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Screening of Rhizobacteria for Plant Growth Promotion and Their Tolerance to Drought Stress

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Rhizobacteria have been known for their capability as plant growth promoter through some mechanisms, directly and indirectly. The purpose of this research to screen rhizobacteria of *Bacillus* spp. and *Pseudomonas* spp. for their drought tolerance as plant growth promoter of maize (*Zea mays*). Screening of rhizobacteria as growth promoter of 47 isolates of *Bacillus* sp. CR and 34 isolates of *Pseudomonas* sp. CRB resulted 24 and 9 isolates were able to stimulate the growth of maize sprouts, respectively. Further screening of those growth promoter of the rhizobacterial isolates to drought tolerance resulted 7 isolates of *Bacillus* sp. CR and 6 isolates of *Pseudomonas* sp. CRB that were able to grow on medium with osmotic pressure -1 and -2 MPa, respectively. Potential rhizobacterial isolates of growth promoter and drought tolerance were tested for antagonist mechanisms which aims to determine ability to live together in one carrier medium if to be made formulation. Both non antagonist rhizobacterial isolates were evaluated for their potential in producing exopolysaccharide (EPS) revealing that CRB 19 and CR 90 exhibited the highest activity of EPS production up to 0.346 mg mL^{-1} on medium with -2.0 MPa and 0.107 mg mL^{-1} on medium with -0.73 MPa, respectively. Based on 16S rRNA sequence analysis, it revealed CRB 19 and CR 90 had highest similarities to *Pseudomonas aeruginosa* strain B2 and *Brevibacillus brevis* B33, respectively. Those growth promoter and drought tolerant of *Bacillus* sp. CR and *Pseudomonas* sp. CRB had potency to be developed as inoculants in dry land agriculture.

Key words: *Bacillus* sp. CR, drought tolerant, growth promoter, *Pseudomonas* sp. CRB, rhizobacteria

Rizobakteria diketahui memiliki kemampuan sebagai pemacu tumbuh tanaman melalui beberapa mekanisme, baik secara langsung maupun tidak langsung. Penelitian ini bertujuan untuk menyeleksi rizobakteria *Bacillus* spp. dan *Pseudomonas* spp. toleran kekeringan sebagai pemacu pertumbuhan tanaman jagung (*Zea mays*). Penapisan rizobakteria pemacu tumbuh dari 47 isolat *Bacillus* sp. CR dan 34 isolat *Pseudomonas* sp. CRB mendapatkan masing-masing 24 dan 9 isolat yang secara signifikan memacu pertumbuhan. Isolat pemacu tumbuh selanjutnya diseleksi terhadap toleran kekeringan, lalu didapatkan 7 isolat *Bacillus* sp. CR dan 6 isolat *Pseudomonas* sp. CRB yang dinyatakan toleran kekeringan. Masing-masing isolat mampu tumbuh pada media dengan tekanan osmotik -1 dan -2 MPa. Isolat potensial pemacu tumbuh toleran kekeringan diuji sifat antagonisnya untuk mengetahui kemampuannya hidup bersama pada satu media pembawa jika dibuat formula. Isolat yang tidak saling antagonis selanjutnya dievaluasi potensinya dalam menghasilkan eksopolisakarida. CRB 19 dan CR 90 masing-masing menghasilkan eksopolisakarida tertinggi hingga 0.346 mg mL^{-1} pada medium -2.0 MPa dan 0.107 mg mL^{-1} pada medium -0.73 MPa. Berdasarkan sekuens gen 16S rRNA, CRB 19 memiliki kemiripan tertinggi dengan *Pseudomonas aeruginosa* strain B2 dan CR 90 dengan *Brevibacillus brevis* B33. *Bacillus* sp. CR dan *Pseudomonas sp.* CRB pemacu tumbuh dan toleran kekeringan tersebut berpotensi untuk dikembangkan sebagai inokulan di lahan kering.

Kata kunci : *Bacillus* sp. CR, pemacu tumbuh, *Pseudomonas* sp. CRB, rizobakteria, toleran kekeringan

Rhizospheres are containing microorganisms that could increase plant growth. These bacteria are call plant growth promoting rhizobacteria (PGPR) that have a significant effect for plant growth, directly and indirectly. PGPR have been capability in producing indole acetic acid (IAA) hormone, exopolysaccharide (EPS), ACC-deaminase, as phosphate solubilizing, and biocontrol agents of pathogenic fungi (Dey *et al.* 2004; Kaci *et al.* 2005; Husen *et al.* 2011). IAA hormones is one of the plant growth hormone that plays a role in several aspects of plant growth, including cell division and elongation, differentiation, tropisms, apical dominance, aging, abscission, and flowering (Zhao *et al*. 2001).

