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Isolation and Molecular Identification of Endophytic Bacteria from the Arils of Durian (*Durio zibethinus* Murr.) var. Matahari

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Endophytes are plant-associated microorganisms able to form colonies in internal tissue and considered as an important component of biodiversity. However, information about the existence of naturally fruits-associated endophytic bacteria at different life-history stages of hosts is very limited. Durian (*Durio zibethinus* Murr.) is an exotic tropical fruits with a high economical value, but the occurrence and functional role of associated endophytes remains unexplored. A total of sixteen endophytic bacterial isolates were identified by 16S rRNA sequence analysis from ripe and unripe stages of durian fruits var. Matahari. These isolates belonged to the genus *Staphylococcus, Bacillus*, *Enterobacter*, *Moraxella*, *Gordonia*, *Salmonella*, *Rhizobium*, *Brachybacterium*, *Kocuria*, and *Klebsiella*. This is the first report of an endophytic bacterial spesies residing in durian arils. This research also indicated potency of culturable endophytes from durian fruits in plant growth promotion.

Key words: 16S rRNA gene, Durian, endophytes

Endofit adalah mikroorganisme asosiatif pada tanaman yang mampu berkoloni dalam jaringan internal tertentu dan menjadi komponen penting dalam biodiversitas. Akan tetapi, informasi mengenai keberadaan bakteri endofit pada bagian organ buah di tahap perkembangan yang berbeda masih sangat terbatas. Durian (*Durio zibethinus* Murr.) merupakan buah tropis dengan nilai ekonomis yang sangat tinggi. Namun, studi mengenai keberadaan dan peranan bakteri endofit pada buah durian masih belum tereksplorasi dengan baik. Enam belas isolat bakteri endofit yang berasal dari *arillus* Durian var. Matahari mentah dan matang telah berhasil diidentifikasi dengan menggunakan analisis sikuen gen 16 rRNA. Isolat tersebut terdiri dari genus *Staphylococcus, Bacillus*, *Enterobacter*, *Moraxella*, *Gordonia*, *Salmonella*, *Rhizobium*, *Brachybacterium*, *Kocuria*, dan *Klebsiella*. Penelitian ini adalah studi pertama mengenai identifikasi spesies bakteri endofit yang berada pada *arillus* buah Durian. Selain itu, hasil penelitian ini pun menunjukkan bahwa bakteri endofit yang berasal dari buah Durian berpotensi dalam pertumbuhan tanaman.

Kata kunci: Durian, endofit, gen16S rRNA

Bacteria may be living in plant tissue as pathogen, saprophytic, or endophyte. Bacteria that colonize healthy plant tissue without causing or produce obvious injuries to the host is defined as an endophytic bacteria (Bacon and Hinton 2007). Endophytic bacteria may exhibit a plant growth promotion (PGP) ability through an extensive mode of action such as ACC deaminase production, phytohormones synthesis, phosphate solubilization, siderophore production and nitrogen fixation (Rosenblueth and Martínez-Romero 2006; Malfanova 2013). By colonizing plant tissues, bacterial endophytes can help their host more efficiently than those that bind exclusively to the plant's rhizosphere.

Of many studies on endophytic bacteria, very few shows their occurrence and functional role in plant reproductive organs such as seeds, flowers, or fruits (Kukkurainen *et al.* 2005; de Melo Pereira *et al.* 2012; Compant *et al.* 2011; Audipudi *et al.* 2014). Fruits are unique habitat for microbial growth in the phyllosphere region because of their sugar content. As the bacteria proliferate inside the plant tissue, they are likely to develop interaction with the host and many of them have shown plant growth-promoting (PGP) effect. These abilities were mediated through direct effect of PGP ability or through competition with phytopathogens. Direct PGP effect mediated by endophytes is mostly based on providing essential nutrients and production or regulation of phytohormones (Malfanova 2013). de Melo Pereira *et al.* (2012) demonstrated that *Pseudomonas*, *Enterobacter*, and *Bacillus* isolates from strawberry fruits were capable to produce indole acetic acid (IAA), fix N_2, and secrete siderophore. These abilities also have been confirmed *in planta* to enhance strawberry

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growth. However, our understanding of the role and potential of endophytes under different stages of fruits development is still very limited.

