BULETIN VETERINER UDAYANA

pISSN 2085-2495
eISSN 2477-2712

Received: 7 May 2026; Accepted: 25 May 2026; Published: 5 June 2026

IN VITRO STUDY OF THE ANTIMICROBIAL ACTIVITY OF HONEY AGAINST *ESCHERICHIA COLI* AND ITS POTENTIAL TO SUPPORT THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS*

Studi *In vitro* Aktivitas Antimikroba Madu terhadap *Escherichia coli* dan Potensinya dalam Mendukung Pertumbuhan *Lactobacillus acidophilus*

Marlin Cindy Claudya Malelak[1]*, Agnesia Endang Tri Hastuti Wahyuni[2], Mariana Reslinda Putri[1], Christin Melkianus[1], Aletha Yuliana Mandala[1], Yunita Apriana Milla[1], Yohanes Timbun Raja Mangiut Ronael Simarmata[1]

[1]Veterinary Medicine Study Program, Faculty of Medicine and Veterinary Medicine, Nusa Cendana University, Jl. Adi Sucipto, Kupang, East Nusa Tenggara 85111, Indonesia

[2]Faculty of Veterinary Medicine, Gadjah Mada University, Bulaksumur, Depok, Sleman Regency, Special Region of Yogyakarta 55281, Indonesia

*Corresponding author email: marlin.malelak@staf.undana.ac.id

How to cite: Malelak MCC, Wahyuni AETH, Putri MR, Melkianus C, Mandala AY, Milla YA, Simarmata YTRMR. 2026. *In vitro* study of the antimicrobial activity of honey against *Escherichia coli* and its potential to support the growth of *Lactobacillus acidophilus*. *Bul. Vet. Udayana*. 18(3): 535-544. DOI: https://doi.org/10.24843/bulvet.2026.v18.i03.p05

Abstract

Antibiotics are widely used in livestock production to promote growth, improve productivity, and prevent disease. However, the continuous use of antibiotics as Antibiotic Growth Promoters (AGPs) may lead to several adverse effects, including the development of antimicrobial resistance, accumulation of antibiotic residues in animal products and the environment, and disruption of beneficial intestinal microflora. Therefore, natural alternatives are increasingly being explored, with honey emerging as a promising candidate. This study aimed to evaluate the antibacterial activity of honey against *Escherichia coli* and its potential to stimulate the growth of the lactic acid bacterium *Lactobacillus acidophilus*. Three types of 100% pure honey were tested: Kupang honey, Lombok honey, and commercial honey. Antibacterial activity against *E. coli* (1.5×10^8 CFU/mL) was assessed using the Kirby–Bauer disc diffusion method, while the effect on *L. acidophilus* (1.5×10^8 CFU/mL) was evaluated using a modified agar disc diffusion assay for growth stimulation. The results showed that all honey types exhibited antibacterial activity against *E. coli*, although with relatively small inhibition zones. Lombok honey produced the largest inhibition zone (6.85 mm), followed by Kupang honey (6.26 mm) and commercial honey (6.25 mm). In contrast, all honey samples significantly stimulated the growth of *L. acidophilus*, as indicated by prominent growth-stimulation zones. Lombok honey showed the largest stimulation zone (26.97 mm), followed by commercial honey (26.88 mm) and Kupang honey (26.66 mm). These findings indicate that the tested honeys possess selective antibacterial activity against *E. coli* while also promoting

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

the growth of beneficial bacteria. Therefore, honey has strong potential as a natural and sustainable alternative to conventional AGPs for supporting animal health.

Keywords: Antibacterial, Antibiotic Growth Promoter, *Escherichia coli*, Honey, *Lactobacillus acidophilus*, Prebiotic

