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Nitrogen Fixing Potential of Endophytic Bacteria Isolated from *Aloe barbadensis* Miller and *Aloe* sp.

RAHAYU FITRIANI WANGSA PUTRIE*, TIWIT WIDOWATI, SYLVIA J.R. LEKATOMPESSY, AND HARMASTINI SUKIMAN

Plant Symbiotic Microbes Laboratory, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI)
Jalan Raya Bogor KM. 46, Cibinong 16911, Indonesia

Aloe is a crassulacean acid metabolism (CAM) species that are known to live in extreme enviroment such as drought condition. Nitrogen fixation procces influenced by the ability of plants to adapt in drought condition. Endophytic bacteria from Aloe and their ability for nitrogen fixation were little reported, but potential and its relationship between the ability for nitrogen fixing with resistance to drought conditions have not been reported. This research aimed study the endophytic bacteria from two varieties of aloe, namely *Aloe barbadensis* Miller and *Aloe* sp. in their ability on conducting the nitrogen fixing process and its relationship with resistance to drought. Characterization of endophytic bacteria were carried out by morphological observation of colony, Gram staining and molecular identification. Screening of nitrogen fixation was done using nitrogen-free semisolid NFb malate medium. Endophytic bacteria from *Aloe* sp. more than *A. barbadensis* in their potency of nitrogen fixation which related with habitat where their planted. A total of 40% of the endophytic bacteria isolates from the leaves of the aloe var. *A. barbadensis* and 62.5% of isolates from var. *Aloe* sp. are known to have a better ability to fixing nitrogen than the others. Isolates *A. barbadensis* AB 12 and *Aloe* sp. AS 8 were the best isolates from each varieties on ability for nitrogen fixation. Based on 16S rRNA gene analysis those two selected isolates were similar to *Bacillus methalotropicus* strain DA 16-5 and *Bacillus aryabhattai* strain B8W22.

Keyword : aloe, endophytic bacteria, nitrogen fixation

Lidah buaya merupakan salah satu spesies tanaman *crassulacean acid metabolism* (CAM) yang dapat hidup pada lingkungan ekstrim seperti kekeringan. Kemampuan adaptasi terhadap kekeringan dipengaruhi oleh kemampuan fiksasi nitrogen. Bakteri endofit dari lidah buaya dan kemampuannya dalam memfiksasi nitrogen telah sedikit dilaporkan, namun potensi dan hubungannya antara kemampuan fiksasi nitrogen dengan ketahanan terhadap kekeringan belum dilaporkan. Penelitian ini bertujuan untuk mengetahui dan membandingkan kemampuan bakteri endofit dari dua varietas lidah buaya, yaitu *Aloe barbadensis* Miller dan *Aloe* sp. dalam memfiksasi nitrogen serta hubungannya dengan ketahanan terhadap kekeringan. Karakterisasi bakteri endofit dilakukan dengan pengamatan morfologi koloni, pewarnaan Gram dan identifikasi molekuler. Penapisan fiksasi nitrogen dilakukan dengan menggunakan medium nitrogen-free semisolid NFb malate. Bakteri endofit yang berasal dari *Aloe* sp. lebih banyak yang dapat memfiksasi nitrogen dibandingkan dengan *A. barbadensis* dimana kemampuan ini memiliki hubungan dengan habitat tumbuhnya. Sebanyak 40% isolat bakteri endofit dari daun lidah buaya var. *A. barbadensis* dan sebanyak 62.5% isolat var. *Aloe* sp. diketahui memiliki kemampuan yang lebih dalam proses penambatan nitrogen dibandingkan dengan isolat lainnya. Isolat AB 12 dan AS 8 adalah isolat penambat nitrogen terbaik dari setiap varietas. Berdasarkan analisis gen 16S rRNA isolat tersebut mempunyai kemiripan yang tinggi dengan *Bacillus methalotropicus* strain DA 16-5 dan *Bacillus aryabhattai* strain B8W22.