Durian *(Durio zibethinus* Murr) is an economically important fruit in Southeast Asia. The major importers are Taiwan and Hong Kong for fresh durian, while USA, Australia, and Hong Kong for frozen durian (Perez 2013). As a result, the demand for durian is always increasing every year, especially for favorite cultivars. This climacteric fruit has a strong smell and a unique taste. Durian normally has five locular units and each locular contains 1–5 pulp units. The pulp unit consists of seed, which is completely covered by a creamy, white, yellow or golden yellow aril, the edible portion of the fruit. In spite of their unique characteristics, durian also has great nutritions and antioxidant activities (Poovarodom *et al*. 2010). Nevertheless, the information about microorganisms in durian is rather limited, expecially in the term of endophytic bacteria. The aim of this study was to identify endophytic bacteria found in the arils of durian fruits under natural condition using the culture-dependent method and 16S rRNA sequence analysis for its characterization.

MATERIALS AND METHODS

Samples. Durian fruit (*Durio zibethinus* Murr.), var. Matahari were collected from an agricultural field belonging to the Kebun Percobaan, Balai Penelitian Buah, Subang, West Java, Indonesia. The cultivar Matahari was chosen because it produced high quality fruit, based on some characteristics like the thickest flesh, the highest percentage of edible portion, and yellow colour of the fruit flesh (arillus). Unripe and ripe fruits were selected from the same tree and brought to the laboratory in sterile polyethylene bags. To avoid contamination and to isolate endophytic bacteria only from arillus part, the suture and the stalk of the fruits were covered with wax.

Isolation of Endophytic Bacteria. The fruits surface (epicarp) was sterilized by 70% ethanol for 4 min, 2.5% sodium hypochlorite for 4 min, and 70% ethanol for 4 min, followed by three rinses in sterile deionized water. After surface disinfection, 1 g of Durian arillus from three different locular unit site were mixed with 1 mL 0.85% NaCl and followed by serial dilutions. After that, 0.1 mL aliquotes from appropriate dilutions were plated into Luria Bertani agar medium. Triplicate plates were incubated at 37 ºC for 48 h for the total plate counts. Colonies with unique morphological appearence was selected from the plates. A single pure colony was obtained from approximately 11 times subcultures process. Gram staining was also performed to confirm the purity of the colony.

Biochemical Characterization. Isolates were tentatively grouped according to their morphological and cultural characteristics of the colonies on plates (Cappuccino and Sherman 2005). For biochemical analysis, all isolates was grown aerobically on Luria–Bertani (LB) plates overnight at 37 ºC. A total of eighteen biochemical tests were performed to all isolates, including those for sugars fermentations, oxidase, catalase, cell motility, and IMViC test using standard methods as described by Cappuccino and Sherman (2005). The isolated endophytic bacteria were identified by biochemical characterization based on Bergey's Manual Of Systematic Bacteriology (Garrity 1984).

Identification of Endophytic Bacteria using 16S rRNA gene. Single colony of bacteria was grown in liquid LB medium for 16-18 hours for DNA extraction. Bacterial DNA was obtained according to the boiling procedure reported by Yang *et al.* (2008). Universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used in order to amplify approximately 1500 bp of 16S rRNA gene (Turner 1999). A total PCR volume of 50 µL contains 2 µL DNA template (25 ng μL^{-1}), 25 µL Go*Taq* Green® Master Mix (Promega), 1.5 µL primer forward and 1.5 µL primer reverse (25 pmol μL^{-1}), and 20 µL nuclease free water. The PCR was performed with a thermal profile of 95 ºC for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 45 s, and extension at 72 °C for 2 min and a final elongation at 72 °C for 7 min (Applied Biosystems, 2720 Thermal Cycler). A 5 µL of PCR products then visualized by electrophoresis in 1% agarose (Bioline) in 1x TAE buffer for 25 min, 100V. PCR products were then purified using kit from GeneAid. Sequencing of PCR products were performed in Macrogen Inc., Korea. The nearly fulllength 16S rRNA gene sequence was compiled using CLC Genomics Workbench 3 and compared with those from the National Centre of Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program. Alignment and clustering were performed by Kimura-2 parameter (Tamura 1992) and Neighbor-joining methods. Phylogenetic trees and molecular analyses were conducted using MEGA version 5.2 (Tamura *et al.* 2011), where 1000 replication of bootstrap analyses were performed (Soltis and Soltis 2003).

Bioassay of Plant Growth Promoting (PGP) Properties. Furthermore, sixteen isolates then evaluated in term of PGP properties to measure their potency in growth promotion.

