BIODIVERSITAS
Volume 21, Number 7, July 2020
Pages: 3391-3397

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210764

Isolation, identification and antimicrobial activities of Lactic Acid Bacteria from fruits of wild plants in Tambrauw Forest, West Papua, Indonesia

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Manuscript received: 14 April 2020. Revision accepted: 29 June 2020.

Abstract. *Dinoto A, Rosyidah A, Susilo ARPS, Julistiono H. 2020. Isolation, identification and antimicrobial activities of Lactic Acid Bacteria from fruits of wild plants in Tambrauw Forest, West Papua, Indonesia. Biodiversitas 21: 3391-3397.* Presence of culturable lactic acid bacteria (LAB) in fruits of wild plants and their antimicrobial activities has not been widely reported. The purposes of this study were to isolate LAB from the fruits of wild plants found in the Tambrauw forest area, West Papua Indonesia, and to evaluate their antimicrobial activities. Isolation of LAB from fruit was conducted using MRS medium supplemented with 1% $CaCO_3$. Isolates of LAB were identified based on 16S rRNA gene using BLAST analysis. Antimicrobial assays were carried out by determining the minimum inhibitory concentration (MIC) based on thiazolyl blue tetrazolium blue (MTT) using indicator microorganisms *Escherichia coli, Staphylococcus aureus,* and *Mycobacterium smegmatis*. The results showed that total of fourteen isolates of LAB with different characteristics was successfully isolated from 8 of 14 collected wild plants. Based on 16S rRNA sequences, isolates had closest relationships with *Lactococcus lactis, Lactococcus garvieae, Weissella confusa, Weissella oryzae,* and *Enterococcus faecalis* with the similarity of 99%. All 16S rRNA nucleotides of these strains have been deposited in the GenBank. Assays for antimicrobial activities were demonstrated by the highest inhibition of supernatant of *Lac. lactis* HM 1.1 from fruit plant *Donax canniformis* and *W. confusa* H14.2 from fruit plant *Capparis* sp. against *E. coli, S. aureus,* and *M. smegmatis* even though the MIC values of those strains were lower than that of bacterial strain from the commercial probiotic product. This study showed that wild fruit from Tambrauw forest harbor beneficial lactic acid bacteria that could be important for health of animals and humans as well. In addition, this study provided basic information on indigenous LAB for promoting further development of medicinal antibacterial compounds.

Keywords: Antimicrobial activity, *Escherichia coli*, lactic acid bacteria, *Mycobacterium smegmatis, Staphylococcus aureus,* wild fruits

Abbreviations: LAB: lactic acid bacteria; MIC: minimum inhibitory concentration; MTT: thiazolyl blue tetrazolium blue

INTRODUCTION

Lactic acid bacteria are a group consisting of microbial strains that ferment sugar predominantly to lactic acid, Gram positive, typically non- sporulating rod or coccus shaped (Ray et al. 2014; Ali 2010). The presences of LABs were reported in many plant-based fermented products of vegetables and also in flower and fruits (Karyawati et al. 2018; Mangunwardoyo et al. 2016; Sulistiani et al. 2014; Swain et al. 2014). LAB formed association with both animal and plant niches and play an important role in the production of fermented food (Makarova et al. 2006). The LAB originated from forests that have good potential to be explored. The application of LAB as biocontrol and probiotic agents are examples of its potential (Hwanhlem et al. 2014). Probiotics are dietary supplements consisting of living bacteria with various health benefits (Gowri et al. 2016). Nonetheless, probiotics could be also beneficial for animals.

LABs are found in wild animals as well as gorillas (Tsuchida et al. 2014). The presence of LAB in the natural environment could be an object of study on ecology considering the interaction between LAB-plants and animals. This speculation as proposed by Choi et al. (Choi et al. 2016) showed that LAB in *Citrus medica* was able to attract wild nematodes (*Caenorhabditis elegans*) due to the diacetyl produced from fermentation by LAB which was detected by diacetyl odor receptor (ODR-10). Whereas other studies of *Lactobacillus casei* showed that *C. elegans* proved to be useful as probiotics that protected nematodes from oxidative stress (Kamaladevi et al. 2013) and improved the phase II detoxification system caused by in vivo malathion pesticides (Kamaladevi et al. 2016).