Kata kunci : bakteri endofit, fiksasi nitrogen, lidah buaya

Some plant species have specific pathway which allow them to survive under extreem conditions such us drought stress. The best known is the crassulacean acid metabolism (CAM) plants, particularly the species of the genera Opuntia, Agave, and a Liliaceous species, one of them is aloe. Genus of aloe are known have around 400 species including *Aloe pollyphyla, A. vera* Linn syn. *A. barbadensis* Miller*, A. ferox* Miller*, A. arborecens, A. brevifolia, A. microstigma, A. buhrii, A. hereroensis, A. humilis, A. maculata*, *A. chinensis* Baker, *A. indica* Royle, *A. perryi* Baker and others (Rodriguez-Garcia *et al.* 2007; UCDBC 2009; Rajeswari *et. al*. 2012; Silva *et al.* 2014). Aloe is a CAM species that naturally survive to drought conditions and high temperatures. Salinity and drought stress affected to the plant height, number of leaves, leaf length, leaf thickness, aerial fresh yield, leaf fresh weight, and gel weight (Shams *et al*. 2015).

Nitrogen fixation procces influenced by the ability of plants to adapt in drought condition (Dinh *et al.* 2013; Serraj 2003). Drought cause a significant decreases in nodule dry weight and amount of nitrogen fixing by the plant. Peanut genotypes that planted

*Corresponding author; Phone: 021-8754587/8754588, Email:rahayufwputrie@gmail.com

under well-watered condition and under drought stress were significantly different for nitrogen fixation. Drought tolerant genotypes had higher SPAD Chlorophyll Meter Reading (SCMR), fixed more nitrogen and achieved higher pod yield than sensitive genotypes (Dinh *et al.* 2013).

Biological nitrogen fixation (BNF) in agriculture are most promising on supporting the growth and productivity of plant. Plant growth promoting rhizobacteria (PGPR) had the ability to fix atmospheric nitrogen by symbiotic and non-symbiotic mechanism and provide it to plants (Saharan and Nevra 2011; Ahemad and Kibret 2014; Gupta *et al*. 2015). BNF were contribute 180 $\times 10^6$ metric tons/year globally, 80% from symbiotic association and the rest from free-living or associative systems. A number of bacterial species belonging to genera of PGPR viz. *Rhizobium, Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and cyanobacteria (Saharan and Nevra 2011; Gupta *et al*. 2015). Crops inoculation by PGPR provide an integrated approach for disease management, growth promotion activity, maintain the nitogen level in agricultural soil (Ahemad and Kibret 2014; Gupta *et al*. 2015)

Plant-growth-promoting bacterial endophytes (PGPBEs) have been known for positively influencing plant growth in limited field conditions. Bacterial root endophytes reside in a vast number of plant species are a part of the root microbiome. Those endophyte community structure (species diversity: richness and relative abundances) were influenced by abiotic and biotic factors of environment (Gaiero *et al.* 2013). Nitrogen source in the atmosphere are known about 79% of the total atmospheric gases. Although nitrogen is very abundant in nature, it was often limiting plant productivity because atmospheric nitrogen is only available to organisms symbiotically associates with higher plants and non-symbiotically (Khan *et al.* 2008; Gulati *et al.* 2011; Ahemad and Kibret 2014). The abilitiy of endophytic bacteria from various plant as plant growth promoters, to fixed nitrogen and could be support in drought stress have been reported (Ngoma *et al.* 2013; Nogkhlaw and Joshi 2014; Ngoma *et al.* 2014; Miliute *et al.* 2015).

Exploration of endophytic bacteria from drought tolerant plant, specially leaves, stem and roots of aloe as a potential agents for antifungal activity againts *Fusarium oxysporum* and a vast source of extracellular enzymes such as amylase, cellulase, chitinase, pectinase, lipase, and urease also have been reported (Yadav *et al.* 2015). The crude and ethyl acetate fractions of the metabolites of six isolates endophytic from aloe had broad spectral antimicrobial activities against pathogenic *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus cereus*, *Salmonella Typhimurium*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pyogenes*, and *Candida albicans* (Akinsanya *et al.* 2015a).

On the other hands, the ability of endophytic bacteria from aloe as a nitrogen fixing were little reported. Investigate for endophytic bacteria assosiated with aloe from the pristine subtroprical forest in Meghalaya India, *Herminiimonas saxobsidens* AA JQ770186 showed that their ability for IAA production, phosphate solubilisation and nitrogen fixation which are beneficial to host plant (Nongkhlaw and Joshi 2014). Although there have been reports related to endophytic bacteria from aloe in their ability for nitrogen fixation, but potential and its relationship between the ability for nitrogen fixing with resistance to drought conditions have not been reported.