Drought can affect microorganisms and plant growth since water becomes a limiting factor for plants

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and microorganisms to survive. One way drought adaptation of microorganisms are secretion of exopolysaccharide (EPS) in higher amounts. EPS are structural component of extracellular matrix in biofilms are synthesized by cells to respond physiological stress in the environment (Marvasi *et al*. 2010). EPS were produced as response biotic and abiotic stress factors to adaptation in extreme environments. The main function is to assist the protection against environmental stresses. Microorganisms such as *Agrobacterium* sp., *Alcaligenes faecalis*, *Xanthomonas campestris*, *Bacillus* sp., *Zygomonas mobilis*, *Leuconostoc*, *Pseudomonas* sp., *Acetobacter xylinum*, and several other genera of microorganisms are known to produce EPS (Donot *et al*. 2011). EPS production by *Rhizobium sullae* KYGT207 strain was isolated from dry land in South Algeria (Gassi Touil) and was known to contribute of water absorption and nutrients by roots through modification of physical properties of *Triticum durum* rhizosphere. *In vivo* test which inoculated KYGT207 strain on wheat plants was also known to give significant influence as the plant growth promotion. EPS in sandy soil can protect plants from stress, lack of water and contributes to the formation of soil aggregates (Kaci *et al*. 2005). Previously we have isolated two groups of PGPR, *Bacillus* sp. CR and *Pseudomonas* sp. CRB from rhizosphere of soybean plant of Cirebon field area West Java (Wahyudi *et al.* 2011 a.b). The use of drought tolerance of PGPR in plant growth promotion is an effective and environmentally friendly step. Objective of this study was to screen PGPR *Pseudomonas* sp. CRB and *Bacillus* sp. CR for plant growth promoting of maize under drought stress condition.

MATERIALS AND METHODS

Screening of *Pseudomonas* sp. and *Bacillus* sp. as Growth Promoter of Maize. Initial stage was seed surface sterilization (Somasegaran and Hoben 1985). Sterilized seeds were then soaked in distilled water for 24 h to speed up the germination process. After 24 h, seeds were placed in Petri dishes covered by filter paper that was previously moistened with distilled water for 24 h. Fourty seven isolates of *Bacillus* sp. CR and 34 isolates of *Pseudomonas* sp. CRB were cultured on nutrient broth (NB) (yeast extract 2 g L^{-1}, peptone 5 g L^{-1}, NaCl 5 g L^{-1}) and King's B medium (peptone 20 g L^{-1}, K_2HPO_4 1.5 g L^{-1}, $MgSO_4$ 1.5 g L^{-1}, and glycerol 15 mL L^{-1}) shaked at 120 rpm for 24 h at room temperature, respectively. Seeds that have been germinated with 2-3 mm long of primary roots were transferred to a Petri dish that contains 1% water agar medium and each seed was inoculated with 100 µL culture of *Bacillus* sp. CR or *Pseudomonas* sp. CRB with a cell density of 10^9 cells mL^{-1}. Seeds with medium without inoculum were used as a control. Seeds that had been inoculated with bacteria were incubated for 7 d at room temperature in dark conditions. Growth parameters measured were length of stem, root length, and number of lateral roots (Dey *et al*. 2004). Data obtained from this experiments were statistically analyzed by one-way Analysis of Variance (ANOVA) using SAS 9.1 software and followed by Duncan's test (DMRT) at 5% level.

Screening of Drought Tolerance. *Bacillus* sp. CR and *Pseudomonas* sp. CRB were cultured in nutrient broth (NB) medium. Two hundred µL preculture of each isolates was inoculated in 20 mL of medium containing polyethylene glycol 6000 (PEG 6000) with concentrations of 0, -0.73, -1, -1.5, -2, and -2.5 MPa. Concentration of osmotic pressure arrangements according to PEG 6000 calculated by Michel and Kauffman (1973). Incubation of medium that had been inoculated with bacterial isolate was performed at room temperature for 24 h by shaking at 120 rpm. Optical density (OD) of each bacterial culture was measured using spectrophptometer at a wavelength of 570 nm. Medium without bacterial inoculation was used as a control. Bacteria that were able to grow minimum at -0.73 MPa with OD 0.4 catagorized as drought tolerant (Sandhya *et al.* 2009).

Antagonism Test. The purpose of this test was to determine capability of *Bacillus* sp. CR and *Pseudomonas* sp. CRB to live together in one medium with no competition each other if to be made formulated. Each isolates was grown in LB medium (tryptone 10 g L^{-1}, NaCl 10 g L^{-1}, and yeast extract 5 g L^{-1}) at 120 rpm for 24 h to reach concentration of 10^8 cells mL^{-1}. Antagonism test was done by the following ways: 10 mL of LA medium was inoculated with 100 µL targets culture, then poured into a sterile Petri dish. A total of 1 mL test culture on LB medium with cell density of 10^8 cells mL^{-1} was centrifuged 10 min at 10 000 rpm to obtain the supernatant futher used to antagonism test. Sterile paper discs were given for each supernatant test culture then placed on the dish containing LA medium that had been inoculated with the targets culture for further incubated. Each isolate CR and CRB were used as the test culture and targets culture. Antagonism was shown by a clear zone around the paper disc.

Test of Exopolysaccharide (EPS) Production.

Isolates of *Bacillus* sp. CR and *Pseudomonas* sp. CRB were grown in NB medium added by PEG 6000 to induction of drought stress. Culture incubation was performed in an incubator shaker at room temperature for 72 h, furthermore the cultures were centrifuged to separate the supernatant and pellet. Each supernatant was mixed with 3 mL of cold absolute alcohol and incubated overnight at 4 °C. EPS was obtained by centrifugation for 15 min at 10 000 rpm. Quantity of total carbohydrate content which settles on the EPS was measured using the method described by Dubois *et al* (1956). Optical density of each EPS sample was measured using spectrophotometer at a wavelength of 490 nm.

