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Review Article
Alternative Antibiotics Source from Symbiont of Lactid Acid Bacteria Inside Stomach of Honeybees (*Apis mellifera* and *Apis dorsata*) Against Multiresistant Antibiotics Pathogenic Bacteria

Novra Arya Sandi and Siti Isrina Oktavia Salasia

Department of Clinical Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, JI, Fauna 2, Karangmalang, Yogyakarta 55281, Indonesia

Abstract
Honey is natural food that common used directly by the society. Forest honey produced by wild bees *(Apis dorsata*) while the other type of honey produced by *Apis mellifera*. Beekeeping *A. mellifera* with monoflora or multiflora nectar is generally carried out by various countries including Indonesia. Scientifically, honey containing bioactive compounds with antimicrobial properties but still uncertain which compounds that play a role in these activities. Reported bioactive compounds that have antibacterial activity of honey are inhibine and non-inhibine. Inhibine is forming enzyme and accumulation of hydrogen peroxide (H_2O_2) to dilute honey and nectar. The H_2O_2 has also long been known as an effective antibacterial and major component of penicillin, especially notatin. The types of antibacterial compounds influenced by nectar-source plants with compounds including alkaloids, flavonoids and glycosides. Some researchers reported that beside phytochemicals, antibacterial activity due to presence of Lactic Acid Bacteria (LAB) that produce bioactive compounds with antibiotic-like activity. Some studies reported 3 LAB strains potential to produce bioactive compounds with activity resembles antibiotics, namely *L. kunkeei* Fhon2, *L. kunkeei* Lahm and *L. kunkeei* Yubipro with the greatest inhibition zones than other Lactobacillus. Honeybees are isolated from the stomach LAB capable of producing organic acids, free fatty acids, ethanol, benzoic acid, enzymes, H_2O_2 and antimicrobial peptides. Different character of the bioactive compounds will jointly deliver results on inhibition zone and broad spectrum for various types of microbial pathogens. Microbial pathogens tested including *Serratia marcescens*, *Klebsiella aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and methicilin resistant *Staphylococcus aureus* (MRSA).

Key words: Honey, symbiont of lactic acid bacteria, antibiotic source, pathogenic bacteria, multiresistant antibiotics

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Corresponding Author: Siti Isrina Oktavia Salasia, Department of Clinical Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, JI, Fauna 2, Karangmalang, Yogyakarta 55281, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phenomenon of bacterial resistance becomes increasingly common around the world and considered as one of the major causes of global health crisis (Blair *et al*., 2015). Ishii *et al*. (2015) noted that antibiotic-resistant bacterial mutants will continue to emerge despite the discovery of new antibiotics. Increasing awareness of potential problems-associated antibiotics usage triggers research efforts to identify alternatives approaches.

Consequently, it is also has been recorded by Sandi *et al*. (2015) that scientific efforts and new strategies being studied to develop new compounds that can be used beyond conventional antibiotic therapies. Honey has been used since ancient times for treatment of some respiratory diseases and wounds of skin, therefore it could be used as one of potent sources of antibiotics. Forest honey is one type of forest products that can be used directly by communities around the forest or forest area. Forest honey produced by wild bees (*Apis dorsata*), the type of bee that is not commonly cultivated intensively. The wild honeybees generally live in Indonesia naturally in the forests of Sumatra, Kalimantan, Sulawesi, Java and Nusa Tenggara islands and produce honey that is high enough to potentially be used as a source of livelihood of forest communities. The development of the utilization of forest honey is considered capable of conserving Indonesia's forests management and it is performed traditionally and empirically that people use honey as food and drink, because it has the efficacy to treat a variety of diseases such as respiratory disorders, gastrointestinal infections and various other infections. Scientifically, honey proved to contain organic compounds that have antibacterial activities (Willix *et al*., 1992). Apart from containing antibacterial compounds, honey (including, wild honey) allegedly contains antioxidant compounds, as several studies have shown that honey cultivation contains vitamin C, organic acids, enzymes, phenolic acids, flavonoids and beta carotene are useful as antioxidants (Gheldof *et al*., 2002; Beretta *et al*., 2005; Parwata *et al*., 2010; Giorgi *et al*., 2011). On the other hand, it is known forest honey has antibacterial and antioxidant activity higher than the cultivated honey, because it was harvested from the forest that feed with multiflora nectars as food source for honeybees. Parwata *et al*. (2010) also confirmed that honey has the chemical composition of the compounds vary based on the nectar food resources. The difference in the selectivity of the inhibition of the five types of honey cultivation against some types of bacteria, so it needs to be done at the same comparison study antibacterial activity of honey cultivation and forest honey from Indonesia.