Abstrak

Antibiotik banyak digunakan dalam produksi ternak untuk meningkatkan pertumbuhan, produktivitas, dan pencegahan penyakit. Namun, penggunaan antibiotik secara terus-menerus sebagai *Antibiotic Growth Promoters* (AGPs) dapat menimbulkan berbagai dampak negatif, termasuk perkembangan resistensi antimikroba, akumulasi residu antibiotik pada produk hewani dan lingkungan, serta gangguan terhadap mikroflora usus yang menguntungkan. Oleh karena itu, berbagai bahan alami mulai dikembangkan sebagai alternatif, salah satunya adalah madu. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri madu terhadap *Escherichia coli* serta potensinya dalam menstimulasi pertumbuhan bakteri asam laktat *Lactobacillus acidophilus*. Tiga jenis madu murni 100% yang digunakan dalam penelitian ini adalah madu Kupang, madu Lombok, dan madu komersial. Aktivitas antibakteri terhadap *E. coli* (1,5 × 10^8 CFU/mL) diuji menggunakan metode difusi cakram Kirby–Bauer, sedangkan pengaruh terhadap *L. acidophilus* (1,5 × 10^8 CFU/mL) dievaluasi menggunakan metode modifikasi difusi cakram agar untuk stimulasi pertumbuhan. Hasil penelitian menunjukkan bahwa seluruh jenis madu memiliki aktivitas antibakteri terhadap *E. coli*, meskipun menghasilkan zona hambat yang relatif kecil. Madu Lombok menghasilkan diameter zona hambat terbesar (6,85 mm), diikuti oleh madu Kupang (6,26 mm) dan madu komersial (6,25 mm). Sebaliknya, seluruh sampel madu secara signifikan mampu menstimulasi pertumbuhan *L. acidophilus*, yang ditunjukkan oleh terbentuknya zona stimulasi pertumbuhan yang besar. Madu Lombok menunjukkan diameter zona stimulasi terbesar (26,97 mm), diikuti oleh madu komersial (26,88 mm) dan madu Kupang (26,66 mm). Temuan ini menunjukkan bahwa madu memiliki aktivitas antibakteri selektif terhadap *E. coli* sekaligus mampu mendukung pertumbuhan bakteri menguntungkan. Dengan demikian, madu berpotensi menjadi alternatif alami dan berkelanjutan pengganti AGPs konvensional dalam mendukung kesehatan hewan.

Kata Kunci: Antibakteri, *Antibiotic Growth Promoter*, *Escherichia coli*, *Lactobacillus acidophilus*, Madu, Prebiotik

INTRODUCTION

Resistance to microorganisms due to the use of antibiotics is now a concern for people in all parts of the world. Antibiotics are used in livestock with the aim of increasing growth and production and preventing disease, but repeated use of feed as a *Growth Promoter (*AGP*)* can have negative effects such as microorganism resistance to antibiotics, accumulation of antibiotic residues in animal and environmental products, normal microflora imbalances, and microflora reduction in both the intestine. Therefore, the Indonesian government has officially prohibited the utilization of AGPs as livestock feed additives through the Ministry of Agriculture Regulation Number 14/PERMENTAN/PK.350/5/2017 concerning the classification of veterinary medicines (Kementerian Pertanian Republik Indonesia, 2017). At present, the use of natural ingredients such as honey is considered to have minimal side effects with a low level of toxicity, and is easier to obtain.

Honey is a natural sweet substance that bees produce by transforming flower nectar or other sweet secretions of plants. Depending on the raw material used by the bees, honey may be classified as nectar, honeydew, or mixed nectar–honeydew. The physicochemical parameters of natural honeys, such as moisture, saccharose and hydroxymethylfurfural (HMF) contents,

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

acidity and specific conductivity, are strictly defined and constitute the quality indicators characterising individual honey varieties (Juszczak *et al.*, 2009). According to SNI 3545: 2013, honey is a liquid that has a sweet taste and is produced by honey bees (*Apis* Sp) from plant flower extracts (floral nectar) or other parts of plants (extra flora). Honey has four characteristics, namely high sugar content, low moisture content, gluconic acid (acidic environment pH 3.2 - 4.5), and hydrogen peroxide, which causes honey to inhibit the growth of pathogenic bacteria (Al-Sayaghi *et al.*, 2022). Research by Almasaudi (2021) stated that specialized honeys, including bitter varieties, possess potent antibacterial activity against both Gram-negative and Gram-positive test bacteria due to their elevated phenolic compounds and hydrogen peroxide accumulation. In addition, honey also contains oligosaccharide sugar, which is largely indigestible, so honey has the potential as a prebiotic to increase the growth of probiotics (Mustar, 2022a). Fratianni *et al.* (2023) stated that normal microflora, such as *Lactobacillus* and *Bifidobacteria*, will ferment indigestible oligosaccharides for the benefit of their localized bacterial metabolism. Indonesia has various types of honey with various types of flora, which are the source of its nectar (Riswahyuli, Setyabudi, & Raharjo, 2020). The diversity of honey types, both locally and commercially, is now beginning to be explored and studied so that the utilization of this natural material can be carried out maximally. The different sources of nectar from each honey can affect the nature of the honey produced by bees, such as the color, taste, and components of honey, so that the antibacterial activity possessed may also differ.