Phospate Solubilization Test. Test of phosphate solubilization was performed by standard methods. *Pseudomonas* sp. CRB or *Bacillus* sp. CR was grown by streaking on Pikovskaya Agar plate medium containing tricalcium phosphate (glucose 10 g L^{-1}, NaCl 0.2 g L^{-1}, KCl 0.2 g L^{-1}, $MgSO_4.7H_2O$ 0.1 g L^{-1}, $MnSO_4.2H_2O$ 2.5 mg L^{-1}, $FeSO_4.7H_2O$ 2.5 mg L^{-1}, yeast extract g L^{-1}, $(NH_4)_2SO_4$ 1 gL-1, and $Ca_3(PO_4)_2$ 5 g L^{-1}). Plates were incubated at room temperature for 72 h. Clear zone formed around the colony indicated the bacteria solubilized phosphate. The clear zone was formed by these bacteria were measured to determine phosphate solubilization index (PSI).

$$\text{Phosphate solubilizing index} = \frac{\text{clear zone diameter - colony}}{\text{colony diameter}}$$

Molecular Identification Based on 16S rRNA Sequence. Bacterial genomes were extracted by the Cetyl Trimetyl Ammonium Bromide (CTAB) method. 16S rRNA gene was amplified by PCR with 63F primers (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWTGGTACAAGGC-3') (Marchesi *et al.* 1998) in total volume of 50 µL. The PCR volume contains of 1 µL of genomic DNA template; 1 µL of primer for each forward and reverse, 25 µL of 2X Phusion Master Mix (Thermo Scientific Phusion High Fidelity), 1 µL of DMSO, and ddH_2O to a volume of 50 µL. Amplification was performed for 30 cycles that include stages of initial denaturation at a temperature of 94 °C for 2 min, denaturation at a temperature of 92 °C for 30 sec, annealing at a temperature of 55 °C for 30 sec, extension at 72 °C for 1 min, and final extension at 72 °C temperature for 7 min. DNA of PCR products were purified and sequenced and analyzed by comparing the sequences with GenBank database using the BlastN program to determine similarity. The DNA sequences were also analyzed their phylogenetic tree using Mega5 and ClustalW software.

RESULTS

Results of this study, 24 out of 47 isolates of *Bacillus* sp. CR and 9 out of 34 isolates of *Pseudomonas* sp. CRB screened for growth promotion revealed that they had capability in promoting growth of maize sprouts. *Bacillus* could increase the growth of shoot length, root length, and number of lateral root of maize sprouts. *Pseudomonas* could increase the growth of root length and stem length of maize sprouts. This results also revealed that *Bacillus* sp. CR 36 was able to increase all parameters of the growth of maize, significantly (Table 1,2).

All of the rhizobacteria selected as plant growth promoting were screened for their drought tolerance. Seven isolates of *Bacillus* sp. CR and six isolates of *Pseudomonas* sp. CRB were classified as drought tolerant, they were able to grow in medium with -1.0 MPa for *Bacillus* sp. CR, and -2.0 MPa for *Pseudomonas* sp. CRB (Table 3 and 4). Rhizobacteria *Pseudomonas* sp. CRB, and *Bacillus* sp. CR which could grow in the highest osmotic presssure were subjected to antagonism test to know their capability to survive in one medium. Results of this experiment are performed in Table 5 and Table 6. Moreover, the potent isolate of rhizobacteria, CRB and CR were examined for their EPS production. Isolate CRB 19 exhibited the highest activity of EPS production to 0.346 mg mL^{-1} in NB medium with -2.0 MPa of osmotic pressure. Average of EPS produced by *Pseudomonas* sp. CRB was higher than *Bacillus* sp. CR. *Bacillus* sp. CR produced EPS within a range of 0.050-0.107 mg mL^{-1} on medium with -0.73 MPa of osmotic pressure and 0.072-0.096 mg mL^{-1} on medium with -1 MPa of osmotic pressure (Fig 1A). *Pseudomonas* sp. CRB was able to produce EPS within a range of 0.062-0.196 mg mL^{-1} on medium with -0.73 MPa of osmotic pressure and within a range 0.055-0.197 mg mL^{-1} on medium with -1 MPa of osmotic pressure (Fig 1B). Majority, *Bacillus* sp. CR and *Pseudomonas* sp. CRB have been known their ability as phospate solubilizing on Pikovskaya agar medium. *Pseudomonas* sp. CRB 10 was known as the highest in phospate solubilizing. Phosphate solubilizing index of *Pseudomonas* sp. CRB and *Bacillus* sp. CR within a range of 0.18-0.67. All those isolates of rhizobacteria were able to solubilyze

