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The Survival of Endophytic Bacteria Isolated from Jerusalem Artichoke In Drought Conditions

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Abstract:Four endophytic bacteria isolated from Jerusalem artichoke had the ability of plant growth promotion. These bacteria, isolate 3.13, 4.43, 5.2, and 5.18, were selected to study the promotion of plant growth in drought conditions. Thus, the survival of the bacteria under drought conditions needed for the investigation. All four isolates showed Nutrient Broth (NB) growth containing 20% Polyethylene Glycol (PEG). Moreover, all four isolates were tested for their survival in NB with 20% PEG and soil by incubating at 30, 35, 40, and 45°C. Reasonable survival was observed in all isolates at 30 to 40°C of both media. Furthermore, isolate 5.18 demonstrated the growth at 45°C and maintained cell numbers at 4.19×10^4 CFU/mL after 29 days and 1.30×10^7 CFU/mL after 57 days in NB with 20% PEG and in the soil, respectively.

Keywords: *Endophytic bacteria, PEG, drought tolerance, Jerusalem artichoke*

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I. INTRODUCTION

Drought is one of the major disasters for food production worldwide and is estimated to impact negatively on national cereal production [1]. Limitation of water supply in drought conditions affects plant growth, leading to decreasing crop yields. Drought is considered to be a big problem for Jerusalem artichoke production in rain-starved areas, as it reduces inulin accumulation in tubers [2, 3].

Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuber crop native to North America, and widespread in Thailand and India. It is a valuable source of inulin [4, 5]. Jerusalem artichoke harvest is normally in early spring season [6]. The tubers are elongated and uneven, typically 7.5-10 cm long and 3-5 cm thick [7]. This tuber plant is helpful for healthy diets, such as reducing the risk of heart disease and diabetes mellitus as well as lowering blood cholesterol level. In addition, improvement of the immune system via the increase of beneficial intestinal bacteria is observed in humans who eats this tuber plant [8]. Moreover, Jerusalem artichoke can be used to produce ethanol in biofuel production, tea in the beverage industry and animal feed in agriculture [9]. The study of Sritongon et al. [10] showed that beneficial bacteria such as Rhizobacteria could promote the growth of Jerusalem artichoke via increasing shoot dry weight, root dry weight, and biomass. There is a possibility that endophyte may

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be able to enhance Jerusalem artichoke performance in drought conditions. In this study, four endophytic bacteria isolates (3.13, 4.43, 5.2 and 5.18) isolated from Jerusalem artichoke in Thailand were studied the ability to grow or survive in drought conditions. If they grow under drought condition, they may be grow in Jerusalem artichoke and help Jerusalem artichoke in drought stress. The preliminary study of endophytic bacteria survival in drought condition will lead to apply in promoting Jerusalem artichoke growth under drought stress.

II. LITERATURE REVIEW

A. *Endophytic Bacteria*

Endophytic bacteria that are considered beneficial bacteria for plants have been found in every part of the plant. They colonize in plant apoplast, including the intercellular spaces of the cell walls and xylem vessels of plant roots, stems and leaves, and they are also found in tissues, flowers, fruits and seeds [11]. Population densities of endophytic bacteria are extremely variable in different plants and tissues and have been shown vary from hundreds to reaching as high as 9×10^9 of bacteria per gram of plant tissue [12, 13, 14]. Endophytic bacteria have been reported in several plants such as wheat [15], potato [16], banana[17], papaya [18]. They can be defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host [19, 20]. They can improve plant performances in stress environments by producing indole-3-acetic acid (IAA) [21], proline [22], ACC (1-Aminocyclopropane-1-Carboxylate) deaminase and exopolysaccharides [23]. A study of [24] in *Festuca arundinacea* grasses containing endophytic bacteria showed that, when dehydrated, the osmotic pressure adjustment was increased to maintain plant cell proliferation; the plant could maintain both physiological and biochemical functions.

