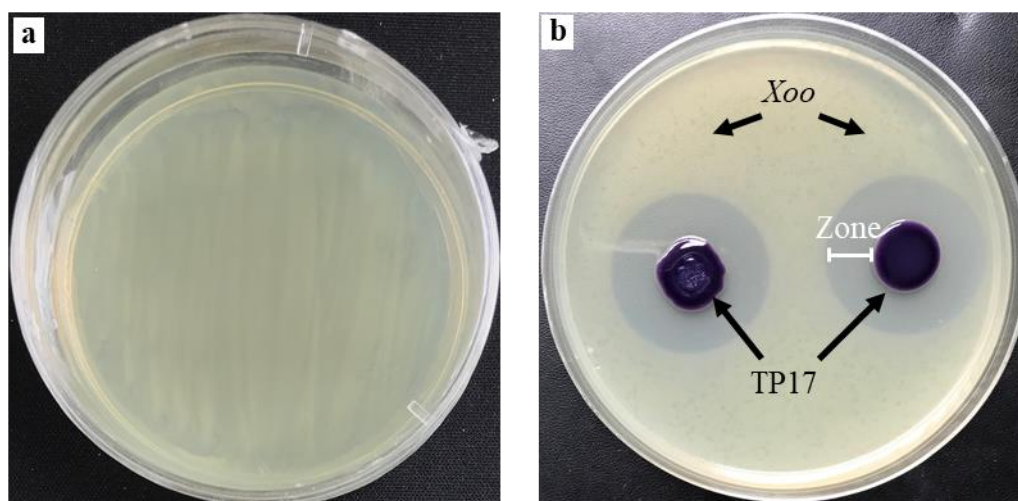


Figure 3. Antibacterial effect on growth of *Xoo* strain X19.2. (a) Control plate (no EB) with normal growth of *Xoo*; (b) Inhibition zone formed by TP17 isolate against *Xoo* strain X19.2 using plate diffusion method

Taken together, in this study, 13 isolates (TP3, TP5, TP7, TP8, TP9, TP10, TP11, TP12, TP13, TP15, TP17, TP21, and TP23) showed strong inhibition against bacterial phytopathogen *Xoo* *in vitro*. In addition, the previous study reported that these isolates possess other plant growth promoting traits such as IAA, siderophore, and ammonia production, phosphate solubilization and antifungal activity against *Magnaporthe oryzae* (Nguyen Van Phuong et al., 2020). Following this path, we can expect to come up with biocontrol strategies and bioinoculant formulations that are not only more sustainable but also more efficient.

## CONCLUSION

In the present study, the rice root endophytic bacterial isolates were described for ability to produce exo/endo-enzymes,

which hydrolyze gelatin, starch and hydrogen peroxide. Among endophytes that have ability to produce gelatinase, amylase and catalase, thirteen isolates (TP3, TP5, TP7, TP8, TP9, TP10, TP11, TP12, TP13, TP15, TP17, TP21 and TP23) showed antibacterial activity against *Xoo* strains. The isolate TP17 showed the highest inhibition percent (58.98%) against strain X19.2. Considering the great potentials of the endophytic strain TP17, further investigations in plants under different growth conditions are required to clearly confirm the use of this endophytic bacteria for development of biocontrol agent.

**Acknowledgements:** This study is granted by Graduate University of Science and Technology (GUST), Vietnam Academy of Science and Technology (VAST) under the Project number: GUST.STS.ĐT2017-SH05.

The authors would like to thank the Mixed International Laboratory LMIRICE-2 and Agricultural Genetics Institute for providing the strains *Xanthomonas oryzae* pv. *oryzae*. The authors also dedicated the gratefulness to Department of Plant Cell Biotechnology, Institute of Biotechnology, VAST and the University of Science and Technology of Hanoi, VAST for their facilitations to perform this research.

## REFERENCES

- Ahemad M., Kibret M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King. Saud. Univ. - Sci.*, 26: 1–20.
- Cappuccino J. G., Welsh C., 2017. Microbiology: A Laboratory Manual, 11<sup>th</sup> edition, Global edition. Pearson Education Limited, England, 560.
- Choodamani M. S., Hariprasad P., Sateesh M. K., Umesha S., 2009. Involvement of catalase in bacterial blight disease development of rice caused by *Xanthomonas oryzae* pv. *oryzae*. *Int. J. Pest. Manag.*, 55: 121–127.
- El-shakh A. S. A., Kakar K. U., Wang X., Almoneafy A. A., Ojaghian M. R., Li B., Anjum S. I., Xie G., 2015. Controlling bacterial leaf blight of rice and enhancing the plant growth with endophytic and rhizobacterial *Bacillus* strains. *Toxicol. Environ. Chem.*, 97: 766–785.
- Fitri L., Ismail Y. S., Putriani P., Warzatullisna W., 2020. Application of Rice Root Endophytic Bacteria in Ciherang Variety Rice (*Oryza sativa*) Seeds. *Biosaintifika*, 12: 21–27.
- Hameed A., Iqbal N., 2014. Chemo-priming with mannose, mannitol and H<sub>2</sub>O<sub>2</sub> mitigate drought stress in wheat. *Cereal. Res. Commun.*, 42: 450–462.
- Inoue K., Suzuki T., Ikeda K., Jiang S., Hosogi N., Hyong G., Hida S., Yamada T., Park P., 2007. Extracellular matrix of *Magnaporthe oryzae* may have a role in host adhesion during fungal penetration and is digested by matrix metalloproteinases. *J. Gen. Plant Pathol.*, 73: 388–398.
- Jeung J. U., Heu S. G., Shin M. S., Vera Cruz C. M., Jean K. K., 2006. Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology*, 96: 867–875.
- Kennedy I. R., Choudhury A. T. M. A., Kecskés M. L. and Rose M. T., 2008. Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers. Proceedings of a project (SMCN/2002/073) workshop held in Hanoi, Vietnam, 12–13 October 2007. ACIAR Proceedings No. 130, pp. 49–59.
- Leboffe M. J., Pierce B. E., 2010. Microbiology laboratory theory and application, 3rd ed. Morton Publishing Company, Englewood, CO.
- Ly Ngoc Thanh Xuan, Tran Van Dung, Ngo Ngoc Hung, Cao Ngoc Diep, 2016a. Isolation and characterization of rice endophytic bacteria in acid sulphate soil of Mekong delta, Vietnam. *World J Pharm Pharm Sci.*, 5: 301–317.
- Ly Ngoc Thanh Xuan, Tran Van Dung, Ngo Ngoc Hung, Cao Ngoc Diep, 2016b. Isolation and characterization of rhizospheric bacteria in rice (*Oryza sativa* L.) cultivated on acid sulphates soils of the Mekong delta, Vietnam. *World J Pharm Pharm Sci.*, 5: 343–358.
- Mew T. W., Alvarez A. M., Leach J. E., Swings J., 1993. Focus on bacterial blight of rice. *Plant Dis.*, 77: 5–12.
- Moronta-Barrios F., Gionechetti F., Pallavicini A., Marys E., Venturi V., 2018. Bacterial microbiota of rice roots: 16S-based taxonomic profiling of endophytic and rhizospheric diversity, endophytes isolation and simplified endophytic community. *Microorganisms*, 6: 14.
- Niño-Liu D. O., Ronald P. C., Bogdanove A. J., 2006. *Xanthomonas oryzae* pathovars:

- Model pathogens of a model crop. *Mol. Plant. Pathol.*, 7: 303–324.
- Nguyen Dac Khoa, Nguyen Dang Ngoc Giau, Tran Quoc Tuan, 2016. Effects of *Serratia nematodiphila* CT-78 on rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Biol. Control*, 103: 1–10.
- Nguyen Huynh Nha Uyen, Nguyen Thi Cam Van, Nguyen Dac Khoa, 2018. Comparison of the disease-reducing effects of the antagonistic *Bacillus* sp. *Serratia nematodiphila* against *Xanthomonas oryzae* pv. *oryzae* causing rice bacterial leaf blight. *Can Tho Univ. J. Sci.*, 54(9B): 59–66. (in Vietnamese with English summary).
- Nguyen Ngoc Lan, Vu Van Dung, Nguyen Thi Kim Lien, Nguyen Kim Thoa, Do Huu Nghi, Nguyen Huy Hoang, 2019. Isolation and identification of indole acetic acid producing bacteria from the coasts of Ben Tre and Tra Vinh provinces. *Academia Journal of Biology*, 41(4): 55–67.
- Nguyen Van Phuong, Mai Thi Phuong Nga, To Thi Mai Huong, Mai Duc Chung, Chu Hoang Ha, Le Tran Binh, 2020. *In vitro* screening for plant growth promoting traits and antifungal activity against *Magnaporthe oryzae* from submerged rice root endophytic bacteria. Proceedings of 2020 Vietnam National Conference on Biotechnology. Publishing House Hue University, pp. 568–574.
- Olanrewaju O. S., Glick B. R., Babalola O. O., 2017. Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol.*, 33: 1–16.
- Phan Thi Cong, Tran Dang Dung, Tran Minh Hien, Nguyen Thanh Hien, Abu T. M. A. Choudhury, Mihaly L. Kecskes, Ivan R. Kennedy, 2009. Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. *Eur. J. Soil Biol.*, 45: 52–61.
- Reinhold-Hurek B., Hurek T., 2011. Living inside plants: Bacterial endophytes. *Curr. Opin. Plant Biol.*, 14: 435–443.
- Sen S., Chandrasekhar C. N., 2014. Effect of PGPR on growth promotion of rice (*Oryza sativa* L.) under salt stress. *Asian J. Plant Sci. Res.*, 4: 62–67.
- Sofa A., Scopa A., Nuzzaci M., Vitti A., 2015. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int. J. Mol. Sci.*, 16: 13561–13578.
- Hoang Minh Tam, Cao Ngoc Diep, 2017. Isolation and characterization of endophytic bacteria isolated from the sugarcane cultivated on acrisols of Tay Ninh province, Vietnam. *Int. J. Innov. Eng. Technol.*, 8: 222–236.
- Trinh Thanh Trung, Dinh Thi Tuyet Van, Nguyen Phuong Lien, Dao Thi Luong, Duong Van Hop, 2017. Potential application on preparation for bio-fertilizer using *Bacillus velezensis* strains isolated from various regions in Vietnam. *J. Biotechnol.*, 15: 169–179 (in Vietnamese with English summary).
- Van Thi Phuong Nhu, Cao Ngoc Diep, 2014. Isolation, characterization and phylogenetic analysis of endophytic bacteria in rice plant cultivated on soil of Phu Yen province, Vietnam. *Am. J. Life Sci.*, 2: 117–127.
- White J. F., Kingsley K. L., Zhang Q., Verma R., Obi N., Dvinskikh S., Elmore M. T., Verma S. K., Gond S. K., Kowalski K. P., 2019. Review: Endophytic microbes and their potential applications in crop management. *Pest. Manag. Sci.*, 75: 2558–2565.
- Zhao L. F., Xu Y. J., Lai X. H., 2018. Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. *Brazilian J. Microbiol.*, 49: 269–278.