Table 1 Effect of *Bacillus* sp. isolates in some growth parameters of maize

Treatment	Shoot length (cm)	Root length (cm)	Number of lateral root
CR 61	**15.5** $_{a}$	13.8 $_{n}$	14.0 $_{lmnopqr}$
CR 39	**15.0** $_{ab}$	15.0 $_{lnm}$	**24.4** $_{c}$
CR 6	**14.6** $_{abc}$	16.8 $_{ijkl}$	24.8 $_{c}$
CR 59	**14.5** $_{abcd}$	19.7 $_{defgh}$	12.6 $_{pqr}$
CR 32	**14.3** $_{abcd}$	17.4 $_{hijk}$	14.8 $_{klmnopq}$
CR 69	**14.2** $_{abcd}$	**25.2** $_{b}$	20.2 $_{de}$
CR 42	**13.9** $_{abcde}$	15.1 $_{lnm}$	11.0 $_{r}$
CR 12	**13.7** $_{abcdef}$	22.5 $_{c}$	19.6 $_{defg}$
CR 36	**13.5** $_{abcdefg}$	**25.5** $_{b}$	28.0 $_{ab}$
CR 3	**13.3** $_{abcdefgh}$	**25.7** $_{b}$	12.2 $_{pqr}$
CR 51	**13.1** $_{abcdefgh}$	21.1 $_{cdef}$	19.4 $_{defgh}$
CR 81	12.9 $_{bcdefghij}$	20.8 $_{cdef}$	13.6 $_{mnopqr}$
CR 79	12.8 $_{bcdefghij}$	**29.6** $_{a}$	26.0 $_{bc}$
CR 30	12.5 $_{bcdefghijk}$	17.7 $_{hijk}$	13.4 $_{nopqr}$
CR 83	12.4 $_{bcdefghijk}$	**28.8** $_{a}$	17.8 $_{efghijk}$
CR 22	12.4 $_{bcdefghijk}$	22.3 $_{c}$	13.8 $_{mnopqr}$
CR 86	12.3 $_{bcdefghijk}$	14.4 $_{mn}$	**29.8** $_{a}$
CR 31	12.2 $_{cdefghijk}$	**25.2** $_{b}$	**21.4** $_{d}$
CR 74	12.2 $_{cdefghijk}$	22.5 $_{c}$	16.4 $_{ghijklmn}$
CR 54	12.1 $_{cdefghijk}$	18.4 $_{ghij}$	16.2 $_{hijklmn}$
CR 2	12.1 $_{cdefghijk}$	**25.1** $_{b}$	19.4 $_{defgh}$
CR 56	12.1 $_{cdefghijk}$	18.8 $_{fghi}$	13.2 $_{nopqr}$
CR 66	12.0 $_{cdefghijk}$	21.7 $_{cd}$	16.2 $_{hijklmn}$
CR 25	12.0 $_{cdefghijk}$	21.9 $_{cd}$	15.2 $_{jklmnop}$
CR 90	11.9 $_{cdefghijk}$	**25.5** $_{b}$	17.6 $_{efghijk}$
CR 21	11.9 $_{cdefghijk}$	21.8 $_{cd}$	**24.4** $_{c}$
CR 88	11.8 $_{defghijk}$	16.2 $_{jklm}$	17.2 $_{efghijkl}$
CR 34	11.8 $_{defghijk}$	17.0 $_{ijkl}$	**26.6** $_{bc}$
CR 15	11.7 $_{defghijk}$	19.1 $_{efghi}$	11.8 $_{qr}$
CR 29	11.4 $_{efghijk}$	20.8 $_{cdef}$	19.0 $_{defghi}$
CR 26	11.4 $_{efghijk}$	22.2 $_{cd}$	15.0 $_{jklmnopq}$
CR 17	11.2 $_{efghijk}$	20.7 $_{cdefg}$	12.6 $_{pqr}$
CR 64	11.1 $_{fghijk}$	21.5 $_{cd}$	18.2 $_{efghij}$
CR 50	11.0 $_{fghijk}$	15.7 $_{klmn}$	12.8 $_{opqr}$
CR 27	10.9 $_{ghijk}$	**25. 1** $_{b}$	11.2 $_{r}$
CR 13	10.7 $_{ghijk}$	14.2 $_{nm}$	20.0 $_{def}$
CR 91	10.7 $_{ghijk}$	22.7 $_{c}$	14.0 $_{lmnopqr}$
CR 23	10.7 $_{ghijk}$	17.0 $_{ijkl}$	18.0 $_{efghij}$
CR 75	10.7 $_{ghijk}$	21.4 $_{cde}$	18.6 $_{defghi}$
CR 55	10.6 $_{hijk}$	22.2 $_{c}$	16.0 $_{ijklmno}$
CR 8	10.6 $_{hijk}$	**25.2** $_{b}$	12.6 $_{pqr}$
CR 67	10.4 $_{ijk}$	22.1 $_{cd}$	**24.6** $_{c}$
Control	10.3 $_{ijk}$	20.2 $_{cdefg}$	17.4 $_{efghijk}$
CR 38	10.2 $_{jk}$	18.8 $_{fghi}$	19.0 $_{defghi}$
CR 24	10.2 $_{jk}$	22.2 $_{cd}$	14.0 $_{lmnopqr}$
CR 46	10.1 $_{jk}$	**25.0** $_{b}$	24.6 $_{c}$
CR 33	10.0 $_{k}$	**27.9** $_{a}$	13.6 $_{mnopqr}$
CR 47	9.70 $_{k}$	21.7 $_{cd}$	16.8 $_{fghijklm}$

Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α=0.05). Bold indicated growth promoter isolates compared with control.

phosphate, except CR 67, CR 90, and CRB 4.