Based on the background above, evaluating the antibacterial activity of various honey types against pathogenic bacteria and their potential benefits in supporting lactic acid bacteria (LAB) is essential. Consequently, this study aims to compare the antibacterial activity of several types of honey against *Escherichia coli*, as well as to determine and compare their capability to support the growth of LAB. The results of this study are also expected to provide the most effective honey information, which can then be tested as an alternative ingredient to replace the antibiotic growth promoter (AGP).

RESEARCH METHODS

Research Object

The objects of this study were the pathogenic bacterium *Escherichia coli* ATCC® 11775 and the probiotic bacterium *Lactobacillus acidophilus*, both obtained from the Inter-University Center (PAU), Gadjah Mada University, Yogyakarta. The study also evaluated three distinct types of honey samples, namely Honey A (Kupang, NTT), Honey B (Lombok, NTB), and Honey C (commercial honey). To ensure standardized experimental conditions, pure colonies of *Escherichia coli* ATCC® 11775 and *Lactobacillus acidophilus* were suspended in a sterile saline solution (0.9% NaCl). The turbidity of each bacterial suspension was carefully adjusted until it matched the McFarland 0.5 standard, which corresponds to an approximate bacterial density of 1.5×10^8 CFU / ml, following the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. This standardized inoculum was used immediately to ensure optimum cell viability during the assays.

Research Design

This study was conducted using an experimental laboratory method structured under a Completely Randomized Design (CRD). The antibacterial activity of the honey samples against *Escherichia coli* was evaluated using the standard Kirby-Bauer disc diffusion assay. Concurrently, the prebiotic potential of the honey samples toward *Lactobacillus acidophilus was* evaluated using a modified agar disc diffusion growth stimulation assay.

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal
Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

Data Collection Method

Data were collected through laboratory experimental procedures. The antimicrobial activity against *E. coli* was evaluated using the standard Kirby-Bauer disc diffusion method, while the evaluation of honey toward *L. acidophilus* was performed using a modified agar disc diffusion growth stimulation assay following the approach described by Klangpetch *et al.* (2017). *E. coli* was cultured in Brain Heart Infusion (BHI) media, while *L. acidophilus* was cultured in MRS broth and incubated at 37°C for 24 hours under microaerophilic conditions. Bacterial suspensions were prepared in Phosphate Buffered Saline (PBS) at a concentration of CFU/mL. Sterile blank paper discs with a diameter of 6 mm were soaked in 20 µL of 100% honey samples for 15–30 minutes until completely saturated. These discs were then placed on Mueller-Hinton Agar (MHA) media for *E. coli* and MRS agar media for *L. acidophilus*, which had been previously inoculated with the respective test bacteria. Chloramphenicol discs (30 µg) were used as a positive control, while sterile distilled water discs were used as a negative control. After incubation at 37°C for 24 hours, observations were made by measuring the diameter of inhibition zones for *E. coli* and the diameter of dense growth stimulation zones around the discs for *L. acidophilus*. Each test was performed in triplicate to ensure data reliability.

Data Analysis

The data obtained were tabulated in the form of inhibition zone diameters for *E. coli* (mm) and growth stimulation zone diameters for *L. acidophilus* (mm) and presented as mean values. Data analysis was performed descriptively by comparing the average diameters among treatments.