As typical rain forest, Tambrauw forest area located in West Papua, Indonesia is an area with the most diverse organisms (Robiansyah 2018). Microbial diversity in this area especially for LAB is less observed. In addition, although fruit is one source of feeds, the impact of the presence of LAB on fruits on animals consuming the wild fruit has not been studied. Considering the reports on the presence of LAB on fruits (Yu et al. 2017; Chen et al. 2010; Leong et al. 2014), the effects of LAB on animals that consume the aforementioned fruits are possible and lead to the core idea of LAB roles in biodiversity studies of

forest ecosystems. The study on presence of LAB on wild fruits in the Tambrauw forest is expected to be preliminary data on the study of LAB-animal-plant interaction in the forest ecosystem. In addition, the finding of LAB which has probiotic properties such as antimicrobial activity against pathogens was expected to be applied on animals and humans. By collecting the indigenous LAB and investigating the potential properties of pathogens inhibition may lead to the further development of medicinal antibacterial compounds.

The purposes of this study were to isolate lactic acid bacteria (LAB) from the fruits of wild plants found in the Tambrauw forest area, West Papua Indonesia, and to evaluate their antimicrobial activities. Several antimicrobial activities of selected LAB isolated from wild fruits in Tabrauw forest were investigated against three indicator microbes including *Escherichia coli* (Gram-negative), *S. aureus* (Gram-positive) and *M. smegmatis* (*Mycobacterium* spp. model). *E. coli* and *S. aureus* are enteropathogenic bacteria and able to infect wild animals (de Godoy et al. 2016; Iovine et al. 2015). *Mycobacterium* spp. could promote infection in the gastrointestinal tract (Sangari et al. 2001) and be found in the oropharynx of some wild mammals (Albertti et al. 2014).

MATERIALS AND METHODS

Microorganisms used in this study

Target microbes used as pathogen models were *Escherichia coli* InaCC B5, *Staphylococcus aureus* InaCC B4, and *Mycobacterium smegmatis* NBRC 3082 which are obtained from Indonesian Culture Collection (InaCC). The three target microbes were inoculated on nutrient broth (Himedia). Incubation was performed at room temperature on shaker (100 rpm) for 24 hours (*E. coli* and *S. aureus*) or for 72 hours (*M. smegmatis)* on nutrient agar (Himedia). Bacterial strain isolated from the commercial probiotic product was used as reference microorganisms in antimicrobial assays, in which *Lactobacillus casei* is recognized from the product label. This strain from commercial probiotic product was maintained on de Man Rogosa and Sharpe broth (MRS Broth-Oxoid).

Sample collection and bacterial isolation

Sampling was conducted in Tambrauw forest area, West Papua, Indonesia on April 2016. Plants specimens were identified and deposited in Herbarium Bogoriense (BO), Indonesian Institute of Sciences, Bogor, Indonesia. Fruit samples were picked from the tree and cut to small pieces (0.4 cm x 0.4 cm x 0.4 cm) with a 70% ethanol-sterilized knife. The pieces of fruits were swiftly placed on 5 ml of MRS agar (Oxoid) in a 15 ml "corning" tube with or without the addition of 7 ml of sterilized cooking oil. The addition of oil on the surface of the medium was performed to reduce the amount of air oxygen that could dissolve in the MRS agar media thus inhibiting the growth of LAB in the field. Media containing pieces of fruits were left for about 10 days in the field before further isolation in the laboratory. LAB isolation was carried out by serial dilution (up to 10^8 dilution series) with sterilized 0.85% NaCl solution and poured onto MRS agar (Oxoid) containing 1% $CaCO_3$ in petri dish. Incubation was conducted at 37^0 C for 24 hours. The formation of LAB colonies was indicated by a clear zone around the colony on MRS agar containing $CaCO_3$. Selected colonies were transferred to a new medium for purification and the purified isolates were then preserved both in slanted agar and in the glycerol medium at -80^0C deep freezer.