Futhermore, eight potential isolates of rhizobacteria *Pseudomonas* sp. CRB and *Bacillus* sp. CR classified as growth promoter of maize and drought tolerant, were

Table 2 Effect of *Pseudomonas* sp. isolates in some growth parameters of maize

Treatment	Root length (cm)		Shoot length (cm)		Treatment	Root length (cm)		Shoot length (cm)	
Group 1					Group 4				
Control	10.8	abc	17.5	abc	Control	6.6	abc	12.7	abcd
CRB 42	9.6	abc	12.1	abc	CRB 64	9.2	cdef	18.9	d
CRB 55	10.1	abc	13.1	abc	CRB 85	6.1	ab	12.8	abcd
CRB 107	11.7	abc	15.4	abc	**CRB 71**	**10.2**	**ef**	18.8	d
CRB 88	11.4	abc	19.4	abc	CRB 3	9.3	cdef	18.0	cd
Group 2					**CRB 4**	**10.6**	**f**	15.0	abcd
Control	10.9	ab	10.1	a	CRB 11 2	8.9	cdef	19.1	d
CRB 34	12.4	abc	**16.2**	**b**	CRB 90	8.7	bcdef	13.8	abcd
CRB 111	11.9	ab	14.4	ab	CRB 25	7.5	abcde	11.2	ab
CRB 19	14.2	bcd	**16.9**	**b**	CRB 104	5.7	a	10.4	a
CRB 24	**18.7**	**d**	**15.6**	**b**	CRB 76	8.0	abcdef	13.9	abcd
CRB 115	7.9	a	13.1	ab	CRB 113	8.7	bcdef	13.4	abcd
CRB 47	**17.8**	**cd**	**14.7**	**b**	CRB 96	7.6	abcde	12.9	abcd
CRB 77	9.3	ab	10.1	a	CRB 33	8.7	bcdef	12.0	abc
CRB 58	10.4	ab	12.6	ab	CRB 36	8.1	abcdef	15.7	abcd
CRB 10	12.3	abc	**16.9**	**b**	CRB 69	8.8	bcdef	17.4	bcd
Group 3					CRB 63	8.2	abcdef	16.9	bcd
Control	11.3	a	10.2	a	CRB 40	9.1	cdef	17.9	cd
CRB 23	14.0	a	**16.6**	**b**	CRB 92	7.1	abcd	18.6	cd
CRB 98	13.0	a	**16.5**	**b**					
CRB 100	9.6	a	9.8	a					

Numbers within a column followed by the same letter are not significantly different at 5% level by. DMRT ($\alpha = 0.05$). Bold indicated growth promoter isolates compared with control.

Table 3 Optical density values of *Bacillus* sp. isolates in osmotic pressure of 0, -0.73, -1, and -1.5 Mpa at λ 570 nm

Isolates code	Osmotic pressure			
	0 MPa	**-0.73 MPa**	**-1 MPa**	**-1.5 MPa**
CR 33	**1.514**	**0.504**	**0.400**	0.071
CR 61	1.273	0.400	0.290	0.000
CR 69	1.011	0.681	0.271	0.000
CR 36	**1.425**	**0.471**	**0.412**	0.064
CR 83	**1.073**	**0.758**	**0.464**	0.017
CR 39	**1.118**	**0.763**	**0.483**	0.056
CR 51	1.317	0.496	0.138	0.000
CR 46	**1.056**	**0.547**	**0.476**	0.022
CR 67	**0.95**	**0.503**	**0.432**	0.033
CR 31	1.069	0.698	0.329	0.000
CR 90	**1.063**	**0.682**	**0.559**	0.028
CR 32	1.11	0.763	0.326	0.000

Bold of figures and letters indicated drought tolerant isolates. MPa: Mega Pascal

molecularly indentified based on 16S rRNA gene and sequence (Table 7 and 8). Phylogenetic tree analysis using neighboor joining method (Fig 2 A,B).

DISCUSSION

Some studies suggest that *Bacillus* sp. and

Table 4 OD values of *Pseudomonas* sp. isolates in osmotic pressure of 0 MPa to -2.5 MPa at λ 570 nm

Isolates Code	Osmotic pressure					
	0 MPa	**-0.73 MPa**	**-1 MPa**	**-1.5 MPa**	**-2 MPa**	**-2.5 MPa**
CRB 4	1.742	1.362	1.254	0.966	0.792	0.490
CRB 10	1.084	0.773	0.727	0.843	0.725	0.173
CRB 19	1.789	1.058	1.017	1.086	0.872	0.563
CRB 23	1.719	1.654	1.331	0.506	0.431	0.000
CRB 47	1.709	1.249	1.151	0.771	0.630	0.000
CRB 98	1.713	1.092	1.042	1.015	0.831	0.664

OD value ≥ 0.4 indicated drought tolerant isolates. MPa: Mega Pascal

Table 5 Inhibition zone formation by *Bacillus* sp. (CR) as target bacteria against *Pseudomonas* sp. (CRB) as test bacteria

Target Isolates	Test isolates						
	CR 33	**CR 39**	**CR 83**	**CR 67**	**CR 90**	**CR 36**	**CR 46**
CRB 98	+	+	-	-	+	+	-
CRB 23	-	+	-	-	+	-	-
CRB 10	-	-	-	-	-	+	-
CRB 47	-	-	-	-	-	+	-
CRB 4	-	-	-	-	-	+	-
CRB 19	-	-	+	-	-	+	-

CR : *Bacillus* sp. (+) : no clear zone
CRB : *Pseudomonas* sp. (-) : no clear zone

Table 6 Inhibition zone formation by *Pseudomonas* sp. (CRB) as target bacteria against *Bacillus* sp. (CR) as test bacteria

Target isolates	Test isolates					
	CRB 98	**CRB 23**	**CRB 10**	**CRB 47**	**CRB 4**	**CRB 19**
CR 33	-	-	-	-	-	-
CR 39	+	+	-	-	-	-
CR 83	-	-	-	-	-	+
CR 67	-	-	-	-	-	-
CR 90	+	+	-	-	-	-
CR 36	+	-	+	+	+	+
CR 46	-	-	-	-	-	-