Previously, we have succeeded on isolating the culturable endophytes microbes from Aloe which could grown in Nutrien Agar (NA), Potato Dextrose Agar (PDA), and Cornmeal Malt Extract (CMM) Agar. A total of 43 isolates of endophytic microbes were isolated from the leaves of the aloe var. *A. barbadensis* and 28 isolates from var. *Aloe* sp., respectively. Endophytic microbes from *A. barbadensis* more than *Aloe* sp. As many as 58% microbes derived from *A. barbadensis* are bacteria, 42% fungi and *Aloe* sp. as many as 86% are bacteria, 14% fungi. This showed that the majority culturable endophyte symbiosis on the leaves of the aloe is a bacteria (Putrie and Sukiman 2015). Based on the case, this research aimed to study endophytic bacteria from two varieties of aloe, namely *A. barbadensis* and *Aloe* sp. and identifying isolates that are known had the best ability of of nitrogen fixation from each variety also its relationship with resistance to drought condition.

MATERIALS AND METHODS

Morphology Characteristic of Endopythic Bacteria Colony. Culturable endopytic bacteria were isolated from the leaves of the aloe var. *A barbadensis* and from var. *Aloe* sp. (Putrie and Sukiman 2015). Sample of aloe used in this research, both of them derived from an experimental garden Research Center for Biotechnology LIPI but there were differences on

place of planted. *A. barbadensis* planted in pots whereas *Aloe* sp were planted in soil directly. Isolates were purified by streak quadrant on nutrient agar (NA) (23 g L^{-1}) subsequently incubated at room temperature 24 h to optimize the growth of culture. Each of colony growth were observed. Those morphological were observed include colour, size, edge of the colony, the colony shape, and condition dry or slimy of colonies.

Gram Staining. Gram staining is an important and useful technique to catagorize the bacteria included in Gram-positive or Gram negative based on their morphology and differential staining properties. The method of Gram staining was described by Bartholomew (1962). The beginning stage was done by making heat-fixed smear slides of bacteria. Crystal violet as a main dye and mordant solution (Lugol's iodine) dropped for ± 1 min, respectively. Ethanol 95% dropped until ethanol fall colored clear and not excessive (overdecolorize). Safranin as last dye dropped for ± 45 sec. Each after given dye, smear slides washed with distilled water, then drain flow. Gram positive bacteria stain blue-purple and Gram negative bacteria stain red.

Nitrogen Fixing Assay. Endophytic bacteria as many as 25 isolates from *A. barbadensis* and 24 isolates from *Aloe* sp. were inoculated in a nitrogen-free semisolid NFb malate medium (K_2HPO_4 0.5g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, NaCl 0.1 g L^{-1}, $CaCl_2$ 0.02 g L^{-1}, trace element 2.0 ml L^{-1} [$Na_2MoO_4.2H_2O$ 0.2 g L^{-1}, $MnSO_4$ 0.235 g L^{-1}, H_3BO_3 0.2 g L^{-1}, $CuSO_4.7H_2O$ 0.24 g L^{-1}], bromthymol blue 0.5% 2.0 ml L^{-1} solution [dissolved in 0.2 N KOH], Fe EDTA [1.64% solution] 4.0 ml L^{-1}, and vitamin solution 1.0 ml L^{-1} [biotin 0.01 g L^{-1}, pyridoxin 0.02 g L^{-1}] pH adjusted to 6.8 with KOH, semi solid agar 1.75 g L^{-1}) (Okon *et al.* 1977). This assay aimed to known ability of the isolates to fix atmospheric nitrogen in medium by stab inoculation. Incubation conducted for 3-5 d. Score positive are showed by growth of the isolates in variable depth and change in colour under the surface medium. Uninoculated NFb medium was kept as control on this assay (Nogkhlaw and Joshi 2014).

Identification Based on 16S rRNA Gene Analysis. The best isolates from each varieties on ability for nitrogen fixation procces subsequently molecularly identified. One colony of isolate were taken with a sterile toothpick then inserted into eppendorf tube containing 100 mL dH_2O subsequently it were vortex. A total of 1 mL suspension were used for the amplification with polymerase chain reaction (PCR) technique. Amplification of 16S rRNA gene by PCR with primer 27 F (5'-AGAGTTTGATCCTGGCT CAG-3') and 1492 R (5'-GGTTACCTTGTTACGAC TT-3') (Weisburg *et al.* 1991) in total volume 50 µL. The PCR volume contains of 1 µL DNA template, 2 µL of primer for each forward and reverse, 25 µL of 2X KAPA Taq Ready Mix (KAPA Biosystem) and ddH_2O 20 µL. Amplification was performed for 30 cycles that included initial denaturation stage at a temperature 96 °C for 5 min, denaturation at a temperature 96 °C for 30 sec, annealing a temperature 55 °C for 30 sec, extension at a temperature 72 °C for 1 min, final extension at a temperature 72 °C for 7 min. DNA of PCR products were purified and sequenced in two directions. Sequences were analyzed by comparing the sequences with GenBank database using the Blastn (http://www.ncbi.nlm.nch.gov) programe of the National Center for Biotechnology Information to determine similarity. The length of base used for Blastn between 500-1500 bp (Clarridge 2004).