RESULTS AND DISCUSSION

Results

Antibacterial Activity of Honey against *E. coli*

The antibacterial activity of honey was assessed using the standard Kirby-Bauer disc diffusion assay by observing the presence of inhibition zones (clear zones) formed around the discs containing each test honey at 100% concentration. The inhibition zone diameter was compared with the positive control (chloramphenicol 30 µg) and the negative control (sterile distilled water). The standard positive control using chloramphenicol exhibited a strong inhibition zone diameter of 6.85 ± 0.030 mm, followed by honey A (Kupang honey) with 6.26 ± 0.025 mm, and honey C (commercial honey) with the smallest diameter of 6.25 ± 0.015 mm, whereas the negative control using sterile distilled water showed no inhibition zone (0 mm). The results of the antibacterial activity test are presented in Table 1. Based on Table 1, all three honey types at 100% concentration were able to inhibit the growth of *E. coli*. Honey B (Lombok honey) produced the largest average inhibition zone diameter of 6.85 ± 0.030 mm, followed by honey A (Kupang honey) with 6.26 ± 0.025 mm, and honey C (commercial honey) with the smallest diameter of 6.25 ± 0.015 mm. Given that the standard paper disc diameter used in this assay is 6 mm, these measurements demonstrate that the actual clear zone extending beyond the disc margin was 0.85 mm for Lombok honey, 0.26 mm for Kupang honey, and 0.25 mm for commercial honey. Although these zones of clearing are narrow, they distinctly confirm that all three honey types possess measurable antibacterial efficacy against *E. coli*. Differences in inhibition zone diameter and color among honey types can be observed in Figure 1.

Benefits of Honey on the Growth of *L. acidophilus*

The prebiotic potential of honey was assessed using a modified agar disc diffusion growth stimulation assay by observing the presence of dense growth stimulation zones (turbid zones) formed around the discs containing each test honey at 100% concentration against *L.*

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

acidophilus. The larger the growth stimulation zone diameter produced, the better the ability of honey to support bacterial growth. In this assay, the negative control using sterile distilled water discs showed no growth stimulation zone (0 mm). In contrast, the positive control using chloramphenicol discs (30 μg) produced a complete zone of inhibition, preventing any bacterial growth around the disc, and was therefore not measured for stimulation. Meanwhile, the growth stimulation zones produced by the honey treatments were led by honey B (Lombok honey) at 26.97 ± 0.072 mm, followed by honey C (commercial honey) at 26.88 ± 0.015 mm, and honey A (Kupang honey) at 26.66 ± 0.025 mm. The results are presented in Table 2. Based on Table 2, all three honey types were able to support the growth of *L. acidophilus* with varying growth stimulation zone diameters. Given that the standard paper disc diameter is 6 mm, these measurements demonstrate that the actual enhanced bacterial proliferation zone extending beyond the disc margin was 20.97 mm for Lombok honey, 20.88 mm for commercial honey, and 20.66 mm for Kupang honey. The modified disc diffusion test results for *L. acidophilus* growth stimulation can be observed in Figure 2.

Discussion

Antibacterial Activity of Honey against *E. coli*

Honey has natural antibacterial activity due to several components, including high sugar content, acidity, and hydrogen peroxide, which are formed through glucose oxidation during the honey ripening process. Hydrogen peroxide is sensitive to heat and light, which denaturates glucose oxidase endogenously (Al-Sayaghi *et al.*, 2022; Masoura *et al.*, 2020; Prabhavathi *et al.*, 2023). The acidity of honey (pH 3.2–4.5) greatly affects bacterial growth, as a drop in pH to the lowest limit for bacterial growth will not only stop bacterial cell growth but also cause bacteria to lose their ability to survive. Low pH increases hydrogen ion concentration, disrupting the proton membrane gradient of bacterial cells (Al-Sayaghi *et al.*, 2022). Honey also contains phenolic compounds that exert antibacterial effects by poisoning the protoplasm, damaging and penetrating cell walls, and depositing microbial cell proteins (Almasaudi, 2021). In addition, honey contains the enzyme catalase, which causes bacteria to be exposed to hydrogen peroxide that is subsequently converted into water and oxygen (Mustar, 2022a). The high sugar content in honey, consisting of approximately 30.30% glucose, 38.40% fructose, and 1.30% sucrose, creates osmotic pressure that can inhibit bacterial growth and cause the death of unicellular organisms such as bacteria (Prabhavathi *et al.*, 2023). These findings are consistent with Almasaudi (2021), who confirmed that honey has antibacterial activity against both Gram-negative and Gram-positive bacteria.

The variation in inhibition zone diameters among the three honey types may be attributed to differences in nectar sources, which influence the physicochemical characteristics, color, taste, aroma, and bioactive compositions of each individual variety (Juszczak *et al.*, 2009). In this study, Honey B (Lombok honey) exhibited the highest antibacterial efficacy against *E. coli* compared to Kupang and commercial honeys. As supported by Escuredo *et al.* (2013), this superior activity is highly correlated with the distinct floral source and unique physicochemical composition of Lombok honey, which may include a higher total phenolic content or a lower pH that works synergistically to enhance its antibacterial potency.