Antibacterial agents for treatment of infectious diseases caused by pathogenic bacteria are reported that have been resistant to various antibiotics, which requiring treatment with a new drug that has high potential. Assessment of the bioactive compounds are useful as antibacterial needs to be done in order to find antimicrobal compounds that could potentially inhibit or kill the pathogenic bacteria that are resistant to antibiotics. One of the alternatives that can be taken is to utilize bioactive compounds with bacteriostatic/bactericidal activity as contained in natural ingredients such as honey. This study expected can generally provide information to the community and in particular on research about the benefits of forest honey and cultivated honey which has been shown to have potential as an antibacterial. Such information would be useful to researchers for the development of natural products as an alternative source of antibiotics for treatment of infection by various pathogens.

The purpose of this study is to analyse some of the results from previous studies that have been performed physico-chemical analysis of forest honey that produced by *Apis dorsata* and cultivated honey produced by *Apis mellifera*. Forest honey was studied previously were originated from the province of Sumbawa and Sentarum Lake area in West Kalimantan, as well as Tesso Nilo, Riau province, Sumatra. The forest honey antibacterial activity has been tested against a variety of pathogenic bacteria that cause infections such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella thypi* and *Pseudomonas aeruginosa*. Other studies were also carried out by Olofsson *et al*. (2014a, b) in Sweden related to the discovery of symbiont responsible for the antibacterial properties from Lactic Acid Bacteria (LAB) which were isolated from the abdomen of honeybee *Apis mellifera*. This article is aimed to discuss and compare the results of previous studies related to antimicrobial activity contained in the honey and the honey bee's body against pathogenic bacteria infection and explain the possibility of its potential as an alternative source of antibiotics.

BODY OF THE REVIEW

Taxonomy of honeybee: Honeybees have long been known by the people of Indonesia, this state can be known by the various names of bees in different local languages, for example nyiruan (Sundanese), tawon (Javanese), labah (Minang) ans loba (Batak) (Apiari Scout Beekeeping Center, 2003).

In general, the classification of honeybees can be explained as follows:

Kingdom : Animalia
Phylum : Arthropods
Class : Insecta
Order : Hymenoptera
Family : Apidae
Genus : Apis
Species : *Apis andreniformis*, *Apis cerana*, *Apis dorsata*, *Apis florae, Apis laboriosa*, *Apis mellifera*

According to Hailm and Suharno (2001) honeybees consists of five types, namely *Apis florae*, *Apis trigona*, *Apis cerana*, *Apis mellifera* and *Apis dorsata*. However, not all honey bees can be cultivated (Apiari Scout Beekeeping Center, 2003). It has been known the average production of honey Indonesia is only about 2,828 t year^{-1} and most of the honey by beekeeping produced in the province of Central Java, East Java, Nusa Tenggara Timur, West Nusa Tenggara and Riau.