Indole-3-Acetic-Acid (IAA) Production Assay. The bacterial isolates were inoculated into 20 mL of nutrient broth supplemented with 0.2 % (v/v) of L-tryptophan and incubated for 24 h at 37 ºC. After incubation, the culture was centrifuged at 3000 rpm for 20min and the supernatant was used for analyzing indole 3 acetic acid production (Rahman *et al.* 2010). Initially one mL supernatant was mixed with 2 mL of Salkowski reagent and tubes were incubated in the dark for 30 min. IAA production in the cultured medium was evident by characteristic indication of reddish to pinkish colour in the solution. Uninoculated growth medium was used as negative control.

Phosphate Solubilization Assay. Phosphate solubilization was measured by the procedure described by Jasim *et al.* (2013). Plates containing Pikovskaya (PVK) medium ($C_6H_{12}O_6$ 10 g L^{-1}, $CaHPO_4$ 5 g L^{-1}, $(NH_4)_2SO_4$ 0.5 g L^{-1}, NaCl 0.2 g L^{-1}, $MgSO_4.7H_2O$ 0.1 g L^{-1}, KCl 0.2 g L^{-1}, yeast extract 0.5 g L^{-1}, $MnSO_4.H_2O$ 0.002 g L^{-1}, $FeSO_4.7H_2O$ 0.002 g L^{-1}) containing 2.4 mg mL^{-1} bromophenol blue were inoculated with a streak of each bacterial culture. Plates inoculated with the isolates were incubated for 48 h at 37 ºC and observed for the transparent "halos" around each colony due to the utilization of tricalcium phosphate present in the medium. Uninoculated growth medium was used as negative control.

RESULTS

In order to determine the existence of endophytic bacteria from durian fruit, we isolated bacteria from three different locular units (sites) of each fruits life-history stages. A total of thirty five bacterial isolates were isolated from unripe and ripe stages. In contrast, no colonies appeared in both of control plates, indicating that aseptic handling and cultivation of the endophytic bacteria were successful. Furthermore, we chose bacterial colonies from LB plate according to their morphological differences. Sixteen morphologically different colonies were selected; four isolates from unripe stage (A1-A4) and twelve isolates from ripe stage (B1-B12). The ripe stage of durian fruits exhibited the highest number of cultivable bacteria.

A total of sixteen bacterial isolates were characterized by 16S rRNA gene analysis. The phylogenetic tree (Fig 1) was obtained using the neighbor-joining method after calculation of a Kimura two-parameter substitution model with the software Mega 5.2 and evaluated by 1000 bootstrap. The most common endophytic bacteria associated with durian arillus belong to the genera *Klebsiella, Enterobacter*, *Bacillus, Moraxella*, *Gordonia*, *Salmonella*, *Rhizobium*, *Staphylococcus*, *Brachybacterium*, and *Kocuria* (Fig 2). *Enterobacter* and *Salmonella* from *γ-proteobacteria* class could be isolated from both fruit developmental stages. Meanwhile, *Bacillus* and *Staphylococcus* from *Firmicutes* class were only found in ripe durian. Of all isolates, 37.5% were Gram-negative and 62.5% were Gram-positive. All isolates showed various biochemical characteristic (Table 1). These characteristics were important, as they supported the molecular identification based on some unique features of each genus.

Bioassays were performed to assess the plant growth promoting properties of each endophytic bacteria based on their IAA production and phosphate solubilization abilities. Sixteen endophyte isolates were subjected to IAA assay using Salkowski's reagent for colorimetric IAA detection in liquid culture. As many as eight isolates (50%) had been shown to produce IAA. All isolates from unripe stages were able to produce IAA, whereas only four isolates showed the ability in ripe stage. A qualitative test of inorganic phosphate solubilization was also accomplished to assess the ability of all isolates to solubilize inorganic insoluble phosphate. Among these isolates, nine isolates (56.2%) had the ability to solubilize phosphate (Table 2). The highest number of phosphate solubilizing bacteria was found in the ripe stage and predominated by *Enterobacter.*