DNA extraction

Genomic DNA extraction of each isolate was performed following method of phenol treatment (Saito and Miura 1963) with some modifications. Purified LAB colonies (2 days) were put in microtube containing 500 µL TE 1x, subsequently homogenized and then centrifuged at 9,000 *xg*, 4°C, for 5 min to obtain pellet. Pellet was added with 50 µL TE 1x and 300 µL buffer extraction, homogenized using a vortex, and added with 150 µL 3M sodium acetate pH 5.2. After incubation at room temperature for 10 min, the mixture was centrifuged at 9,000 *x g*, 4°C, for 5 min. Supernatant was then added with isopropanol (1:1, v/v) and centrifuged at 9,000 *xg*, 4°C, for 5 min. Pellet was resuspended with 500 µL of ethanol 70% and centrifuged again at 9,000 *xg*, 4°C, for 5 min. Washed pellet in microtube was then dried, resuspended with 50 µL TE1x, and stored in the freezer at -20°C.

PCR amplification and phylogeny analysis

The universal 16S rDNA primers, forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5′-ACCTTGTTACGACTT-3′) (Lane et al. 1985) prepared by Integrated DNA Technologies were used in PCR amplification. The PCR amplification was carried out in a reaction mixture containing 1 µL genomic DNA as template (100 µg/ul), 1 µL forward (10 pM), 1 µL reverse primer (10 pM), 0.5 µL DMSO (Sigma), 12.5 µL GoTaq mix (Promega), and nuclease-free water (Promega) up to the final volume of 50 µL reaction mixture. PCR amplification was performed using Thermal Cycler (Takara) with the following cycles: 90 sec initial denaturation at 94°C; 30 sec denaturation at 95°C; 30 sec primer annealing at 55°C; 90 sec elongation at 72°C; and a final extension of 10 min at 72°C. PCR cycle used was 35 cycles., Electrophoresis of PCR amplified products was conducted on 1.2% (w/v) low-EEO agarose gel in 1X TBE buffer (45 mM Tris-borate, pH 8.3, and 1 mM Na 2 EDTA) at 100 V for 20 min. The gel was stained with ethidium bromide (0.5 µg/mL) and visualized under exposure of UV light. The amplified 16S rRNA gene of isolates was then analysis for sequencing by Macrogen Korea. The 16S rRNA sequences were subsequently aligned with the multiple alignment method using Clustal X version 2.0 (Thompson et al. 1997) and BLAST was performed with the database available on NCBI (http: //www.ncbi.nlm.nih.gov) on April 8, 2019. The phylogenetic trees were constructed using Clustal X version 2.0 software with neighbor-joining method of 1000 bootstraps (Saitou and Nei 1987).

Screening of LAB antimicrobial activities

In the initial screening for antimicrobial activity of LAB, the "disk diffusion" method was applied (Wanger 2007). Supernatants from the 48-hour LAB were prepared by microcentrifugation (miniSprin, Eppendorf) at 10,000 rpm for 10 min). Centrifugation was performed aseptically and the cell-free supernatant was obtained. After incubation, the target microbial suspension was taken and diluted 10 times. About 100 µl of sample was taken and poured over the NA medium; then on it was placed a paper disc that previously dipped in the LAB supernatant. Depending on the cultures, after incubation for 24 hours (*E. coli* and *S. aureus*) and 72 hours (*M. smegmatis)*, the formation of clear zones was measured using a ruler. The measurement was performed in duplicate.

The assay of minimum inhibition concentration

Antimicrobial activities of cell culture supernatants were measured by determining minimum inhibition concentration (MIC) using MTT (thiazolyl blue tetrazolium blue) (El Baz and Shetaia 2005; Moodley et al. 2014). MIC assay was performed on 96-well microplate at room temperature. The test of MIC of supernatant against target microbes was conducted by inoculating culture (1/100 initial density) on NB medium as much as 100 µl with a series of 40, 20, 10, 5, and 0% supernatant concentrations. Samples were shaken at 100 rpm for 24 hours or 72 hours. After incubation, each suspension was added with 10 µl of MTT solution (5 mg/mL) and incubated for 2 hours. After incubation, cell suspension was added with 11 µl of propanol containing 0, 04 M HCl, and incubated again for 2 hours; formazan formed from the reduction of MTT by enzymes in the cell was then read with a microplate reader (Bio-rad iMark) at a wavelength of 595 nm. MIC was determined at the same absorbance value of the NB media value, the value at the time of the absence formazan due to no detection of the activity by the reducing enzyme.