Phytochemical analysis and honey benefits: Phytochemical analysis can identify the compounds which are secondary metabolites that qualitatively indicated by the intensity of the color produced by phytochemical reagents (Rita, 2010). This analysis was conducted to identify bioactive compounds on forest honey that play the role in honey with potential as antibacterial and antioxidant. Based on results of phytochemicals test (Table 1), honey contains active compounds were positively identified saponins, alkaloids, phenolics, flavonoids, triterpenoids and glycosides. The function of each of bioactive compound among them such as alkaloid could be used to treat microbial infections, saponins can be used as an anti-fungal, anti-influenza and anti-inflammation of the throat (Harborne, 1996). The triterpenoids substance can be classified into four classes of compounds, namely: Triterpene, steroid, saponins and glycosides that have been efficaciously lowering blood cholesterol levels, also reported as an antibiotic and fungicidal but some are poisonous.

Table 1: Phytochemical analysis from 3 types of honey in Indonesia

	Result of biochemical compounds detection		
Type of biochemical compounds	KB 1	SM1	SB1
Saponin	+	+	+
Alkaloid	+	+	+
Tannin	-	-	-
Phenolics	+	+	+
Flavanoid	+	+	+
Triterpenoid	+	+	+
Steroid	-	-	-
Glycoside	+	+	+

Description: (+) positive/contain bioactive compounds, (-) negative does not contain bioactive compounds, KB1: Honey from West Kalimantan, SM1: Honey from Riau province, Sumatra, SB1: Honey from Sumbawa, West Nusa Tenggara

The large diameter of the inhibition zones formed from the results of the antibacterial assay allegedly affected by the presence of the bioactive compounds in honey including alkaloids, flavonoids, triterpenoids and phenolic glycosides (Table 1). The bioactive compounds play the role in term of the destruction from bacterial's cell wall resulting in lysis or inhibit of protein synthesis, or changes in the permeability of cytoplasmic membrane that lead to leakage of nutrients from within the cells. Therefore, it could lead to protein denaturation of cell's membrane and interrupting the cell metabolism by inhibiting the action of the its intracellular enzyme. According to Bogdanov (1997), the antibacterial activity of the honey comes from plant food sources. There have been reported that there is certain plant species contains complex mixture of alkaloid compounds which have classification based on nitrogen ring and alkaloid biosynthesis including true alkaloids, protoalkaloid and pseudoalkaloid. Alkaloids may play roles as an antibacterial and there are known about 5500 alkaloids that are scattered on different families of plants as a source of nectar honeybee (Harborne, 1996).

Antibacterial bioactive compounds in honey: Honey bioactive compounds that have antibacterial activity were clasified into inhibine and non-inhibine compounds. Inhibine was declared as forming enzyme and the accumulation of hydrogen peroxide (H_2O_2) in dilute honey and nectar (White *et al*., 1963). The H_2O_2 has long been known as an effective antibacterial and is the main component of some penicillin especially notatin (glucose oxidase). Various bacteria are very sensitive to inhibine and Gram-negative bacteria are more sensitive than Gram-positive bacteria (Willix *et al*., 1992). However, inhibine is very sensitive to heat and its presence in honey is determined by the type, age, condition and processing of honey. Non-inhibine compounds are bioactive compounds that have the potential of honey as an antibacterial. The types of antibacterial compounds belonging to the compound group include alkaloids, flavonoids, glycosides and other compounds (Table 1). The content of bioactive compounds each type of honey are different between one with another, this difference is influenced by the source of nectar for honeybees (Bogdanov, 1997). The honeybees feed on nectar differences may affect differences in the characteristics of honey such as color, flavor and aroma as well as the antibacterial activity of honey. Differences inhibition of honey to some of tested pathogenic bacteria was thought to be influenced by differences in the levels and types from one group of bioactive compounds, so that testing on different species of bacteria can produce different inhibition that characterized by differences in the diameter of inhibition zone.