IAA is important for plant growth and development. Various plant species inoculated with IAA producing bacteria increased root growth and enhanced formation of lateral roots and roots hairs [25] which were resulted from increasing water and nutrient uptake [26]. Inoculation of canola seeds with *Pseudomonas putida* GR12-2, which produced low levels of IAA, resulted in two- or three-fold increases in the length of seedling roots [27].

Proline is an important osmoregulator, accumulated as a consequence of drought stress. Creus et al. [28] found that *Azospirillum* stimulated the growth of wheat seedlings grown in darkness under osmotic stress, together with a significant decrease in osmotic potential.

ACC deaminase-producing bacteria can cleave the plant ethylene precursor which is ACC, thereby lowering the level of ethylene in stress plants. The lowering of ethylene concentration in root vicinity is helpful for promoting root growth [29]. Mayak et al. [30] studied in plant growth promoting bacteria that had ACC deaminase activity; the bacteria were isolated from soil samples taken from the Arava region of southern Israel. One of these strains, *Achromobacter piechaudii* ARV8 significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. In addition, the bacterium reduced the production of ethylene of tomato seedlings, following water stress. Bal et al. [31] reported that inoculation with selected PGPR isolates had considerable positive impacts on different growth parameters of rice including germination percentage, shoot and root growth and chlorophyll content compared to uninoculated control. Inoculation with the ACC deaminase producing strains reduced ethylene production under salinity stress.

Exopolysaccharide (EPS) production by bacteria protects themselves from inhospitable conditions and enables their survival. The capsular material of *Azospirillum brasilense* Sp245 contains high molecular weight carbohydrate complex responsible for the protection and enhanced survival under drought stress [32].

III. METHODOLOGY

A. *The testing Ability of Endophytic Bacteria in Drought Conditions*

The potential isolate 3.13, 4.43, 5.2 and 5.18, were selected to use in this study as they could produce IAA, ACC deaminase, potassium and phosphorus solubilization which are a good property for helping plant growth. The four isolates were grown in nutrient broth at 30°C with shaking 150 rpm for 24 hrs as a starter. To create the stress condition, polyethylene glycol 6000 (PEG) was added into nutrient broth medium with various concentrations at 10% 20% and 30% (w/v). The media were then inoculated with 1% of overnight starter, incubated at 30°C with shaking 150 rpm. The growth of bacteria was determined at day 0, 3, 5 and 7 by comparing with a different number of McFarland standard (Table 1).

B. *Survival of Endophytic Bacteria in Drought Conditions and High Temperature*

Isolate 3.13, 4.43, 5.2 and 5.18 showed the dominant growth in nutrient broth containing 20% PEG. Therefore, 1% of bacterial starter were inoculated in nutrient broth with 20% PEG, performing in triplication, and incubated at 30, 35, 40 and 45 °C.

The sample was determined at 0, 5, 10, 15 and the final period of 60 days. 0.1 mL of suitable dilution was sp-

read onto NA, incubating at 30°C for 24 h before counting the colony.

TABLE 1
MCFARLAND STANDARDS [33]

McFarland Standard No.	0.5	1	2	3	4	5	6	7	8	9	10
Absorbance*	0.08	0.1	0.2	0.4	0.5	0.65	0.85	1.0	1.1	1.2	1.4
Approximate Cell Count Density ($\times 10^8$ cells)	1.5	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0

*at wavelength of 600 nm

C. *Survival of Endophytic Bacteria in Soil at a High Temperature*

Five grams of soil were placed into a plastic bag (covered cotton plug), with triple preparation, and sterilized with autoclave at 121°C for one hr. The bags were left at room temperature for 24 h and then autoclaved again. For endophytic bacteria, the four isolates grew in NB at 30°C, 48 h and then centrifuged to keep the cell at 5,000 rpm, for 10 min. The cell pellet was resuspended in 0.85% NaCl by adjusting cell concentration of 108 CFU/mL. Each plastic bag was inoculated with 5 mL of cell suspension which had a cell density approximate 108 CFU/g of soil and 5 mL of 0.85% NaCl as a control. The humidity of the soil was adjusted by sterilized distilled water, reaching 50% of moisture. The bag was incubated at 30, 35, 40 and 45°C and samples were determined at 0, 5, 10, 15 and a final period of 60 days. 45 mL of sterile 0.85% NaCl diluent was transferred into the plastic bag, and 0.1 mL of suitable dilution was spread on NA, incubating at 30°C for 24 h before counting the colony (Modified method from [34]).