The effect of high temperature on antimicrobial activity

Supernatants predicted containing bacteriocin were tested for their stability during heating in accordance with (Noonpakdee et al. (2002) and Bungenstock et al. (2020). The supernatant was heated at 80°C for 15 minutes to investigate the presence of supernatant active ingredients that are sensitive to heat. The MIC of heated supernatant was tested. The antimicrobial activity of the isolated LAB was compared to the antimicrobial activity of commercial LAB which was isolated from probiotic products containing *Lb. casei*. The treatment was performed with three replications.

RESULTS AND DISCUSSION

Lactic acid bacteria in fruits of wild plants

Total of fourteen samples of fruit obtained as source of LAB were successfully been identified as *Donax canniformis* (G.Forst.) K.Schum., *Dysoxylum parasiticum* (Osbeck) Kosterm., *Tabernaemontana aurantiaca* Gaudich., *Tetrastigma papillosum* Planch., *Ficus arfakensis* King., *Pinanga* sp., *Lasianthus* sp., *Dracaena angustifolia* (Medik.) Roxb., *Syzygium* sp., *Myristica subalulata* Miq., *Helicia moluccana* (R, Br) Blume., *Galearia celebica* Koord., *Cordyline* sp., and *Capparis* sp.; representing fourteen different families (Table 1.) As many fourteen isolates were obtained from both media containing oil (4 isolates) and without oil (10 isolates).

In this study, LAB was actually found in all collected fruits and could grow in liquid medium (MRS broth) during the first step of isolation in the field of Tambrauw forest. Unfortunately, when cell suspensions arrived at the laboratory after 10 days of incubation, not all of them could grow well on solid media (MRS Agar). Probably, it was related to the sensitivity to oxygen or other unidentified factors. In addition, the length of time between initial isolation in the field and in the laboratory contributed to the failure of the isolation. We observed that LAB isolates were not obtained from fruits of *Dysoxylum parasiticum* (Osbeck) Kosterm. (No. 2), *Ficus arfakensis* King. (No. 5), *Pinanga* sp. (No. 6), and *Dracaena angustifolia* (Medix) Roxb. (No. 8) (Table 1).

LAB identification was performed by amplifying and sequencing the universal region of 16S rRNA gene. PCR amplification of all isolates was successfully conducted based on observation on electrophoresis gel at about 1500 bp. As result, the contig sequences obtained from all isolates were about 1349-1370 in base pairs length, and they were sufficient to compare with the collection of rRNA sequences of the NCBI GenBank. BLAST analysis demonstrated the similarity percentage of all isolates were 99% (Table 2) to well several known LAB species (type strains). Furthermore, the phylogenetic analysis of 1000 bootstraps showed that fourteen isolates are clustered in four different clades (Figure 1), including clade of *Lactococcus lactis* (9 isolates), *Lactococcus garviae* (1 isolate), *Enterococcus* (1 isolate), and *Weisella* (3 isolates) with the confident level (>500 bootstraps). In general, isolates having closest relationship of 16S rRNA gene with *Lactococcus lactis* were frequently found in the collected fruits of Tambrauw forest (64%). Another LAB isolate of genus *Lactococcus* was identified as *Lactococcus garvieae* (Table 2). The obtained nucleotide sequences were deposited in GenBank (ncbi.nlm.nih.gov) and assigned the following accession numbers: MK759906, MK759948, MK760562, MK760566, MK760928, MK761005, MK761013, MK761065, MK761134, MK761150, MK761192, MK761194, MK771836, and MK771857.