Previous comparative study about antibacterial activity assays from three types of forest honey and other types of cultivated honey against *S. thypi* and *E. coli* indicates that all three types of wild honey has better antibacterial activity rather than cultivated honey. Honey that commonly used is honey obtained from the cultivation of honeybee species *A. mellifera* that feed with one type of nectar (monoflora) and several types of nectar (multiflora). Previous research that forest honey from Sumbawa, West Nusa Tenggara (SB1) have the highest antibacterial activity against *E. coli* and *S. thypi* compared with other types of honey, with the largest diameter of inhibition zone are 32.5 and 27.5 mm, respectively. While forest honey obtained from Kalimantan Barat (KB1) have the highest antibacterial activity against *S. thypi* compared with other types of cultivated honey with diameter of zone of inhibition is 12.8 mm. Results from zones of inhibition of forest honey from Sumatra (SM1) are known to have the highest antibacterial activity against *E. coli* and equivalent to the honey from rubber tree which has the highest antibacterial activity against *E. coli* among the types of other cultivated honey and were classified as very strong with the diameter of the inhibition zone is 25 mm.

General guidance for treatment of bacterial pathogens infections: Antibiotics were commonly used for treatment of infections caused by pathogenic bacteria with different types and different spectrum. In general, according to Mills *et al*. (1987) is based on the discovery of the pathogen causing the infection, then antibiotic usage can be divided into two, namely empiric therapy and definitive therapy. Empirical therapy in many cases of infection from microorganisms that cause the infection can not be known or ascertained at the time of firstly antibiotic used for therapy. Selection of antibiotics are given based on estimates possibility of infecting organism, based on decent experience or epidemiological patterns of local bacteria. Selection of antibiotic is also very influential in the treatment of infections due to bacterial pathogens and in determining the choice of antibiotic have to be used based on certain principles that can reduce the severity of the infectious diseases. Grahame-Smith and Aronson (1992) explained the negative impact of irrational use of antibiotics might lead to occurrence of microbial resistance, emerging of resistant strains microorganisms and increasingly in side effects and toxicity of antibiotics. These things can occur directly because of the influence of antibiotics concerned or due to the condition of superinfection and high costs due to excessive use of antibiotics in some cases that actually do not requires antibiotics. Therefore, these might lead to the failure of expected optimal clinical benefits in the prevention and treatment of infectious diseases. Rakholiya *et al*. (2015) explained that prolonged usage of antibiotic with broad-spectrum effect has led into the emergence of microbial resistance and this has been a major concern which causes high economic loss globally. O'Neill (2014) explained that bacterial resistance is a phenomenon that is very detrimental in terms of health through increased mortality and morbidity and economic loss is predicted to reach $ 100 trillion if continued until 2050. Recently have been reported that there are at least three main species of bacteria that multi-drugs resistance are globally concerned including, *Klebsiella pneumoniae* (Akter *et al*., 2014), *E. coli* and *S. aureus* (MRSA) (O'Neill, 2014) and also MRSA originated from humans, dairy cows and goats (Widianingrum *et al*., 2016). Tawfick and Gad (2014) suggested that microbial resistance phenomenon has made many researcher eargerly to search in various ways that cost effective for the treatment of infectious diseases. One way to overcome this obstacle is being intensively improved through variety of research to find alternative antibiotics from natural substances that have potential as an alternative source of antibiotics.

DISCUSSION

Previous study was reported results (Fig. 1) which shows that forest honey KB1 was clasified as weak antibacterial activity at concentrations of 25% and a moderate at concentration of 50% against *Pseudomonas aeruginosa*. Both forest honey SB1 and SM1 at concentration of 25% has an antibacterial activity against *E. coli* with moderate classification and concentration of 50% were classified as strong antibacterial. The activities of all types of honey were classified as weak antibacterial against *S. aureus* at both concentration of 25 and 50% but at concentration of 100% were classified as moderate. The highest antibacterial activity of tested three types of forest honey was SB1 with the classification of a very strong antibacterial properties against *S. thypi* and *E. coli*. In general, the test results showed that Gram-negative bacteria (*P. aeruginosa*, *E. coli* and *S. thypi*) are more susceptible to bioactive compounds that plays a role as antibacterial from honey rather than Gram-positive bacteria (*S. aureus*). This is assumed base on the composition of honey which is composed largely of compounds that are polar, such