RESULTS

Endophytic bacteria, both of *A. barbadensis* and *Aloe* sp. were optimatelly growth on 24 h after incubated. Morphology of all bacteria colony from *A. barbadensis* and *Aloe* sp., had several similarity (Table 1, Table 2 and Fig 1). Both of them, majority of endophytic bacteria colony were pigmented, moderate, smooth of colony edge, round shape, slimy and opaque. A total of 56% isolates AB and 79.2% isolates AS out of each total bacterial isolates varieties were shown a slime colony, respectively. All isolates, both it from *A. barbadensis* and *Aloe* sp. then classified with Gram staining. Based on the result, all isolates included to Gram positive bacteria. Gram staining result showed that isolates stain blue-purple. After that, for those isolates were conducted nitrogen fixing assay used nitrogen free semisolid NFb malate medium. Endophytic bacteria from *Aloe* sp. more frequently than *A. barbadensis* in their potency for nitrogen fixation (Table 3). Positive results were marked by changes in media (Fig 2).

A total of 92% isolates AB and 96% isolates AS had abillity to nitrogen fixing in medium, but their ability for each isolates were different. Only 40% out of 92% isolates AB and 62.5% out of 96% isolates AS. were showed a better ability to fixed nitrogen compared to the others isolates. One isolates from each varieties that shown the best ability to fixed nitrogen, AB 12, and AS 8 subsequently indentified by

Table 1 Morphology of endophytic bacteria from *Aloe barbadensis*

No.	Isolates code	Part of leaf	Pigment	Morphological colony				
				Size	Edge of the colony	Shape	Dry/slimy	Transparantly
1.	AB 1	pole	old cream	moderate	smooth	round	dry	transpatant
2.	AB 2	pole	milky white yellowish	moderate	smooth	round	slimy	opaque
3.	AB 3	pole	milky white	small	smooth	round	thin slimy	opaque
4.	AB 4	pole	old cream	small	smooth	round	dry	opaque
5.	AB 5	pole	old cream	small	rough	round	dry	opaque
6.	AB 6	pole	old beige	moderate	smooth	round	dry	opaque
7.	AB 7	pole	milky white yellowish central part whiter	moderate	smooth	round	slimy	opaque
8.	AB 8	pole	bright white milk	moderate	smooth	round	thick slimy	opaque
9.	AB 9	pole	milky white yellowish	moderate	smooth	round	slimy	opaque
10.	AB 10	pole	creamy white in the middle	point	smooth	round	slimy	transparantly in the point
11.	AB 11	pole	old beige-gray (more beige than AB 6)	moderate	smooth	round	dry	opaque
12.	AB 12	pole	dull beige	moderate	smooth	round	dry	transparantly in the point
13.	AB 13	pole	cream	small	smooth	round	dry	opaque
14.	AB 14	pole	cream	small	smooth	round	slimy	opaque
15.	AB 15	center	yellow	small	smooth	round	Thin slimy	opaque
16.	AB 16	center	milky white	moderate	smooth	round	Thick slimy	opaque
17.	AB 17	center	beige-gray	moderate	smooth	round	dry	opaque
18.	AB 18	center	white	point	smooth	round	thin slimy	opaque
19.	AB 19	center	creamy white	moderate	rough	round	thick slimy	opaque
20.	AB 20	center	old beige-gray	moderate	rough	round	dry	opaque
21.	AB 21	tip	white	small	smooth	oval	dry	opaque
22.	AB 22	tip	white	point	smooth	round	slighty dry	transparantly
23.	AB 23	tip	milky white	moderate	smooth	round	slimy	opaque
24.	AB 24	tip	milky white	moderate	smooth	round	thin slimy	opaque
25.	AB 25	center	yellow	point	smooth	round	slimy	opaque

molecular identification. Based on 16S rRNA sequence gene analysis isolat AB 12 and AS 8 belonged to *B. methalotropicus* strain DA 16-5 and *Bacillus aryabhattai* strain B8W22, respectively (Table 4).