IV. RESULTS

All four isolates, 3.13, 4.43, 5.2 and 5.18, showed increased growth in drought condition at 20% PEG. The growth of isolate 4.43 and 518 increased 4 fold at three days and slow growth until seven days with 6 and 5 fold of beginning, respectively (Table 2).

TABLE 2
THE TURBIDITY OF BACTERIAL GROWTH IN NUTRIENT BROTH WITH 20% PEG

Isolate/Days	0	3	5	7
3.13	1	3	3	4
4.43	1	4	4	6
5.2	1	3	3	4
5.18	1	4	4	5

While, the isolate 3.13 and 5.2 were able to grow more than 4 fold at seven days. This indicated that they could grow in water limitation. In nutrient broth with 20% PEG, all 4 isolates could survive in temperature at 30-40°C, while the cell numbers of isolate 3.13 and 4.43 were reduced dramatically on 5 days at 45°C and isolate 5.2 were reduced dramatically on ten days at 45°C (Figure 1). The isolate 5.18 could tolerant at 45°C nearly one month before cell reduction. The growth of each isolate at different temperatures showed significant differences during incubation times (Table 3, Table 4, Table 5, Table 6). The cell number decreased similarly in all isolates at 30 and 35°C. Also, similar trend of reduction in isolate 3.13, 5.2 and 5.18 was observed at 40°C, but showed more reduction in the isolate 4.43. The isolate 5.18 was outstanding growth at every temperatures. This indicated that the isolate 5.18 could be tolerant in drought and high temperature.

In a parallel experiment, the isolate 3.13 and 4.43 showed similar results in nutrient broth with 20% PEG. The cell could survive in soil at a temperature of 30-40°C nearly two month, having 8.26×10^5 and 1.16×10^6 CFU/g, respectively, and could survive at 45°C for 15 days (Figure 2). The cell number of all isolates showed significant differences at all temperatures. At 30 and 35°C, the isolate 3.13, 4.43 and 5.2 showed the same reduction of the cell (Table 7, Table 8). The cell number of isolate 5.2 was reduced more than other isolates

at 40°C; at this temperature the cell of isolate 3.13 and 4.43 remained as same as in soil at 30 and 35°C (Table 9). The high temperature had more effects on the isolate 5.2 growth in soil condition. The isolate 5.18 could be tolerant in all temperatures, particularly at 45°C (Table 10), resulting in high cell number remaining at 1.3×10^7 CFU/g.