DISCUSSION

Recently, endophytes are viewed as a new potential source of novel genes, proteins, and natural biochemical compounds. These unique bacteria showed that they can also colonize plant reproductive organs such as seeds, flowers, or fruits (Kukkurainen *et al*. 2005; Pereira *et al*. 2011; Compant *et al*. 2011; Audipudi *et al*. 2014). The present study demonstrates that healthy and field-grown durian fruits were inhabited by various endophytic bacteria. Generally, thirty five colonies were isolated from both fruit stages; nine isolates from unripe stage and twenty six isolates from ripe stage. The largest population and diversity of endophytic bacteria in fruit arilli was observed during

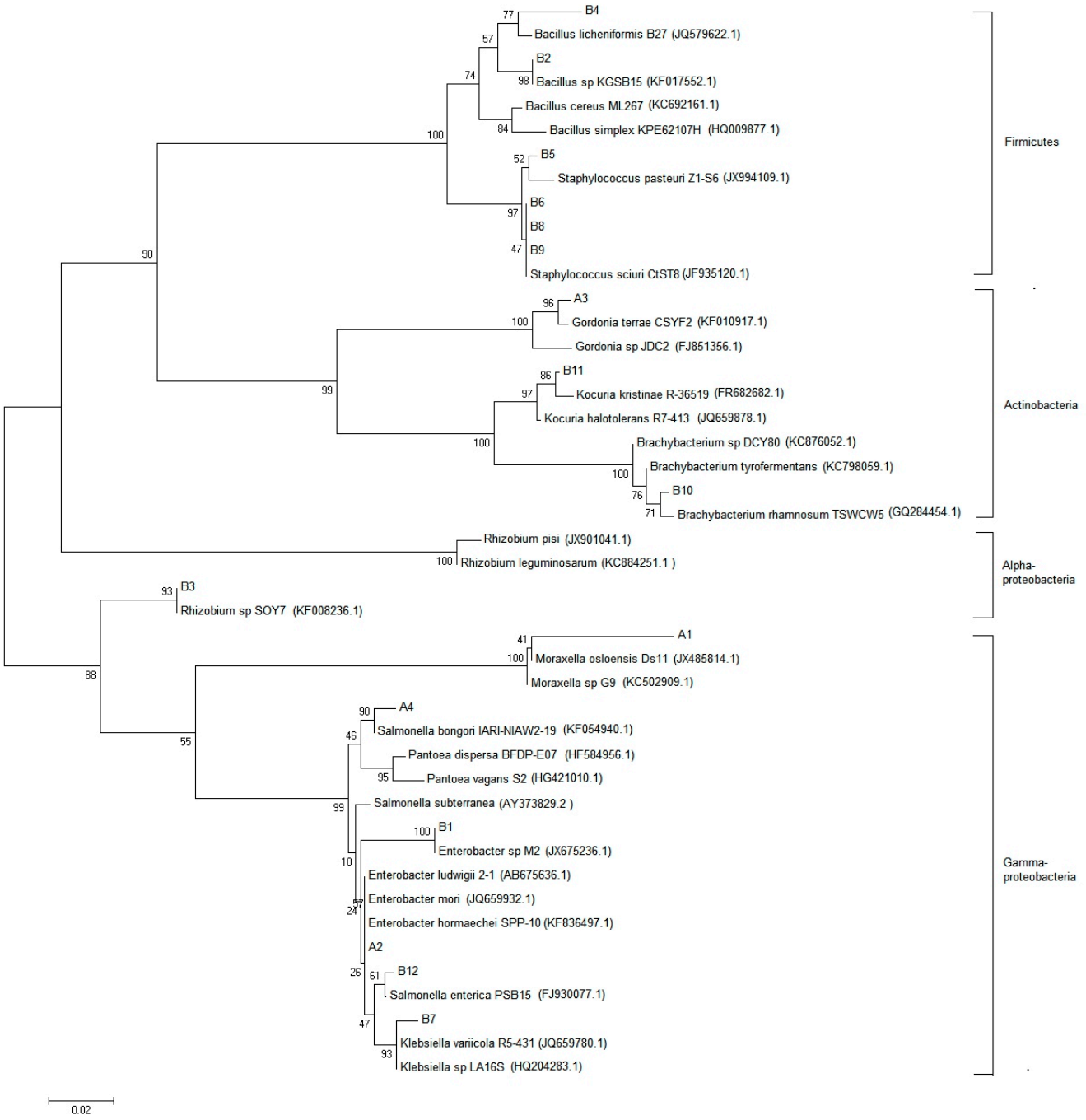

Fig 1 Neighbor-joining tree of the 16S rRNA sequences of durian (cv. Matahari) fruits endophyte . The tree topology was evaluated with Kimura (two parameters). Bar: 2 nucleotide substitutions per 100 nucleotides.

ripe stage. During ripening under natural conditions, the fructose and glucose contents were significantly increased in durian arillus, which may be one of the factors that contributes to the differences in the endophyte diversities between the developmental stages (Voon *et al*. 2006). The monosaccharide content was attributed by the hydrolysis of sucrose in the unripe stage and may lead to the enhancement of endophyte growth in the ripe stage.