Information :
CR : *Bacillus* sp. (+) : no clear zone
CRB : *Pseudomonas* sp. (-) : no clear zone

Pseudomonas sp. have been capability to increase plant height by producing IAA hormones (Patten and Glick 2002; Leveau and Lindow 2005; Wahyudi *et al.* 2011a,b). Rhizobacteria as growth promoter in this research previously also had been tested in producing IAA hormone within a range 2.82-22.79 ppm (Wahyudi *et al.* 2011a,b). Other isolates, 23 strains of *Bacillus* and 25 strains of *Pseudomonas* did not have ability as growth promotion of maize sprouts. This might be caused by their IAA concentration is too high, for example isolate CR 55 producing IAA up to 44.66 ppm (Wahyudi *et al*. 2011a). The highest of IAA concentration was known to stimulate the growth of maize using biological fertilizer within a range 54.55 ppm in leaves and 22.68 ppm in roots, respectively. Leaf tissue contains a higher IAA concentration rather than root tissue (Wibowo 2008). High levels of IAA hormone would promote formation of ethylene and

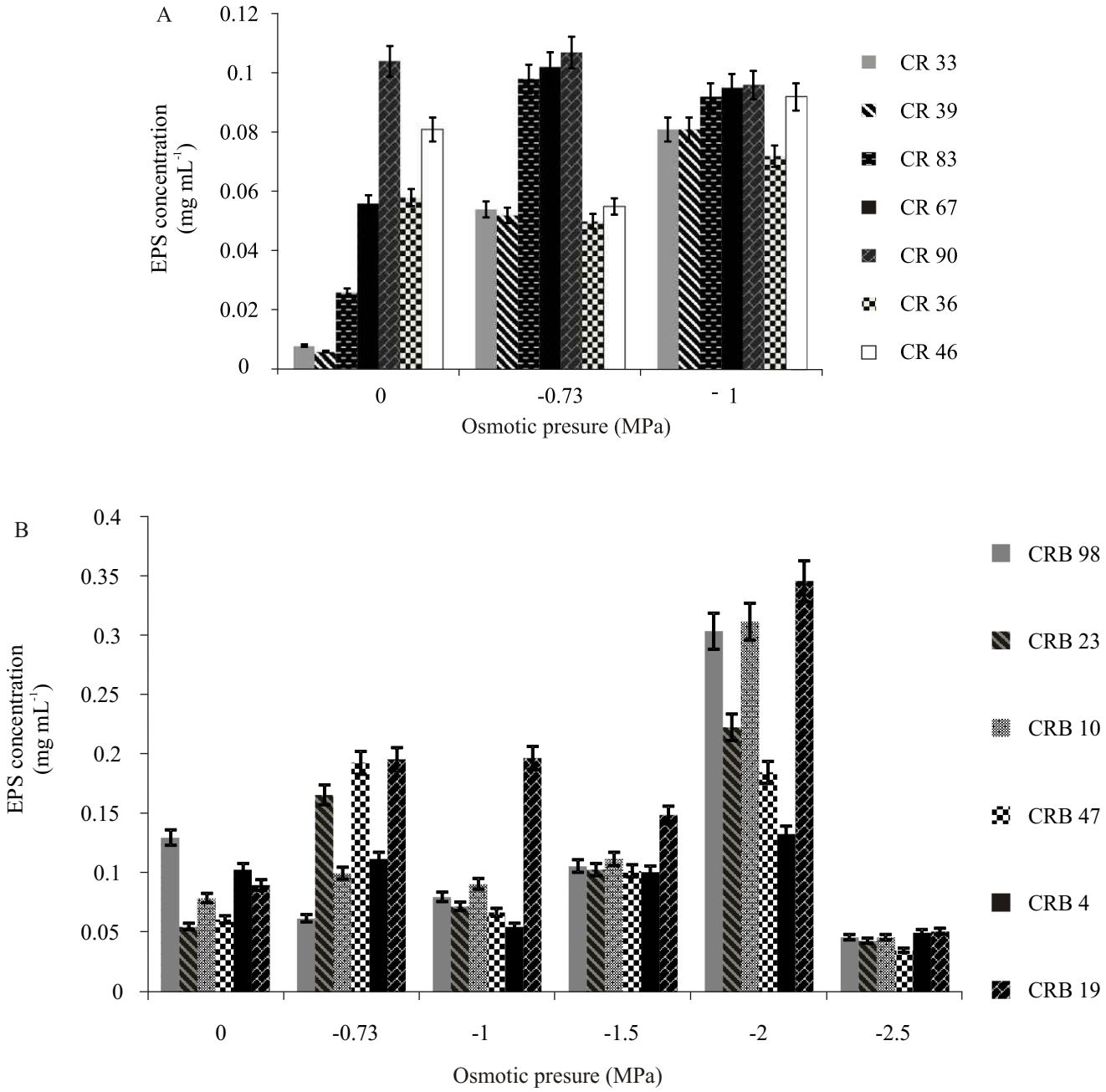

Fig 1 EPS concentration produced by *Bacillus* sp. CR (A) and *Pseudomonas* sp. CRB (B) at different osmotic pressure.