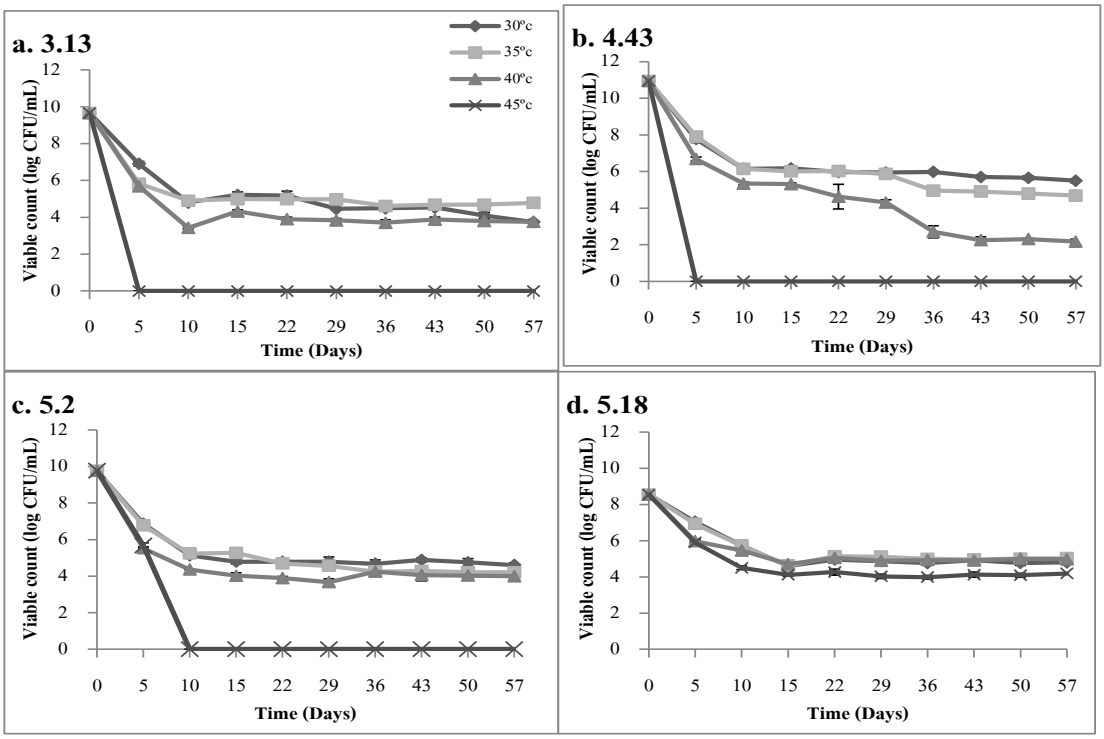

Fig. 1. Survival of endophytic bacteria isolates 3.13(a), 4.43(b), 5.2(c) and 5.18(d) in nutrient broth containing 20% PEG in different temperatures