The profiles of the LAB in the wild fruits in Tambrauw forest were previously not clearly known. Previous study reported LAB in domesticated fruit such as new species of *Lactobacillus musae* sp. nov. from bananas (Chen et al. 2017). The existence of *Lactobacillus* spp. and *Weisella* spp. dominated the LAB in bananas from Taiwan have been also reported (Yu et al. 2017). *W. cibaria* was reported as the most commonly found species in mulberry of several plantation areas, as well as *Lactobacillus plantarum* and *Lactococcus lactis* subsp. *lactis* (Chen et al. 2010). In this study we did not find the presence of *Lactobacillus* in fruits, however, several strains of *Lactococcus* have been observed. A study conducted on

coffee plantations in Taiwan demonstrated a tendency for different LAB distributions depending on the high level of the area. Species of *Lactococcus lactis* subsp. *lactis* was commonly found in the coffee cherry on farms at the altitude of 1,200 m above sea level, while the heterofermentative *Leuconostoc* spp. and *Weissella* spp. frequently found in the lower areas of 800 m above sea level (Leong et al. 2014). In this study, however, we did not specifically study the distribution of LAB in fruit at different altitudes due to fruit plants distributed unevenly in the Tambrauw forest area. However, the presence of several LAB genera identified in this study including *Lactococcus* (2 species), *Weissella* (2 species), and *Enterococcus* (1 species) harboring in 10 fruit plants of Tambrauw forest area was the record of biological resources in West Papua, Indonesia.

We only found the isolates belonging to genus of *Weissella* with high relationship to *W. oryzae* and *W. confusa*. No species *W. cibaria* was observed in all samples of this study. The presence of *W. confusa* and *W. cibaria* isolated from fruits were reported previously. By nested PCR analysis, the occurrence of *W. confusa* and *W. cibaria* was quite frequently found with a level of approximately 13-18% (Emerenini et al. 2013). *W. oryzae* was originally reported not from fruit, but from fermented Japanese (*Oryza sativa* L. subsp. *japonica*) (Tohno et al. 2013). The phenomenon of the discovery of this species in fruits showed considerable distribution in various plant habitats. In further studies, *W. oryzae* strain of SG25^T has been successfully characterized and was known to have about 2.13 Mbp of the genome. From the comparison with other LABs, it was known that the pathway of arginine deiminase (ADI) is similar to that found in the genus *Lactococcus* rather than other genera of *Weissella*. In addition, this species has a pathway of agmatine deiminase (AgDI) that is not found in other members of the *Weissella* genus indicating the possibility of horizontal gene transfer (Tanizawa et al. 2014). The phenomenon of horizontal gene transfer may be related to the adaptability of a particular microbial strain.