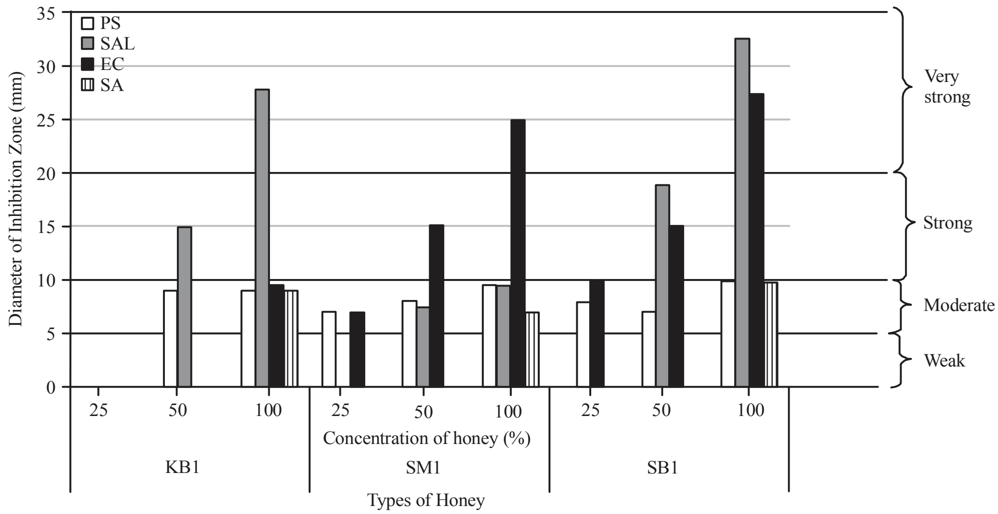

Fig. 1: Graph comparing inhibition zone diameter (mm) antibacterial assay of 3 types of forest honey from Indonesia, KB1: Honey from West Kalimantan, SM1: Honey from Sumatra island, SB1: Honey from Sumbawa (West Nusa Tenggara). PS: *Pseudomonas aeruginosa*, SAL: *Salmonella thypi*, EC: *Escherichia coli*, SA: *Staphylococcus aureus*

as monosaccharide sugars (fructose and glucose), organic acids, phenolic acids, vitamin C, flavonoids and other polar compounds. The simply explanation that the Gram-negative bacteria have hydrophilic group that relatively easier to bind with polar bioactive compounds, whereas the Gram-positive bacteria have the hydrophobic group that relatively easier to bind with non-polar bioactive compounds. Those stucture and conformation make the antibacterial activity pure honey is more effective against the types of Gram-negative bacteria rather than Gram-positive. It is also supported by research conducted by Rio *et al.* (2012) that the Gram-negative bacteria are more sensitive to honey's antibacterial compounds compared with Gram-positive. The antibacterial activity can only give an effect when the honey is not heated, because the honey will lose antibacterial properties when heated (Olofsson *et al.*, 2014a).

Erguder *et al.* (2008) stated that one of the factors that influence the antibacterial activity in honey include the high sugar content of honey and honey high acidity level. Sugar content in honey amounted 81-85% consisting of a mixture of glucose and fructose and acidity in honey, which can affect the osmotic pressure contained in honey (French *et al.*, 2005). A strong interaction between the molecules of sugar and water lead to very limited availability of free water for microorganisms. The free water is called the Water Activity (WA), namely the amount of free water that can be used by the bacteria to grow (Fennema, 1985).