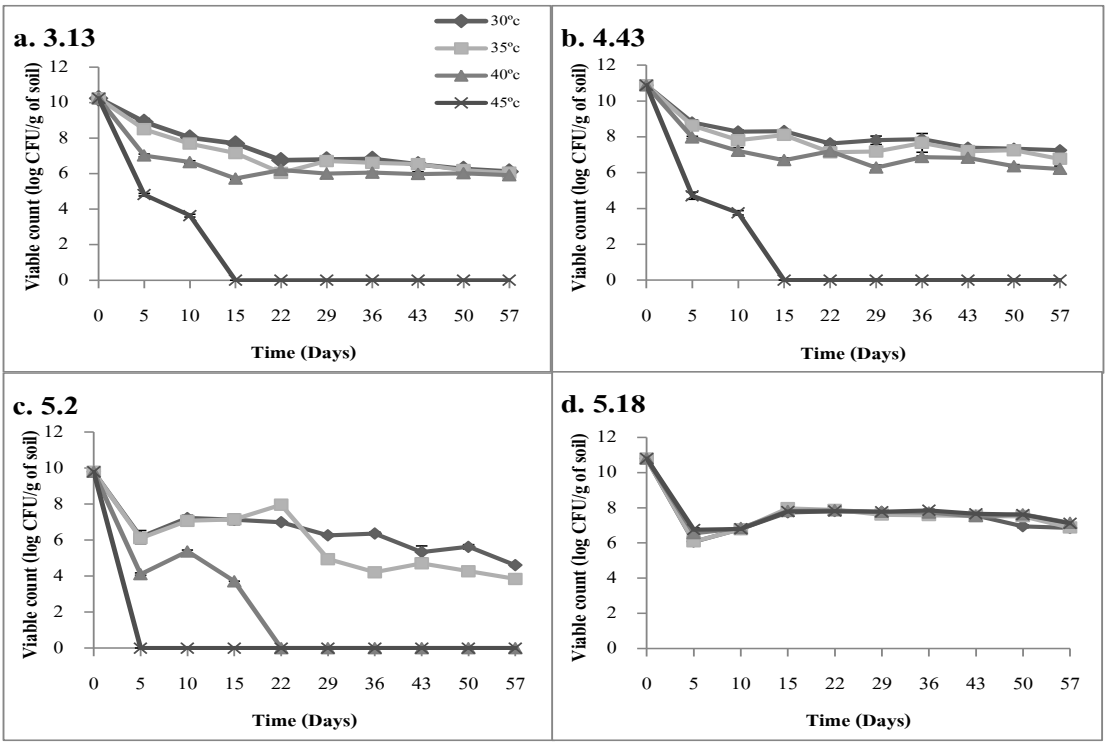

Fig. 2. Survival of endophytic bacteria isolates 3.13(a), 4.43(b), 5.2(c) and 5.18(d) in soil in different temperatures

TABLE 3
SURVIVAL OF ENDOPHYTIC BACTERIA IN NUTRIENT BROTH CONTAINING 20% PEG AT 30°C

Days/Isolate	Viable count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	9.66 ± 0.12^{a}	10.94 ± 0.04^{a}	9.76 ± 0.03^{a}	8.55 ± 0.06^{a}
5	6.90 ± 0.10^{b}	7.77 ± 0.19^{b}	6.86 ± 0.11^{b}	7.04 ± 0.09^{b}
10	4.80 ± 0.19^{d}	6.15 ± 0.04^{cd}	5.13 ± 0.02^{c}	5.74 ± 0.07^{c}
15	5.21 ± 0.16^{c}	6.17 ± 0.10^{c}	4.79 ± 0.12^{de}	4.60 ± 0.29^{e}
22	5.18 ± 0.24^{c}	5.96 ± 0.07^{de}	4.78 ± 0.06^{de}	4.95 ± 0.20^{d}
29	4.46 ± 0.03^{e}	5.94 ± 0.18^{e}	$4.78\pm0.26d^{e}$	4.87 ± 0.17^{d}
36	4.48 ± 0.08^{e}	5.97 ± 0.07^{de}	4.67 ± 0.22^{de}	4.76 ± 0.089^{de}
43	4.53 ± 0.09^{e}	5.70 ± 0.07^{f}	4.88 ± 0.09^{d}	4.91 ± 0.12^{d}
50	4.09 ± 0.16^{f}	5.65 ± 0.10^{fg}	4.75 ± 0.21^{de}	4.76 ± 0.13^{de}
57	3.73 ± 0.15^{g}	5.49 ± 0.11^{g}	4.60 ± 0.12^{e}	4.80 ± 0.13^{de}
F-test	**	**	**	**
%CV	2.72	1.68	2.65	2.72

**: highly significant at $p \leq 0.01$

TABLE 4
SURVIVAL OF ENDOPHYTIC BACTERIA IN NUTRIENT BROTH CONTAINING 20% PEG AT 30°C

Days/Isolate	Viable count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	9.66 ± 0.12^{a}	10.94 ± 0.04^{a}	9.76 ± 0.03^{a}	8.55 ± 0.06^{a}
5	5.83 ± 0.11^{b}	7.90 ± 0.10^{b}	6.77 ± 0.19^{b}	6.93 ± 0.15^{b}
10	4.90 ± 0.15^{cd}	6.14 ± 0.04^{c}	5.23 ± 0.14^{c}	5.72 ± 0.20^{c}
15	4.99 ± 0.08^{c}	6.01 ± 0.14^{c}	5.27 ± 0.14^{c}	4.62 ± 0.15^{e}
22	4.79 ± 0.18^{c}	6.03 ± 0.19^{c}	4.69 ± 0.12^{d}	5.13 ± 0.12^{d}
29	4.97 ± 0.19^{c}	5.86 ± 0.19^{c}	4.56 ± 0.18^{d}	5.12 ± 0.11^{d}
36	4.61 ± 0.18^{e}	4.95 ± 0.25^{d}	4.24 ± 0.11^{e}	4.99 ± 0.15^{d}
43	4.68 ± 0.23^{de}	4.90 ± 0.24^{d}	4.28 ± 0.19^{e}	4.96 ± 0.11^{d}
50	4.68 ± 0.21^{de}	4.79 ± 0.13^{d}	4.21 ± 0.18^{e}	5.01 ± 0.05^{d}
57	$4.78\pm0.11c^{de}$	4.68 ± 0.28^{d}	4.21 ± 0.13^{e}	5.02 ± 0.12^{d}
F-test	**	**	**	**
%CV	3.01	2.88	2.79	2.32