DISCUSSION

Morphology of endophytic bacteria colony showed that majority of colony from both of them are slimy. Endophytic bacteria produce more mucus or exopolisaccharide (EPS) to keep plant from water loss. Abiotic factor such as drought stress tolerance in bacteria were characterized by production of exopoly-saccaride (EPS). Production of EPS were increased by bacteria during a drought as a form physiological adaptation. EPS quantity and composition were influence by genus and species of bacteria, in some cases dependent on environmental conditions for growth (Putrie *et al.* 2013). Based on Gram staining, all isolates from *A. barbadensis* and *Aloe* sp. were Gram positive bacteria. This was possible because adaptation of Gram positive bacteria in extreem environmet, like as drought higher than Gram negative bacteria. Gram positive bacteria could be survive in drought environment by spore. Drought periode could be promote of presence of spore forming bacteria (Meisner *et al.* 2015).

The ability to adapt in drought condition has been known assosiate with nitrogen fixation (Zahran 1999). Nitrogen is generally considered one of the major limiting nutrients in plant growth (Khan *et al*. 2008; France *et al.* 2009). Under drought stress, the ability to maintain high nitrogen fixation could aid peanut genotypes in maintaining high yield (Pimratch *et al.* 2008). Several mechanism of nitrogen fixation were involved in the physicologycal response to drought stress such as carbon shortage and nodule carbon metabolism, limitation of nitrogen and feedback

Table 2 Morphology of endophytic bacteria from *Aloe* sp.

No.	Isolates code	Part of leaf	Pigment	Morphological colony				
				Size	Edge of the colony	Shape	Dry/Slimy	Transparantly
1.	AS 1	pole	yellow	moderate	smooth	round	slimy	opaque
2.	AS 2	pole	cream	moderate	rough	round	slimy	opaque
3.	AS 3	pole	cream	moderate	rough	round	slimy in the middle	opaque
4.	AS 4	pole	old cream of the central part, light cream in tip	moderate	smooth	round	slimy in the middle	transparantly in tip
5.	AS 5	pole	old cream slightly yellow	moderate	smooth	round	little slime	opaque
6.	AS 6	pole	old cream	moderate	smooth	round	little slime	opaque
7.	AS 7	pole	cream slightly yellow	moderate	rough	round	slimy	opaque
8.	AS 8	pole	cream slightly yellow	moderate	smooth	round	slimy	opaque
9.	AS 9	pole	cream -gray	moderate	smooth	round	dry	opaque
10.	AS 10	pole	cream slightly yellow	moderate	smooth	round	dry	opaque
11.	AS 11	center	milky white	moderate	smooth	round	slimy	opaque
12.	AS 12	center	cream	moderate	smooth	round	little slime	opaque
13.	AS 13	center	cream slightly yellow	moderate	smooth	round	little slime	opaque
14.	AS 14	center	yellow	sizeable	rough	round	little slime	opaque
15.	AS 15	point	cream slightly yellow	moderate	rough	round	dry	opaque
16.	AS 16	tip	cream slightly yellow	moderate	rough	round	slimy	opaque
17.	AS 17	tip	milky white	moderate	rough	round	slimy	opaque
18.	AS 18	tip	yellow	moderate	smooth	round	little slime	opaque
19.	AS 19	tip	cream slightly yellow	moderate	rough	round	dry	opaque
20.	AS 20	tip	milky white	moderate	smooth	round	slimy	opaque
21.	AS 21	tip	yellow	a little small	rough	round	slimy	opaque
22.	AS 22	pole	pale white	moderate	rough	round	dry wrinkled	opaque
23.	AS 23	pole	bright white	moderate	smooth	round	slimy	opaque
24.	AS 24	pole	white	moderate	smooth	round	slimy	opaque

regulation by the accumulation of nitrogen fixation products (Serraj 2013). Endophytic bacteria in plant had a metabolism that play a role in the resilience of host plants at extreme environmental conditions such as drought and influence the process of nitrogen fixation in plants. Endophytic bacteria that inhabiting on those plants had ability for fixing nitrogen by nitrogenase enzyme. Those enzyme produced by *nif* genes that contribute to activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme. In diazotrophs, *nif* genes were typically found in a cluster of around 20-24 kb with seven operons encoding 20 different proteins. The complex of molybdenum nitrogenase enzyme had two component proteins encoded by the *nif*DK and the *nif*H genes. The *Nif*DK component were heterotetrameric (α2β2) protein formed by two αβ dimers related by a twofold symmetry. *Nif*DK carried one iron molybdenum cofactor (FeMo-co) within the active site in each a-subunit (*Nif*D) (Ahemad and Kibret 2014).