The isolation was performed by culture-dependent method in aerobic condition. Thus, there is also a possibility to isolate endophytic bacteria anaerobically. However, low endophytic bacterial counts in our study were also largely supported by the fact that the fruit endophytic bacterial populations would decreas because of their distance from the root (Compant *et al*. 2011; Malfanova 2013). Sixteen isolates were selected for further molecular identification and plant growth properties investigations. Each isolate was represented by morphological characteristics that are different from the other culturable endophytic bacteria existed in the sample.

Table 1 Biochemical characteristics of the endophytic bacterial isolates

No	Sources	Isolates	Gram Staining	Motility	Litmus Milk Reaction	Fermentation			H_2S Production	No_3 Reduction	IMVIC Test				Urease Test	Catalase Test	Oxidase Test	Gelatin Liquefaction	Starch Hydrolysis	Lipid Hydrolysis
						Lactose	Dextrose	Sucrose			Indole	Methyl Red	Voges Proskauer	Citrate Test						
1	Unripe Durian	A1	Gram -	-	*alkaline*	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
2		A2	Gram -	+	reduction	+	+	+	-	+	-	+	+	-	-	+	-	-	+	+
3		A3	Gram +	-	*alkaline*	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+
4		A4	Gram -	-	*alkaline*	+	+	-	+	+	-	+	-	+	-	+	-	-	-	+
5	Ripe Durian	B1	Gram -	+	reduction	+	+	+	-	+	-	+	+	-	-	+	-	-	-	+
6		B2	Gram +	+	reduction	-	+	+	-	+	-	+	-	-	+	+	-	+	+	-
7		B3	Gram -	-	reduction	+	+	+	-	+	-	-	-	-	+	+	+	-	-	+
8		B4	Gram +	+	reduction	-	+	+	-	+	-	+	+	-	+	+	-	+	-	+
9		B5	Gram +	+	reduction	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-
10		B6	Gram +	+	reduction	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-
11		B7	Gram -	-	reduction	+	+	+	-	+	-	-	+	+	+	+	+	+	-	-
12		B8	Gram +	+	reduction	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-
13		B9	Gram +	+	reduction	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-
14		B10	Gram +	-	*alkaline*	+	+	+	-	-	-	+	-	-	+	+	+	-	+	+
15		B11	Gram +	+	*alkaline*	-	+	+	-	-	-	+	-	-	+	+	+	-	-	+
16		B12	Gram -	-	*alkaline*	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+

Table 2 Growth promoting capability of the isolated endophytic bacterial isolates

No	Sources	Isolates	Identification	Plant Growth Promoting Properties	
				IAA Production	Phosphate Solubilization
1	Unripe durian	A1	*Moraxella osloensis*	+	+
2		A2	*Enterobacter hormaechei*	+	+
3		A3	*Gordonia terrae*	+	-
4		A4	*Salmonella bongori*	+	-
5	Ripe durian	B1	*Enterobacter sp.*	+	+
6		B2	*Bacillus sp.*	-	-
7		B3	*Rhizobium sp.*	+	+
8		B4	*Bacillus licheniformis*	-	-
9		B5	*Staphylococcus pasteuri*	-	-
10		B6	*Staphylococcus sciuri*	-	-
11		B7	*Klebsiella variicola*	+	+
12		B8	*Staphylococcus sciuri*	-	+
13		B9	*Staphylococcus sciuri*	-	-
14		B10	*Brachybacterium rhamnosum*	+	+
15		B11	*Kocuria kristinae*	-	+
16		B12	*Salmonella enterica*	-	+