Table 7 Identification of 16S rRNA gene sequence homology of isolates of *Bacillus* sp. CR with sequences available in Genbank using BlastN program

Isolates	Species most related	Sequence similarity	Length (bp)	Query Cover	E-Value	Accession number
CR 46	*Bacillus isabeliae* strain CVS-8	83%	846/1018	82%	0.00	NR 042619.1
CR 67	*Brevibacillus brevis* strain NBRC 15304	100%	659/659	100%	0.00	NR 041524.1
CR 83	*Bacillus cereus* ATCC 14579 strain ATCC 14579	100%	566/566	100%	0.00	NR 074540.1
CR 90	*Brevibacillus brevis* strain bB33	97%	1200/1243	92%	0.00	JF772474.1

Table 8 Identification of 16S rRNA gene sequence homology isolates of *Pseudomonas* sp. CRB with sequences available in Genbank using BlastN program

Isolates	Species most related	Sequence similarity	Length (bp)	Query Cover	E-Value	Accession number
CRB 10	*Pseudomonas aeruginosa* strain L1	99%	434/440	94%	0.00	JX292018.1
CRB 19	*Pseudomonas aeruginosa* strain B2	91%	966/1062	98%	0.00	JQ900536.1
CRB 23	*Pseudomonas fragi* strain ATCC 4973	84%	657/780	83%	0.00	NR 024946.1
CRB 98	*Pseudomonas* sp. CL 3.1	97%	1297/1334	99%	0.00	FM173664.1

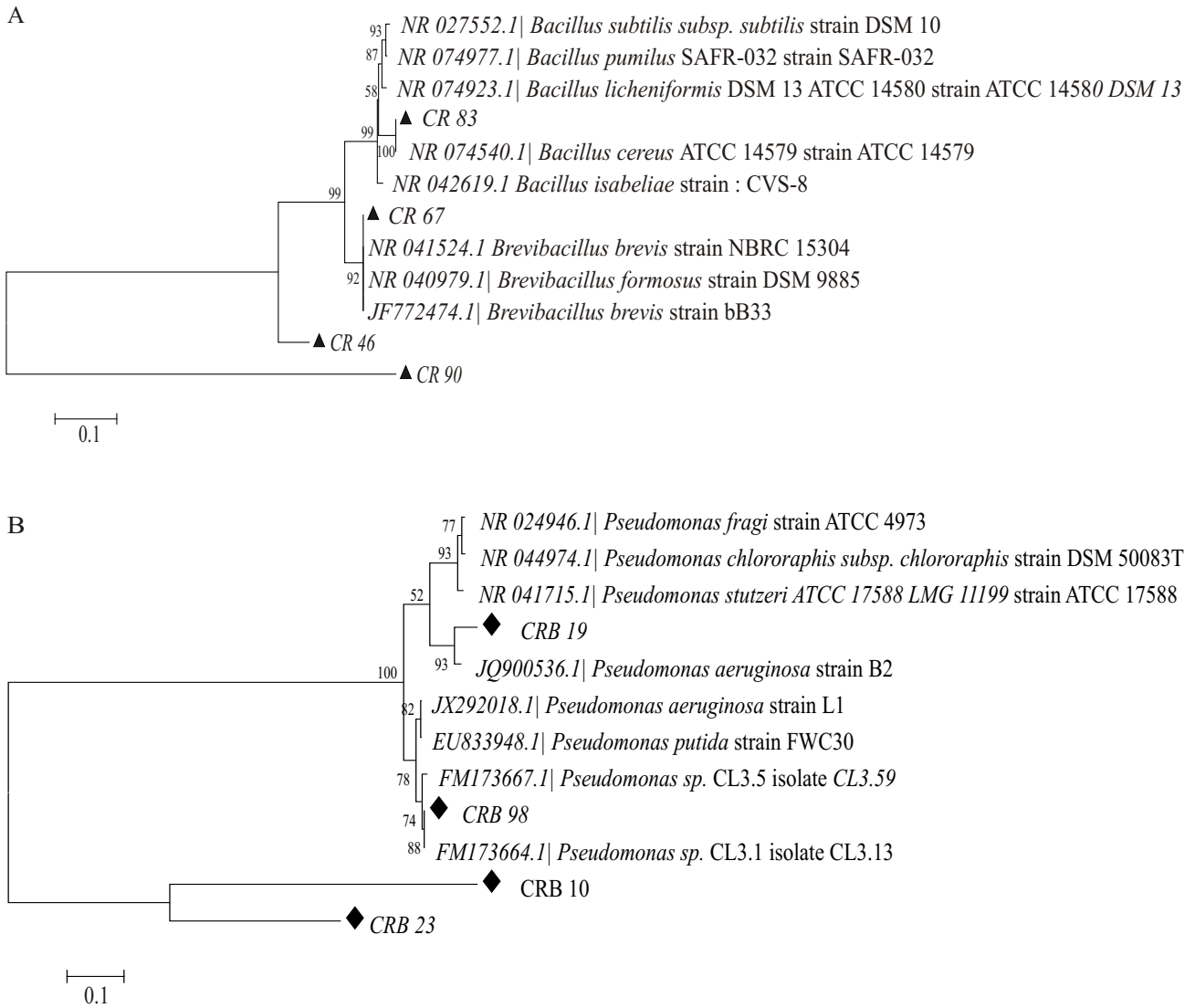

Fig 2 Phylogenetic tree based on 16S rRNA gene of *Bacillus* sp. (A) and *Pseudomonas* sp. (B) compared with 16S rRNA gene of other species. Scale showed that distance evolution on the branch length, while the numbers on the branches indicate bootstrap values.

stop the growth.