Furthermore, the relatively small inhibition zone diameters observed in this study (6.25–6.85 mm, only slightly exceeding the 6 mm disc diameter) suggest a weak but distinct baseline antibacterial activity against *E. coli* at 100% concentration. This narrow zone of clearing may be attributed to the inherent structural resistance of Gram-negative bacteria such as *E. coli*, which possesses a complex lipopolysaccharide outer membrane that acts as a highly selective permeability barrier against many antimicrobial agents, including the phenolic compounds and

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal
Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

hydrogen peroxide present in honey (Nikaido, 2003; Al-Sayaghi *et al.*, 2022). Additionally, physical limitations in disc diffusion efficacy naturally arise when testing high-viscosity and high-density substances like undiluted honey. The high molecular weight sugars significantly limit the molecular diffusion rate of active antimicrobial components through the solid agar matrix away from the paper disc, making the diffusion process less efficient compared to aqueous solutions (Masoura *et al.*, 2020). Therefore, while the visual expression of the antibacterial effect is structurally restricted on solid agar due to these physical and biological constraints, the results successfully confirm the authentic antibacterial capacity of all three tested honeys against *E. coli*.

Benefits of Honey on the Growth of *L. acidophilus*

Honey can support the growth of lactic acid bacteria (LAB) due to its specialized sugar profile, primarily consisting of fructose and glucose, alongside various critical minor bioactive components. The oligosaccharide content of honey is largely non-digestible because the specific spatial configuration of carbon atoms within its monosaccharide units makes the glycosidic bonds highly resistant to enzymatic hydrolysis in the upper digestive tract of humans and animals. These non-digestible oligosaccharides possess a lower sweetness index compared to standard monosaccharides and disaccharides, granting honey a powerful potential as a natural prebiotic substrate to selectively support the proliferation of probiotic bacteria such as *L. acidophilus* (Mustar, 2022a). Fratianni *et al.* (2023) stated that beneficial intestinal microflora, particularly *Lactobacillus* and *Bifidobacteria*, ferment these non-digestible oligosaccharides to fulfill their localized bacterial metabolic requirements. This fermentation process yields short-chain fatty acids (SCFAs), providing several health advantages for the host, including increased probiotic cell mass, competitive inhibition of intestinal pathogen growth, and metabolic appetite regulation. This aligns with Mohan *et al.* (2017), who reported that probiotic strains administered concurrently with specific prebiotic carbohydrates exhibit enhanced stability and robust proliferation. Furthermore, Schell *et al.* (2022) demonstrated that the administration of honey significantly increased the population of lactic acid bacteria within the gastrointestinal tract *in vivo*. These combined findings structurally confirm that honey possesses authentic prebiotic properties capable of maintaining the growth kinetics and functional stability of probiotic species (Mustar, 2022b).

In this study, the modified agar disc diffusion growth stimulation assay revealed massive growth stimulation zones for *L. acidophilus*, extending prominently from 26.66 mm to 26.97 mm. This localized overgrowth occurred because the honey's oligosaccharides successfully diffused outward through the MRS agar matrix, forming a highly concentrated nutrient ring that the aciduric *L. acidophilus* actively metabolized as a carbon source. The variation in the growth stimulation diameters among the three honey types (with Lombok honey being the highest at 26.97 ± 0.072 mm) indicates that the prebiotic capacity is heavily dictated by their respective botanical and geographical nectar sources, which directly alter the exact concentration and diversity of the prebiotic oligosaccharide fractions (Juszczak *et al.*, 2009). Nevertheless, certain research limitations must be acknowledged within the scope of this *in vitro* assay. While the modified disc diffusion method successfully provides rapid visual evidence of direct bacterial growth stimulation on a solid agar medium, it stands as a qualitative screening tool that cannot precisely quantify absolute cell viability or log CFU/mL expansion. Solid agar restricts the physical depth of colony layers, preventing an exact measurement of biomass accumulation over a kinetic timeline. Furthermore, this closed *in vitro* environment does not fully replicate the dynamic, complex physiological conditions of the gastrointestinal tract, such as exposure to varying gastric juices, bile salts, and competing resident microflora

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

(Mohan *et al.*, 2017). Therefore, while these visual growth stimulation zones clearly validate the baseline compatibility and potent prebiotic advantages of the tested local and commercial honeys toward *L. acidophilus*, future quantitative studies utilizing liquid batch fermentations and *in vivo* models are highly recommended to fully map out the dynamic prebiotic index of these honeys.