**: highly significant at $p \leq 0.01$

TABLE 5
SURVIVAL OF ENDOPHYTIC BACTERIA IN NUTRIENT BROTH CONTAINING 20% PEG AT 40°C.

Days/Isolate	Viable count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	9.66 ± 0.12^{a}	10.94 ± 0.04^{a}	9.76 ± 0.03^{a}	8.55 ± 0.06^{a}
5	5.68 ± 0.05^{b}	6.68 ± 0.11^{b}	$5.52\pm0.08b^{b}$	5.97 ± 0.10^{b}
10	3.42 ± 0.06^{f}	5.33 ± 0.08^{c}	4.36 ± 0.00^{c}	5.45 ± 0.15^{c}

**: highly significant at $p \leq 0.01$

TABLE 5 CONTINUEE..

Days/Isolate	Viable count (log CFU/mL)			
	3.13	4.43	5.2	5.18
15	4.31 ± 0.09^{c}	5.31 ± 0.07^{c}	4.02 ± 0.17^{de}	4.70 ± 0.20^{e}
22	3.90 ± 0.11^{d}	4.62 ± 0.67^{d}	3.89 ± 0.08^{ef}	5.04 ± 0.25^{d}
29	3.83 ± 0.11^{de}	4.30 ± 0.15^{d}	3.67 ± 0.18^{f}	4.89 ± 0.18^{de}
36	3.70 ± 0.16^{e}	2.71 ± 0.33^{e}	4.24 ± 0.05^{cd}	4.93 ± 0.09^{de}
43	3.87 ± 0.13^{de}	2.25 ± 0.18^{f}	4.05 ± 0.31^{de}	4.90 ± 0.09^{de}
50	$3.80\pm0.09d^{de}$	2.31 ± 0.03^{ef}	4.03 ± 0.22^{de}	5.00 ± 0.12^{d}
57	3.75 ± 0.11^{de}	2.18 ± 0.12^{f}	3.99 ± 0.20^{de}	4.97 ± 0.16^{d}
F-test	**	**	**	**
%CV	2.37	5.50	3.57	2.80

**: highly significant at $p \leq 0.01$

TABLE 6
SURVIVAL OF ENDOPHYTIC BACTERIA IN NUTRIENT BROTH CONTAINING 20% PEG AT 45∘C

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	9.66 ± 0.12	10.94 ± 0.04	9.76 ± 0.03^{a}	8.55 ± 0.06^{a}
5	0	0	5.67 ± 0.14^{b}	5.89 ± 0.07^{b}
10	0	0	0	4.49 ± 0.10^{c}
15	0	0	0	4.11 ± 0.08^{def}
22	0	0	0	4.26 ± 0.16^{d}
29	0	0	0	4.03 ± 0.13^{ef}
36	0	0	0	3.89 ± 0.10^{f}
43	0	0	0	4.12 ± 0.14^{def}
50	0	0	0	4.09 ± 0.11^{def}
57	0	0	0	4.18 ± 0.02^{de}
F-test	nd	nd	**	**
%CV	4.14	1.20	3.06	2.20