Bacteria PGPR had ability in producing IAA and insert that into pool auxin hormone of plant. Plant roots were the most sensitive organ to fluctuations of IAA levels and its response of increase exogenous IAA widespread in the number of primary root elongation,

lateral root formation and adventitious roots until termination of growth on the plant (Leveau and Lindow 2005). Patten and Glick (2002) also stated that the high of exogenous IAA would increase ethylene production which would effect to inhibition of roots growth. Other mechanism that had capable to support as plant promoting growth by CR and CRB are their ability in phosphate solubilizing which one of the essential nutrients for plants. Phosphate in soils were present in bound form. Phosphate bound with aluminium (Al^{3+}), iron (Fe^{3+}), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Thus, only it a small fraction which could be absorbed by plants (Trivedi and Pandey, 2007), PGPR had been ability to solubilyze bound phosphate so that could be absorbed by plants.

Screening of drought tolerant aimed to determine of isolates which could survive on drought condition so their can survive when applied in the field. The addition of PEG 6000 on medium which bound water molecule were simulated as drought stress so that could reduce of potential water value. Consequently, reduction of potential water value by the high of PEG concentration on medium would cause decreasing survival of bacterial population. Optical density values linearly related to water potential contained on medium. Reduction of water potential values would decrease number of bacterial population. Therefore, PEG 6000 which soluble in water used to reduce of water potential value. Water potential associated with level of drought would affect to capability survive of bacteria. Isolates that had cappability to grow on medium with a certain of potential water value and OD value ≥ 0.4 classified as drought tolerant isolates (Alikhani and Mohamadi 2010).

Majority, the result of antagonism test for plant promoting growth and drought tolerant isolates showed the same result for isolate CR and CRB were used as the test culture and targets culture, respectively. Formation of clearing zone around paper disc occured by production of certain compounds by bacteria test that inhibited growth of bacterial target or while back of test. Isolates with different results founded in isolates CR 33 which wasn't antagonis to CRB 98 when CR 33 was used as target isolates and isolate CR 83 wasn't antagonis to CRB 19 when CR 83 as a target. This result might be caused by differences substance when isolates were used as targets and test isolates. When isolate used as targets, the substances were intact cells while tested back were used supernatant.

Tolerances to the drought stress were characterized by production of EPS. This indicates that production of EPS linearly with stress experienced by cells as a form of physiological adaptation so that cells could survive. Therefore, the capability in producing EPS by bacterial cells used as drought tolerance criteria for bacteria (Sandhya *et al*. 2009). Production of EPS would increased by cells during stress experienced as a form of physiological adaptation so that cells could survive. EPS were produced to protect bacterial cells from drought, heavy metals or other environmental stresses, including host immune response and to produce a biofilm which could increase survival cell in specific ecological niches (Ozturk and Aslim 2010). Quantity and composition of EPS vary greatly depending on genus and species of bacteria, in some cases dependent on environmental conditions for growth. In addition, the carbon sources in medium was functioning as a component of cell formation, source of energy required for synthesis and EPS excretion (Santi 2011).

B. subtilis could produce levan exopolysaccharide within a range 0.32-86.3 g L^{-1} using sucrose as substrate. A total of 16 genes of operon EPS (*yveKyvfF*) involved in the biosynthesis, modification and expenditure. Recently, two genes *eps*G (*yveQ*) and *eps*H (*yveR*) had been identified that might be involved in the biosynthesis. *eps*G encodes a protein that might be involved in EPS polymerization and *eps*H encodes a glycosyltransferase (Marvasi *et al*. 2010). Species of *Pseudomonas* has been known for their capability producing EPS in alginate type. Approximately 24 genes in *P. aeruginosa* were identified. Cluster consisted of 12 structural genes (*alg*D, *alg*8, *alg*44, *alg*K, *alg*e, *alg*G, *alg*X, *alg*L, *alg*l, *alg*J, *alg*F, and *alg*ae) were clustered in a single operon approximately 3.96 Mb. Only *alg*C genes located on separate chromosomes. Those gene encodes for phosphomanno-mutase involved in rhamnolipid and lipopolysaccharide biosynthesis. Operon was containing all the genes that encode proteins involved in the biosynthesis of alginate (*alg*D and *alg*ae for precursor synthesis, *alg*l, *alg*J, and *alg*F for acetylation, *alg*G to epimerisasi, *alg*L for degradation) (Hay *et al*. 2010).

The potent isolates CRB 19 and CR 90 exhibited the highest activity of EPS secretion and based on 16S rRNA analysis revealed CRB 19 and CR 90 belonged to *P. aeruginosa* strain B2 and *Brevibacillus brevis* B33, respectively. Other studies showed that those group was known as promoting growth in plants. *P. aeruginosa* FP6 isolated from rhizosphere had been known for their capability as phosphate solubilizing, producer of IAA hormone, ammonia, sidherophore, and cells wall

degrading enzyme such as cellulase, chitinase, and protease. Inoculation of cowpea (*Vigna unguiculata*) seeds by those bacteria given significantly effect ($P<0.05$) for enhanced seed germination (92%), seedling vigor index, plant height, and also fresh and dry weight in compared with control (Bhakthavatchalu *et al*. 2013). *B. brevis* strain IPC11 also known could increase seed germination, producing phenylalanine ammonia lyase enzyme and reduced cancer disease in tomato (Girish and Umesha 2005). Finally, the potent isolates of *Bacillus* sp. CR and *Pseudomonas* sp. CRB have capability to live in drought condition and could be developed as inoculants in dry land agriculture when it put in medium carrier as biofertilizer.

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