This study has several limitations that should be considered when interpreting the results. First, the antibacterial activity was tested using only 100% honey concentration, which does not reflect practical field application concentrations and may limit diffusion through the agar medium due to the high viscosity of honey. Second, only one strain each of *E. coli* (ATCC 11775) and *L. acidophilus* was used, which may not represent the full spectrum of bacterial responses. Third, while the modified agar disc diffusion assay successfully screens for growth stimulation, solid agar methods are not the absolute standard for fully quantifying prebiotic kinetic parameters; therefore, the dense growth stimulation zones observed around the discs should be interpreted with caution. Fourth, this study was conducted entirely *in vitro* and does not account for the complex gastrointestinal environment, digestive processes, or host immune responses that would occur *in vivo*. Finally, the specific physicochemical composition of the honey samples (e.g., precise phenolic profiles and oligosaccharide fractions) was not chemically characterized in this study, which limits the ability to fully map out the exact compounds responsible for the variations in activity among honey types.

CONCLUSION AND SUGGESTIONS

Conclusions

Honey A (Kupang), Honey B (Lombok), and Honey C (commercial) at 100% concentration exhibit a distinct but narrow antibacterial activity against *Escherichia coli*. This is indicated by the formation of localized zones of inhibition that slightly exceed the standard 6 mm paper disc diameter, with varying measurements heavily dependent on the specific floral origin and physicochemical characteristics of each honey type. However, it is important to note that these inhibition zone diameters (6.25–6.85 mm) were only marginally larger than the standard disc diameter (6 mm), indicating weak antibacterial activity that should be interpreted with caution. In addition, all three types of honey demonstrate a robust capacity to support the proliferation of the lactic acid bacterium *Lactobacillus acidophilus*, as evidenced by the formation of wide, dense growth stimulation zones. These findings structurally confirm that while the high viscosity of pure honey limits its physical diffusion and produces a restricted antibacterial zone against *E. coli* on solid agar, it simultaneously exerts excellent prebiotic properties that selectively enrich beneficial probiotic strains. Therefore, honey possesses dual functional roles as a natural antimicrobial and a prebiotic substrate, highlighting its significant potential as a sustainable, natural alternative to antibiotic growth promoters (AGPs) for enhancing animal health and productivity.

Suggestions

Further quantitative studies are strongly recommended to evaluate the prebiotic and antibacterial efficacy of these honeys using liquid batch fermentation models, which can precisely enumerate absolute bacterial viability via log CFU/mL expansion without the physical diffusion limitations of solid agar assays. It is also suggested to investigate a wider range of honey concentrations (serial dilutions) and broader pathogenic and probiotic bacterial strains. Advanced analytical techniques, such as liquid chromatography, should be utilized to identify the specific phenolic profiles and oligosaccharide fractions responsible for these functional effects. Finally, dynamic *in vivo* trials are necessary to evaluate the safety, stability,

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

and actual efficacy of these local honeys when applied directly as natural feed additives in livestock to successfully replace conventional AGPs.

ACKNOWLEGMENTS

The authors would like to express sincere gratitude to Gadjah Mada University, particularly the Inter-University Center (PAU), for providing the laboratory facilities, bacterial strains, and technical support during this research. Deep appreciation is also extended to all parties who contributed directly or indirectly to the completion of this study and the preparation of this manuscript.

REFERENCES

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences, 28*(4), 2188–2196. https://doi.org/10.1016/j.sjbs.2020.10.017

Al-Sayaghi, A. M., Al-Kabsi, A. M., Abduh, M. S., Saghir, S. A. M., & Alshawsh, M. A. (2022). Antibacterial mechanism of action of two types of honey against *Escherichia coli* through interfering with bacterial membrane permeability, inhibiting proteins, and inducing bacterial DNA damage. *Antibiotics, 11*(9), 1182. https://doi.org/10.3390/antibiotics11091182

Clinical and Laboratory Standards Institute. (2023). *M100: Performance standards for antimicrobial susceptibility testing* (33rd ed.). Clinical and Laboratory Standards Institute.