**: highly significant at $p \leq 0.01$

TABLE 7
SURVIVAL OF ENDOPHYTIC BACTERIA IN SOIL AT 30°C

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	10.23 ± 0.10^{a}	10.87 ± 0.12^{a}	9.79 ± 0.03^{a}	10.79 ± 0.11^{a}
5	8.88 ± 0.20^{b}	8.77 ± 0.15^{b}	6.08 ± 0.37^{c}	6.07 ± 0.02^{e}
10	8.02 ± 0.05^{c}	8.28 ± 0.08^{c}	7.21 ± 0.14^{b}	6.79 ± 0.05^{d}
15	0.76 ± 0.07^{d}	8.32 ± 0.06^{c}	7.09 ± 0.23^{b}	7.71 ± 0.15^{b}
22	6.74 ± 0.07^{e}	7.61 ± 0.06^{e}	6.99 ± 0.05^{b}	7.80 ± 0.05^{b}
29	6.76 ± 0.13^{e}	7.77 ± 0.12^{de}	6.26 ± 0.06^{c}	7.76 ± 0.02^{b}
36	6.81 ± 0.12^{e}	7.86 ± 0.23^{d}	6.36 ± 0.08^{c}	7.65 ± 0.17^{bc}
43	6.52 ± 0.04^{f}	7.39 ± 0.08^{f}	5.23 ± 0.35^{e}	7.55 ± 0.06^{c}

TABLE 7 CONTINUEE..

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
50	6.25±0.08^{g}	7.33±0.06^{f}	5.61±0.11^{d}	6.94±0.07^{d}
57	6.10±0.12^{g}	7.24±0.05^{f}	4.60±0.07^{f}	6.85±0.08^{e}
F-test	**	**	**	**
%CV	1.47	1.38	2.91	1.22

**: highly significant at $p \leq 0.01$

TABLE 8
SURVIVAL OF ENDOPHYTIC BACTERIA IN SOIL AT 35°C

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	10.23±0.10^{a}	10.87±0.12^{a}	9.79±0.03^{a}	10.79±0.11^{a}
5	8.62±0.12^{b}	8.62±0.08^{b}	6.08±0.21^{c}	6.08±0.06^{f}
10	7.67±0.14^{c}	7.80±0.07^{c}	7.07±0.06^{b}	6.77±0.010^{d}
15	7.15±0.11^{d}	8.08±0.15^{c}	7.09±0.29^{b}	7.95ś±.14^{b}
22	6.02±0.16^{f}	7.13±0.11^{d}	3.91±0.27^{f}	7.86±0.11^{b}
29	6.66±0.22^{e}	7.14±0.20^{d}	4.93±0.15^{d}	7.60±0.06^{c}
36	6.60±0.08^{e}	7.96±0.52^{c}	4.26±0.03^{e}	7.57±0.06^{c}
43	6.53±0.06^{e}	7.19±0.08^{d}	4.69±0.15^{d}	7.53±0.03^{c}
50	6.19±0.07^{f}	7.24±0.10^{d}	4.26±0.10^{e}	6.51±0.03^{e}
57	6.04±0.06^{f}	6.76±0.09^{e}	3.84±0.08^{f}	6.86±0.10^{d}
F-test	**	**	**	**
%CV	1.71	2.52	2.94	1.14