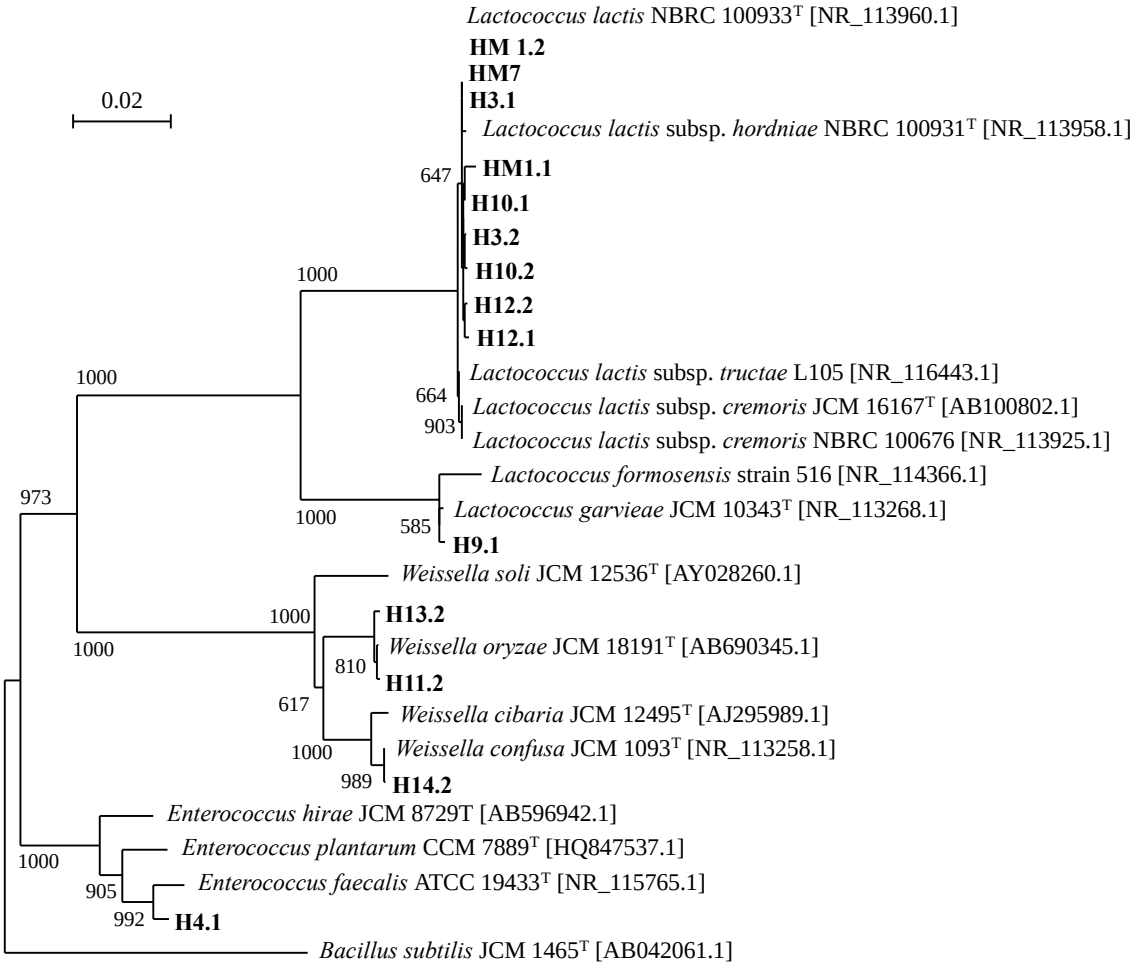

Figure 1. Phylogenetic tree of 16S rRNA sequences of isolates obtained from fruits collected in Tambrauw forest, West Papua. Phylogenetic tree was constructed based on 16S rRNA gene sequencing by neighbor-joining method. Distances were estimated according to the Kimura two-parameter model with bootstrap percentages after 1000 simulations. The bar represents a 2% estimated sequence divergence. *Bacillus subtilis* JCM 1465^T was used as an outgroup.

Table 1. The list of wild plants collected from Tambrauw forest, West Papua, Indonesia

Identified plant		Sampling site	
Species	**Family**	**Location name**	**Coordinate**
Donax canniformis (G.Forst.) K.Schum	Marantaceae	Fef	00°48'50.2"S 132°25'84.44"E
Dysoxylum parasiticum (Osbeck) Kostern	Meliaceae	Fef	00°48'50.2"S 132°25'84.44"E
Tabernaemontana aurantiaca Gaudich	Apocynaceae	Fef	00°48'50.2"S 132°25'84.44"E
Tetrastigma papillosum Planch	Vitaceae	Fef	00°48'50.2"S 132°25'84.44"E
Ficus arfakensis King	Moraceae	Fef	00°47'46.6"S 132°26'23.5"E
Pinanga sp.	Arecaceae	Fef	00°47'46.6"S 132°26'23.5"E
Lasianthus sp.	Rubiaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Dracaena angustifolia (Medik.) Roxb	Dracaenaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Syzygium sp.	Myrtaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Myristica subalulata Miq	Myristicaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Helicia moluccana (R, Br) Blume	Proteaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Galearia celebica Koord	Pandanaceae	Ibbe	00°48'09.8"S 132°25'15.5"E
Cordyline sp.	Asparagaceae	Bamus	00°45'44.5"S 132°15'50.4"E
Capparis sp.	Cappareceae	Bamus	00°45'44.5"S 132°15'50.4"E