Escuredo, O., Míguez, M., Fernández-González, M., & Seijo, M. C. (2013). Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chemistry, 138*(2–3), 851–856.

Fratianni, F., d'Acierno, A., Coppola, R., & Nazzaro, F. (2023). Usage of non-digestible oligosaccharides by health-promoting intestinal bacteria: A review on prebiotic kinetics. *Microorganisms, 11*(2), 345.

Juszczak, L., Socha, R., Rożnowski, J., Fortuna, T., & Nalepka, K. (2009). Physicochemical properties and quality parameters of herbhoneys. *Food Chemistry, 113*, 538–542. https://doi.org/10.1016/j.foodchem.2008.07.098

Klangpetch, W., Phianphak, W., & Whangchai, N. (2017). Development of a modified agar disc diffusion method for screening growth stimulation of probiotic *Lactobacillus* species by agricultural by-products. *Journal of Applied Microbiology and Biotechnology, 5*(2), 112–119.

Masoura, M., Passaretti, P., Overton, T. W., & Lund, P. A. (2020). Use of a model to understand the synergies underlying the antibacterial mechanism of H_2O_2-producing honeys. *Scientific Reports*, 1–14. https://doi.org/10.1038/s41598-020-74937-6

Mohan, A., Quek, S. Y., Gutierrez-Maddox, N., & Shu, Q. (2017). Effect of honey in improving the growth and viability of *Lactobacillus acidophilus* and *Bifidobacterium animalis*. *Journal of Food Science and Technology, 54*(9), 2411–2419.

Mustar, K. (2022a). *Potensi oligosakarida madu sebagai agen prebiotik dan aktivitas enzimatisnya terhadap bakteri asam laktat*. UGM Press.

Mustar, K. (2022b). Peran enzim katalase dan hidrogen peroksida madu dalam modulasi mikrobiota usus. *Jurnal Mikrobiologi Indonesia, 16*(1), 45–52.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews, 67*(4), 593–656.

Prabhavathi, S. J., Subrahmaniyan, K., Kumar, M. S., Gayathry, G., & Malathi, G. (2023).

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

Exploring the antibacterial, anti-biofilm, and anti-quorum sensing properties of honey: A comprehensive review. *Agriculture Archives: An International Journal, 2*(3). https://doi.org/10.51470/AGRI.2023.2.3.10

Riswahyuli, Y., Setyabudi, F. M. C. S., & Raharjo, S. (2020). Characterization of Indonesia wild honey and its potential for authentication and origin distinction. *Food Research, 4*, 1670–1680. https://doi.org/10.26656/fr.2017.4(5).105

Schell, K. R., Shanahan, E., Wilson, I., Blair, S. E., Carter, D. A., & Cokcetin, N. N. (2022). The potential of honey as a prebiotic food to re-engineer the gut microbiome toward a healthy state. *Frontiers in Nutrition, 9*, 957932. https://doi.org/10.3389/fnut.2022.957932

Tables

Table 1. Results of measurement of diamter inhibition zone of Honey against *E.coli*

Types of Honey	Diameter inhibition zone (mm)
Honey A	6,26 mm ± 0,025
Honey B	6,85 mm ± 0,030
Honey C	6,25 mm ± 0,015

Description: A) Kupang Honey; B) Lombok Honey; C) Commercial Honey

Table 2. Results of measuring Diameter of growth stimulation zone against *L.acidophilus*

Type of Honey	Diamter inhibition zone (mm)
Honey A	26,66 mm ± 0,025
Honey B	26,97 mm ± 0,072
Honey C	26,88 mm ± 0,015

Note: A) Kupang Honey; B) Lombok Honey; C) Commercial Honey

Volume 18 No. 3: 535-544
June 2026
https://doi.org/10.24843/bulvet.2026.v18.i03.p05