Antibiotics are very commonly used, especially in the treatment of related infections but reality shows that the problem of infectious diseases still continues. This happens as a result of bacterial resistance to antibiotics, so the traditional drug development efforts are needed to support the promotion of public health. Various studies have been conducted to identify the components in medicinal plants which are useful as an effective antibiotic. Traditional medicine around the world who use herbs can be used as an important source of information to find alternative antibiotics (Samy and Gopalakrishnakone, 2010). Gur *et al.* (2006) explained the benefit from using of components from antibacterial bioactive compounds derived from natural ingredients including medicinal plants expected with less side effects and well-tolerance by patients, economically low cost and also can be used in the long term and can be renewed in nature.

Previous results of researches were using natural ingredients such as honey reported that the antibacterial activity of honey is considered as the effect of the content from various phytochemical which is closely related to the nectar. Nectar that obtained from plants taken by honeybees are known vary and depend on plants that grow in the area of residence of the honeybee. Different results were reported by Olofsson *et al.* (2014a) that there are causes other than phytochemical content, the cause is a Lactic Acid Bacteria (LAB) which produce bioactive compounds that resembles antibiotic activities. The LAB are found around less than one decade ago by Olofsson and Vasquez (2008) on the microbiota from symbiosis that found in the stomach of honeybees and it had never been explained before. Microbiota were found to consist about 40 strains of Lactic Acid Bacteria (LAB) which taxonomically are *Lactobacillus* ssp. (9) and *Bifidobacterium* ssp. (4) (Olofsson *et al.*, 2014b). The LAB is a

Table 2: *In vitro* assay for antibacterial activity of Lactic Acid Bacteria (LAB) from honeybees against a variety of pathogenic bacteria, Olofsson *et al.* (2014a)

				Diameter of inhibition zone									
Honeybee species	LAB strain			*Serratia marcescens* NJ19C 5c		*Klebsiella aerogenes* Clmp R		*S. aureus* 74022 R.R		*P. aeruginosa* LE08		MRSA clinical Isolate 18	*E. coli* V517
Apis mellifera	*L. kunkeei* Fhon2			42*		24*		31*		30°		33	40°
	L. apinorum Fhon13			8*		10$^{\#}$		20*		0		18	12$^{\#}$
	L. kimbladii Hma2			9*		9$^{\#}$		0		10		9°	7$^{\#}$
	L. melliventris Hma8			18*		9$^{\#}$		19*		0		16	12°
	L. Helsingbogensis Bma5			11*		10°		13$^{\#}$		0		6°	12°
	L. kullabergensis Biut2			7*		7°		16°		0		12*	10°
	L. apis Hma11			0		0		0		0		0	0
	L. mellifer Bin4			39*		26*		22°		29°		32*	30°
	L. mellis Hon2			0		0		0				0	0
Apis laboriosa	*L. kunkeei* Lahm 1-13			45*		30*		20°		27°		30	46°
Apis andreniformis	*L. kunkeei* Yubipro 16			45*		32*		34°		30°		30	46°
Antibiotics used	V	V	F	Cx	Cl	F	V	F	Cn	C	Cn	V	A
Standard of diameter (mm)	9	19	29	28	19*	29	19	29*	19	31	21	23	19

MRSA: Methicillin-resistant *Staphylococcus aureus*, Antibiotics that were used, V: Vancomycin, 30 µg, F: Fusidic acid, 10 µg, Cx: Cefuroxime sodium, 30 µg, Cl: Chloramphenicol, 30 µg, Cn: Gentamycin, 10 µg, A: Ampicillin, 10 µg, C: Ciprofloxacin, 5 µg, *Clear edge (sharp) of inhibition zone (mm), °Unclear edge (blurred) of inhibition zone (mm), $^{\#}$Presence of bacterial growth sporadically within inhibition zone, *Clear edge of inhi