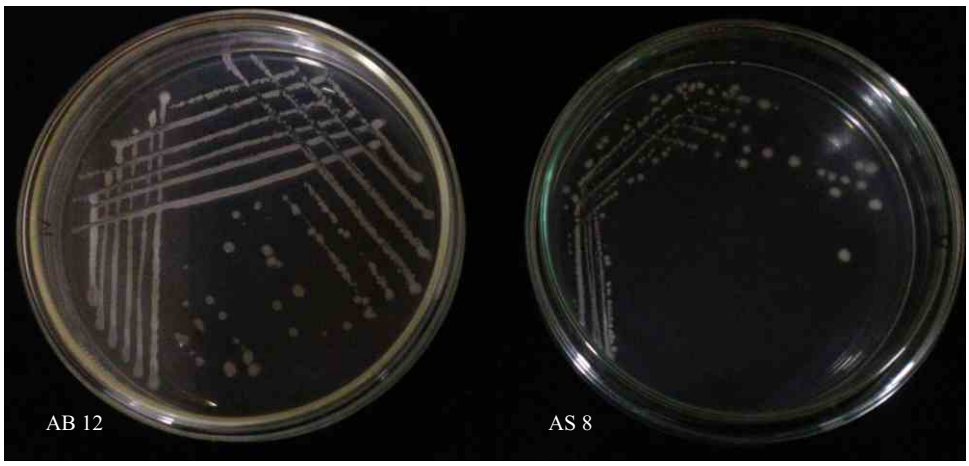
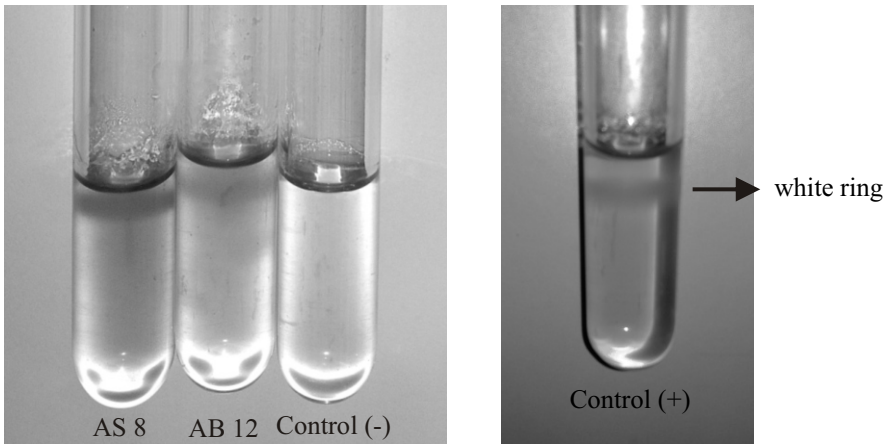


Fig 1 Morphology colony of isolates AS 8 and AB 12.

Table 3 Result of nitrogen fixing test for isolates from *Aloe barbadensis* and *Aloe* sp.

No.	Isolates from *A. barbadensis*	Result	No.	Isolates from *Aloe* sp.	Result
1.	AB 1	++	1.	AS 1	++
2.	AB 2	++	2.	AS 2	+
3.	AB 3	++	3.	AS 3	+
4.	AB 4	+	4.	AS 4	-
5.	AB 5	++	5.	AS 5	+
6.	AB 6	+	6.	AS 6	++
7.	AB 7	+	7.	AS 7	++
8.	AB 8	++	8.	AS 8	++
9.	AB 9	+	9.	AS 9	+
10.	AB 10	+	10.	AS 10	++
11.	AB 11	-	11.	AS 11	++
12.	AB 12	++	12.	AS 12	++
13.	AB 13	+	13.	AS 13	++
14.	AB 14	++	14.	AS 14	++
15.	AB 15	+	15.	AS 15	++
16.	AB 16	++	16.	AS 16	++
17.	AB 17	+	17.	AS 17	+
18.	AB 18	-	18.	AS 18	+
19.	AB 19	++	19.	AS 19	++
20.	AB 20	+	20.	AS 20	+
21.	AB 21	+	21.	AS 21	++
22.	AB 22	+	22.	AS 22	++
23.	AB 23	+	23.	AS 23	+
24.	AB 24	++	24.	AS 24	++
25.	AB 25	+	25.	Control	-
26.	Control	-			