Table 2. BLAST results of 16S rRNA gene of isolates obtained from fruits collected in Tambrauw forest, West Papua, Indonesia

Source of fruit	Isolate	Closest relationship [Accession No]	Similarity (%)
Capparis sp.	H14.2	*Weissella confusa* JCM 1093^T [NR113258.1]	99.9
Cordyline sp.	H13.2	*Weissella oryzae* strain SG25^T [NR114312.1]	99.6
Donax canniformis (G.Forst.) K.Schum	HM1.1	*Lactococcus lactis* NBRC 100933^T [NR113960.1]	99.7
	HM1.2	*Lactococcus lactis* subsp. *hordniae* NBRC 100931^T [NR113958.1]	99.9
Galearia celebica Koord	H12.1	*Lactococcus lactis* NBRC 100933^T [NR113960.1]	99.8
	H12.2	*Lactococcus lactis* NBRC 100933^T [NR113960.1]	99.8
Helicia moluccana (R. Br.) Blume	H11.2	*Weissella oryzae* SG25^T [NR114312.1]	99.8
Lasianthus sp.	HM7	*Lactococcus lactis* subsp. *hordniae* NBRC 100931^T [NR113958.1]	99.9
Myristica subalulata Miq	H10.1	*Lactococcus lactis* NBRC 100933^T [NR113960.1]	99.9
	H10.2	*Lactococcus lactis* NBRC 100933^T [NR113960.1]	99.8
Syzygium sp.	H9.1	*Lactococcus garvieae* JCM 10343^T [NR113268.1]	99.8
Tabernaemontana aurantiaca Gaudich	H3.1	*Lactococcus lactis* subsp. *hordniae* NBRC 100931^T [NR113958.1]	99.9
	H3.2	*Lactococcus lactis* subsp. *hordniae* NBRC 100931^T [NR113958.1]	99.8
Tetrasigma papillosum Planch	H4.1	*Enterococcus faecalis* ATCC 19433^T [NR115765.1]	99.1

Although the existence of microbes on fruits was caused by various factors, such as through air, water, dust of earth, spread by animals, or already exist in the plant seed (Barth et al. 2009). The chemical components of the fruit could the defining factor of the LAB niche. Lactic acid bacteria whose preference is fructose (fructophilic LAB) were commonly found in fruits that contain a great deal of fructose. Fructophilic LAB was found in the digestive tract of insects as well (Endo 2012). Based on16S RNA gene, the LAB isolates were mostly *Lactococcus lactis* type from fruits spread in various locations (Table 1). However, there was only one isolate, *L. lactis* (HM1.1), showed antimicrobial activity. Based on the phylogenetic relationship between *L. lactis* species, the HM1.1 isolate showed a distinct difference (Figure 1). Whereas *W. confusa* was reported to be beneficial as a probiotic, the same species could also infect animals and humans. As well, *Lac. garvieae* was also documented for its pathogenicity (Ferrario et al. 2013; Morita et al. 2011). Thus, precaution must be taken prior application or utilization (Fairfax et al. 2014).

Antimicrobial activity

All isolated lactic acid bacteria demonstrated the antimicrobial activity against three indicator strains, *E. coli*, *S. aureus*, and *M. smegmatis.* However, only two strains are known as HM.1.1 (*Lac. lactis*) and HM 14.2 (*W. confusa*) strongly inhibited the growth of *S. aureus* and *M. smegmatis.* Strain HM1.1 showed a 4.5 mm inhibition zone on *M. smegmatis*, while H14.2 had a 2.0 mm inhibition zone. Isolate HM1.1 had a 2.0 mm inhibition zone on *S. aureus* and H14.2 has a 1.5 mm inhibition zone. Further testing was performed on both cultures. As result of MIC test, supernatant of H14.2 (*W. confusa*) and HM1.1 (*Lac. lactis*) inhibited the growth of three microbial targets; *M. smegmatis* was more sensitive than *S. aureus* and *E. coli* (Table 3). However, the activity was still lower than the antimicrobial activity of strain from commercial probiotics products.