group of bacteria that are functionalist has the ability to produce lactic acid during their fermentative metabolism. In general, certain species are included in the LAB can produce bioactive compounds such as organic acids, free fatty acids, ethanol, benzoic acid, enzyme, hydrogen peroxide (H_2O_2) and antimicrobial peptides. Different characters of bioactive compounds produced will jointly provide the results of inhibition zone and a broad spectrum of various types of microbial pathogens (De Vuyst and Leroy, 2007; Klaenhammer *et al*., 2002). The results in Table 2 shows the inhibition zones varying for 13 LAB studied as microbiota which produce bioactive compounds with antibiotic activities. Lactobacillus that considered have potential for producing antimicrobial peptides are *L. kunkeei* Fhon2, *L. Kunkeei* Lahm and *L. kunkeei* Yubipro that considered to have the largest inhibition zones than other *Lactobacillus* species. Microbial pathogens used in these studies by Estevinho *et al.* (2008) and Olofsson *et al.* (2014a) including *Serratia marcescens* NJ19C 5C, *Klebsiella aerogenes* Clmp R, RR 74 022 *S. aureus*, *P. aeruginosa* LE08, MRSA, *E. coli* V517.

For many decades researches have been struggling to develop new broad-spectrum antibiotics for the treatment of various infectious diseases that caused by pathogens (Rakholiya *et al*., 2015). Researchers are currently being earnestly seeking new putative natural antimicrobial from locations that are ecologically unspoiled as a weapon against pathogenic microorganisms that cause infections (Udwary *et al*., 2011; Donia *et al*., 2011).

It had been known there is a symbiont inside an ecological sites and has been formed to protect the host through the mechanism for the production of bioactive compounds (Demain, 1998; Crawford and Clardy, 2011). Olofsson *et al.* (2014a) reported that there are some metabolites in honey such as 3-hydroxy fatty acids (3-OH FAS) and extracellular protein which produced in large quantities in conjunction with several proteins that considered to play a role as antibacterial. Proteins produced by LAB caused by other bacteria that threaten their existence, so they produce bioactive compounds to protect and defend themselves from other bacterial. The hypothesis described by Olofsson *et al.* (2014a) illustrates that antibacterial activity possessed by LAB is through the production of bioactive metabolites that protects them from bacterial that colonized in plants and cause plant disorders, one of them is *Pseudomonas* spp. found in many plants flower. According to Mundo *et al.* (2004) conducted a study regarding antibacterial activity of honey is known to inhibit the growth of *Bacillus cereus*, *E. coli* O157: H7, *Listeria monocytogenes*, *Salmonella enterica*, *Serovar typhimurium* and *S. aureus* as well as Community-Acquired MRSA (CA-MRSA) that was reported by Maeda *et al.* (2008). These capabilities are known due to the high concentration of sugar (reducing water activity), the presence of H_2O_2 and protein components. However, there may still be bioactive compounds that have not been identified which have antibacterial activities, therefore there is necessary studies and further researches in order to prove and elaborate this theory. Kumbhare *et al.* (2014) explained that the use of antibiotics from natural materials which potent from pharmalogical aspects with the residues that are not harmful to human and animal health, as well as from economic value in terms of easier to obtain and have lower cost production.

CONCLUSION

Honey (forests and cultivated) has potential as an alternative source of antibiotics for the treatment of infection by pathogenic bacteria. The potential is based on scientific facts and results from various studies on honey which states that phytochemicals with antibacterial activity and the presence of LAB that produce bioactive compounds with antibiotic activities. While there are have been reported three strains of LAB known potential to produce bioactive compounds with activity resembles antibiotics, namely *L. kunkeei* Fhon2, *L. kunkeei* Lahm, *L. kunkeei* Yubipro isolated from the abdomen of honeybees. The LAB is capable of producing organic acids, free fatty acids, ethanol, benzoic acid, enzymes, H_2O_2 and antimicrobial peptides. Various microbial pathogens tested on Gram-positive and negative illustrate broad-spectrum antibacterial against various microbial pathogens such as *Serratia marcescens*, *Klebsiella aerogenes*, *E. coli*, *P. aeruginosa*, *S. aureus* and MRSA.

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