The molecular identification of the cultured endophytic bacteria using 16S rRNA gene analysis revealed high bacterial diversity in the arillus of durian fruits. We discovered that bacterial endophytes in ripe durian arillus were more diverse than in unripe stage (Fig 2). Bacterial endophytes from ripe arillus included twelve isolates from eight different genera. Meanwhile, only four isolates belonging to *Moraxella osloensis, Enterobacter hormaechei, Gordonia terrae,* and *Salmonella bongori were isolated* from unripe arillus. Only two genera were found in both developmental stages. This difference might have been caused by various biotic and abiotic parameters (Berg and Smalla 2009). Many identified bacterial genera,

such as *Enterobacter* have also been identified in other studies (Souza *et al.* 2013; Ferrara *et al.* 2011; Wang 2006; Madmony *et al*. 2004). *O*n the other hand, our study is also the first to report the finding of some interesting genera in Durian fruits, such as *Gordonia.* The finding of this interesting genus suggests that Durian fruits are host to unique endophytic bacteria that may possess significant biosynthetic potential.

Members of Enterobacteriaceae family had been isolated from a wide variety of plants, like in maize seeds (Johnston-Monje and Raizada 2011). Our study has revealed the appearance of *Enterobacter* genus in unripe and ripe stages of Durian arillus (Fig 2). Based on their biochemical characteristics (Table 1), *Enterobacter hormaechei* (A2) and *Enterobacter sp.* (B1) should be able to utilize a wide range of sugars. This might contribute to the occurence of these species in fruits. Furthermore, metagenomic studies also referred to *Enterobacter* spp. as one of the "core microbiota" in maize (Johnston-Monje and Raizada 2011). *Enterobacter* does not only reside in the host tissues, but also plays an important role as a plant growth promoting bacteria (Asis and Adachi 2003; Ogbo and Okonkwo 2012).

Salmonella bongori (A4) and *Salmonella enterica* (B12) were also isolated from both stages of durian fruits. Although *Salmonella* is described as human pathogenic bacteria, this genus has recently been reported as a plant endophyte (Schikora *et al.* 2012). Different distribution of genes essential for surface attachment and secretion system, like SPI2 type III transport system, may indicate critical divergences between the strains that infect plants and human (Fookes 2011). According to their biochemical characteristics (Table 1), neither of them can metabolize sucrose nor produce hydrogen sulfide. This

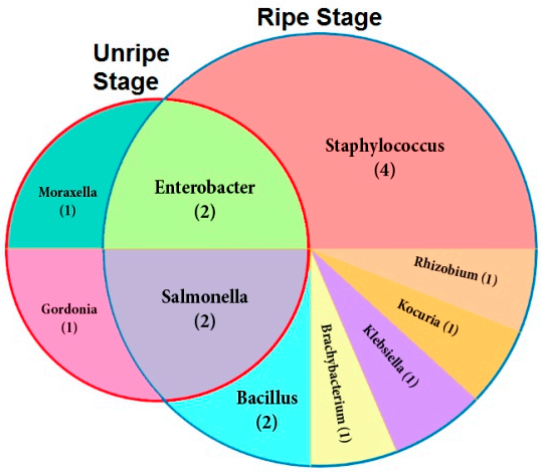

Fig 2 Distribution of endophytic bacterial isolates in Durian (*Durio zibethinus* Murr) cv. Matahari arillus.

finding agrees with former studies (Ellermeier and Slauch 2006), which described specific metabolic characteristics of *Salmonella* genus. Indeed, the genes that play roles in the production of hydrogen sulfide can be used as *Salmonella*-specific molecular markers (Porwollik *et al*. 2002).

Staphylococcus was the most frequently encountered genus in this study. *Staphylococcus* and *Bacillus* are members of phylum *Firmicutes*. Interestingly, *Staphylococcus sciuri* (B6, B8, B9) is the predominant *Firmicutes* in durian arillus. This is different from many studies that showed *Bacillus* spp. as the most common plant endophytic isolates (Souza *et al*. 2013; Kumar *et al*. 2011; Figueiredo *et al*. 2009). *Staphylococcus sciuri* is commonly present on skin and mucosal surfaces of a wide range of wild animals, like bats (Vandžurova *et al*. 2012). Since bats are well known pollinators of durian (Baker 1970; Morton 2004), this finding will be a very interesting topic for further study. Indeed, it has been suggested that some taxa of fruit endophytes may not only be transported from the root environment but also from other sources such as the anthosphere (Compant *et al.* 2011).