**: highly significant at $p \leq 0.01$

TABLE 9
SURVIVAL OF ENDOPHYTIC BACTERIA IN SOIL AT 40°C

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	10.23±0.10^{a}	10.87±0.12^{a}	9.79±0.03^{a}	10.79±0.11^{a}
5	7.01±0.08^{b}	7.96±0.06^{b}	7.11±0.06^{b}	6.54±0.07^{g}
10	6.64±0.08^{b}	7.18±0.19^{c}	5.36±0.08^{c}	6.83±0.10^{f}
15	5.72±0.04b^{c}	6.68±0.12^{d}	3.72±0.00^{d}	7.81±0.05^{bc}
22	6.20±0.04^{b}	7.19±0.08^{c}	0	7.85±0.06^{b}
29	6.00±0.003^{b}	6.26±0.14^{e}	0	7.76±0.05^{bc}
36	6.05±0.07^{b}	6.87±0.06^{d}	0	7.72±0.07^{c}
43	5.96±0.13^{b}	6.81±0.12^{d}	0	7.51±0.02^{d}
50	4.00±0.19^{c}	6.35±0.09^{e}	0	6.62±0.04^{g}
57	5.90±0.11^{b}	6.19±0.15^{e}	0	7.14±0.07^{e}
F-test	**	**	**	**
%CV	1.72	1.65	2.89	0.88

**: highly significant at $p \leq 0.01$

TABLE 10
SURVIVAL OF ENDOPHYTIC BACTERIA IN SOIL AT 45°C

Days/Isolate	Viable Count (log CFU/mL)			
	3.13	4.43	5.2	5.18
0	10.23 ± 0.10^{a}	10.87 ± 0.12^{a}	9.79 ± 0.03	10.79 ± 0.11^{a}
5	4.81 ± 0.08^{b}	4.68 ± 0.20^{b}	0	6.75 ± 0.10^{ef}
10	3.63 ± 0.07^{c}	3.74 ± 0.12^{c}	0	6.79 ± 0.10^{e}
15	0	0	0	7.78 ± 0.08^{bc}
22	0	0	0	7.81 ± 0.05^{b}
29	0	0	0	7.77 ± 0.07^{bc}
36	0	0	0	7.85 ± 0.07^{b}
43	0	0	0	7.65 ± 0.12^{c}
50	0	0	0	6.59 ± 0.14^{f}
57	0	0	0	7.11 ± 0.05^{d}
F-test	**	**	nd	**
%CV	2.51	4.24	0.99	1.21

**: highly significant at $p \leq 0.01$

V. DISCUSSION AND CONCLUSION

The results in the present study provide evidence that endophytic bacteria isolate 3.13, 4.43, 5.2 and 5.18 showed the ability of growth in water limitation, nutrient broth containing 20% PEG. Many endophytic bacteria can grow in medium containing PEG, for example, *Bacillus pumilus* strain DH-11 and *Bacillus firmus* strain 40 in medium containing 10% PEG [35]. *Bacillus thuringiens* is grown under osmotic stress [induced with 40% PEG (equivalent to -3.99 MPa)], decreased cell growth but also certain plant growth promoting abilities [36]. Marulanda et al. [37] studied about microorganisms that could increase drought tolerance to plants growing under water limitation conditions. They found that *Pseudomonas putida*, *Pseudomonas* sp. and *Bacillus megaterium* were grown in osmotic stress caused by 60% PEG. When the osmotic pressure in the surrounding environment increased, cell activated osmoregulation systems to prevent shrinkage and eventual plasmolysis.

Survival test took place in different temperature both in soil and in nutrient broth. Isolate 5.18 was able to grow at 45°C and maintained the cell number at 4.19×10^4 CFU/mL and 1.30×10^7 CFU/mL after 57 days in nutrient broth and in the soil, respectively. Sritongon [10] reported that *Rhodococcus cercidiphylli* SI-903 and *Pseudomonas azotoformans* C2-114 were inoculated the in the soil for survival test until 60 days. The results of the cell occupied in the carriers decreased 2 log units at day 60. Normally, endophytic bacteria grow at 30-35°C, but some strain can grow at high temperatures, i.e., *Microbacterium oxydans* (40°C), *Ochrobactrum intermedium* (40°C), *Stenotrophomo nasmaltophilia* (45°C), *Paenibacillus* sp. (50°C) and *Brevibacillus nitrificans* (50°C). When bacteria grow at high temperature, they have a mechanism to protect themselves. The heat shock response in bacteria is a protective mechanism to cope with heat-induced damage to proteins by synthesizing a specific set of proteins known as heat shock proteins (HSPs) [38, 39].

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