Table 3. Antimicrobial activity of H14.2 and HM1.1 supernatants on *Staphylococcus aureus*, *Escherichia coli*, and *Mycobacterium smegmatis*

LAB supernatant	MIC of supernatant on target microbes (%, v/v)					
	S. aureus		*E. coli*		*M. smegmatis*	
	Unheated	Heated	Unheated	Heated	Unheated	Heated
Strain from commercial probiotic product *(Lb. casei)*	10	20*	20	20	5	5
H14.2 (*W. confusa)*	40	40	40	40	20	40*
HM1.1 (*Lac. lactis)*	40	40	40	40	20	40*

Note: *: activity was decreased after heating at 80°C for 15 min compared to MIC of supernatant before treatment

The application of high temperatures resulted in the decreasing of antimicrobial activity of H14.2 (*W. confusa)* and HM1.1 (*Lac. lactis)* on pathogenic model *M. smegmatic*, indicated by elevated MIC values of heated supernatant compared to non-heated samples (Table 3). The activity of the commercial LAB supernatant did not show any changes on *M. smegmatis*, while the activity on *S. aureus* was reduced. We suggest three possibilities that could explain this different activity of heated supernatant against targeted microorganisms. First, supernatant may contain different bacteriocins so that there will be other bacteriocins that are still active against certain bacteria. Second, bacteriocin might not be completely denatured so that there will be peptide residues that are still active against certain bacteria. Third, supernatant may contain unknown substances whose antimicrobial activity against certain bacteria is not affected by high temperature. However, these suggestions need to be further studied.

Antimicrobial activity is one of LAB probiotics properties. The antimicrobial properties of LAB are affected by hydrogen peroxide, bacteriocin, organic acids formed; such as lactic acid and acetic acid, and antifungal peptides (Reis et al. 2012). To the best of our knowledge, there is limited or no report on LAB isolated or identified from genus of the studied fruits except *Capparis* sp. In natural fermentation of caper berry fruit *Lactobacillus* spp., *Pediococcus* spp., *Enterococcus* spp. were identified (Pulido et al. 2005). Although originally probiotics found in humans, recently it can also be derived from wild fruits and vegetables (Bae et al. 2006; Benavides et al. 2016; Chen et al. 2010). The activity of the supernatant of both LAB *W. confusa* HM14.2 and *Lac. lactis* HM1.1 against *M. smegmatis* decreased after heating at high temperature (Table 3). It indicated the possibility of H14.2 or HM1.1 being anti-*M. smegmatic* due to the content of both BAL that was damaged by high temperature (80°C) for 15 minutes. Peptide is one of the LAB content that plays important role in antimicrobial activity (Cotter et al. 2013; Grosu-Tudor et al. 2014). Several purified bacteriocin peptides were reported previously from *Lac. lactis* (Goyal et al., 2018) and *W. confusa* (Sartono et al. 2019). Peptides usually could not withstand high temperatures, although some are resistant to high temperatures (Maria and Janakiraman 2012). Some bacteriocins are known to kill *M. tuberculosis* with the membrane as a target (Sosunov et al. 2007).

In conclusion, total of fourteen LABs has been successfully isolated from the eight fruits of wild plants in Tambrauw forest area, West Papua. Those LAB strains had closest relationship to species *Lactococcus lactis, Lactococcus garvieae, Weissella confusa, Weissella oryzae*, and *Enterococcus faecalis*. The supernatant of *Lac. lactis* HM 1.1 from *Donax canniformis* and *W. confusa* H14.2 from *Capparis* sp. showed higher the highest inhibition on *E. coli, S. aureus*, and *M. smegmatis.* The data showed that wild fruits from Tambrauw forest harbor beneficial bacteria that could be important for health of animals and humans as well.

ACKNOWLEDGEMENTS

This study was supported financially by DIPA from Research Center for Biology, LIPI, Bogor, Indonesia. Authors thank Mulyadi for samples collection in Tambrauw forest, West Papua, Indonesia and Deni Sahroni for plants identification

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