A B

Fig 2 Nitrogen fixing test (A) endophytic bacteria of Aloe (B) Plant Growth Promoting Rhizobacteria (PGPR) isolate collection of Plant Symbiotic Microbes Laboratory Research Center of Biotechnology LIPI.

Table 4 Identification of AB 12 and AS 8 isolates based on 16S rRNA gene sequence homology by using BlastN program compared with Genbank sequences

Isolates	Species most related	Similarity	Length	Identities	Gaps	Accession number
AB 12	*Bacillus methalotropicus* strain DA 16-5	97%	1437	193/199	1/199 (0%)	KU862327.1
AS 8	*Bacillus aryabhattai* strain B8W22	99%	1533	1161/1171	5/1171 (0%)	NR. 115953.1

The capability endophytic bacteria isolated from *Aloe* sp. to fixed nitrogen was higher than *A. barbadensis*. The high nitrogen fixing were improve the ability of adaptation to drought stress. Nitrogen fixation ability related with habitat where place of the planted. Physiological of the host plant in *Rhizobium*-legume symbiosis have been known strongly impact to N_2-fixing system. Symbiotic N_2 fixation of legumes is also highly sensitive to soil water deficiency. Those condition promote a maximal nitrogen fixation input to the soil system by the *Rhizobium*-legume symbiosis (Zahran 1999).

Isolates AB 12 and AS 8 were the best isolates for nitrogen fixation procces from each varieties subsequently molecularly identified. Based on 16S rRNA gene analysis those isolates belonged to *B. methalotropicus* strain DA 16-5 and *B. aryabhattai* strain B8W22. Genus of *Bacillus* has been known for their potency as plant growth promoters, both directly and undirectly mechanism (Putrie *et al.* 2013; Meldau *et al.* 2012; Francis *et al.* 2010). It was reported that *Bacillus* spp is one of the potential bacteria beside that could fixing nitrogen, their also could produce bioactive compound for biocontrol of numerous plant pathogenic fungi. *B. methalotropicus* strain BC79 were isolated from primeval forest soil in Qinling Mountains, China were able to suppress mycelial growth and conidial germination of numerous plant pathogenic fungi in dual cultures on solid media (Shan *et al*. 2013).

This result may also confirmed and supported our finding that endophytes bacteria which was identified as *Bacillus* spp. have many potentials and one of them is nitrogen fixing ability. *B. methylotrophicus* strain L7 also known as efficient heterotrophic nitrification-aerobic denitrification (Zhang *et al*. 2012). Other species, *B. aryabhattai* strain B8W22 also known as nirogen fixing bacteria. *B. aryabhattai* strain B8W22 were isolated from the roots of tea (*Camellia sinensis* (L.) O. Kuntze) and *Nicotiana attenuata* are known for their potency as diazotropic bacteria in ability to fixed nitrogen and plant growth promoters (Gulati *et al.* 2011; Meldau *et al.* 2012).

The other hand, *Bacillus* are dominant genera of endophytic bacteria in aloe. Twenty-nine culturable bacterial endophytes were isolated from surface-sterilized root, stem and leaf tissues of *A. vera* based on molecularly characterized those belonged to 13 genera i.e. *Pseudomonas*, *Bacillus*, *Enterobacter*, *Pantoea*, *Chryseobacterium*, *Sphingobacterium*, *Aeromonas*, *Providencia*, *Cedecea*, *Klebsiella*, *Cronobacter*,

Macrococcus and *Shigella*. The dominant genera include *Bacillus* (20.7%), *Pseudomonas* (20.7%), and *Enterobacter* (13.8%) (Akinsanya *et al.* 2015a). Next generation sequencing (NGS) technology were captured effectively the metagenomics of microbiota in plant tissues and this can improve our understanding of the microbial-plant host interactions, esspesially in *Aloe vera* by assessing its PCR amplicon of 16S rDNA sequences (V3-V4 regions). The analyses revealed *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteriodetes* also known as the predominant genera (Akinsanya. *et al.* 2015b).

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