Of ten genera found as durian's Matahari endophytes, six of them were *Moraxella* (A1), *Gordonia* (A3), *Rhizobium* (B3), *Klebsiella* (B7), *Brachybacterium* (B10), and *Kocuria* (B11). Most of these isolates were positive for nitrate reduction test (Table 1). Reduction of nitrate is generally an anaerobic respiration, in which an organism derives its oxygen from nitrate. This result may explain how the bacteria adapted to low oxygen levels inside the fruits. *Rhizobium sp.* is a well-known symbiotic nitrogen-fixing bacteria, which can be found as an endophyte in numerous plants (Van Rhijn and Van derleyden 1995). Most *Rhizobium sp.* play a role as plant growth-promoting rhizobacteria (PGPR). Wei (2013) also observed that *Klebsiella variicola* from sugarcane roots was capable to fix nitrogen. In contrast, there was not enough information about the role of the remaining spesies. However, the presence of *Gordonia* is noteworthty, as members of this genus have the capabilities to degrade, transform, and synthesize some organic compounds (Drzyzga 2012).

The remaining isolates were related to the *Bacillus licheniformis* (B4) and *Bacillus sp.* (B2). The *Bacillus* species have been described as endophytic species in banana (Souza *et al*. 2013), maize (Figueiredo *et al*. 2009), and Daedok plants (Kang *et al.* 2013). *Bacillus* plays a role as biocontrol agent in plant and stimulates plant growth. In strawberry plant (de Melo Pereira *et al*.

2012) some *Bacillus* species were able to produced IAA, siderophore, and proven to improve plant growth. In addition, *Bacillus licheniformis* from Daedok plants not only have the capacity to produce plant growth hormones but also showed antifungal activity against plant pathogenic fungi (Kang *et al*. 2013).

Even though the presence of endophytic IAA-producing bacteria has already been reported in other plants, reports from durian fruits is very limited. Interestingly, all unripe endophytic isolates gave positive colour reactions to Salkowski's reagent but only one-third of the ripe isolates exhibited positive response (Table 2). Both of *Enterobacter* spp. from unripe and ripe Durian were able to produce IAA. Our findings are relevant with the results of endohytes study from pollen pines (Madmony *et al*. 2005), which revealed *Enterobacter cloacae* as an IAA-producing bacteria. Durian is a great source of tryptophan (Leontowicz *et al*. 2011) that can be used as the precursor of IAA biosynthesis by microorganism (Gravel *et al*. 2007). In tomato, IAA has been reported to have some crosstalk with ethylene during fruits maturation (McAtee *et al*. 2013). However, until now there has been no direct evidence linking between IAA produced by bacterial endophyte with the modulation of host fruits ripening process.

Availability of organic phosphate (P) for plant nutrition could be a limitation in some soils. Phosphate is an essential nutrient for plant growth and has only limited bioavailability. It is considered to be important elements that limit plant growth. Thus, solubilization and mineralization of P by phosphate-solubilizing bacteria (PSB) is one of the most important bacterial physiological traits in soil (Jeffries *et al*. 2003). Several genera are known to be able to solubilize phosphate such as *Pseudomonas* (Park *et al*. 2009), *Bacillus* (Turan *et al*. 2007), *Rhizobium* (Chabot *et al*. 1996), and *Enterobacter* (Sharma *et al*. 2005). Our result are relevant to these studies (Table 2). Among all endophytic bacteria, 56.25% isolates showed positive results in phosphate solubilizaton. Furthermore, some interesting genera also exhibited this properties, including *Moraxella*, *Klebsiella*, *Staphylococcus, Salmonella,* and *Kocuria*. Although knowledge of the genetics of phosphate solubilization is still scanty, some genes involved in mineral and organic phosphate solubilization have been isolated and characterized (Rodriguez *et al*. 2006). The ability of endophytic bacteria from Durian arils to solubilize phosphate may be employed to boost organic phosphate uptake from the soil.

In conclusion, the results from the study demonstrated the diverse community of endophytic bacteria associated with Durian arillus. We have successfully isolated 35 endophytic bacteria from two developmental stages. To our knowledge, this study constitutes the first report on the isolation and identification of endophytes bacteria in the arillus of Durian fruits, especially var.Matahari. Moreover, metagenomic studies are needed to unravel in depth the structure and function of the endophyte community in Durian fruits. Among these endophytic bacterial isolates obtained, most of them have the ability to produce IAA and perform phosphate solubilization activity. Hence, these isolates can be considered to have potential use in plant growth promoting and development. Further studies are needed to explore some unique isolates to determine their functional roles.

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