Figures

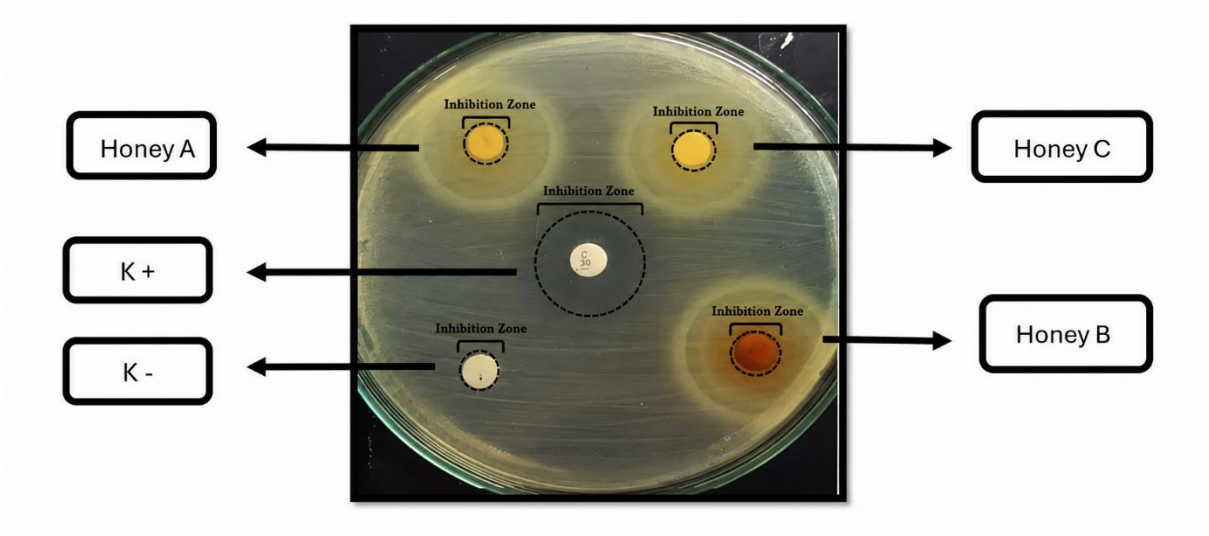

Figure 1. Kirby-Bauer disc diffusion assay results of Kupang honey (A), Lombok honey (B), commercial honey (C), positive control (K+: chloramphenicol 30 µg), and negative control (K-: sterile distilled water) against E. coli. The arrows explicitly indicate the narrow but distinct inhibition zones (clear zones) around the treatment discs and the robust bacterial growth (turbid background) across the Mueller Hinton Agar (MHA) matrix.

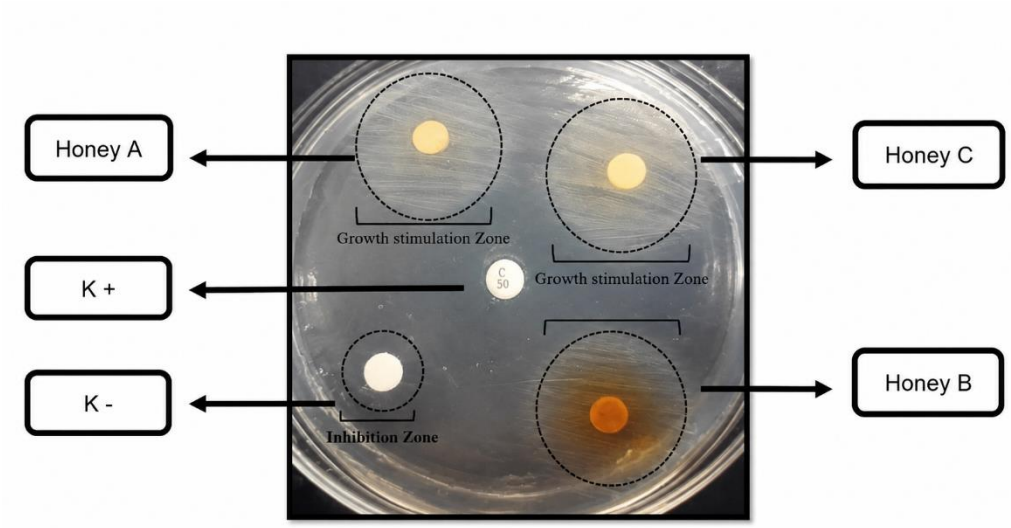

Figure 2. Modified agar disc diffusion growth stimulation assay results of Kupang honey (A), Lombok honey (B), commercial honey (C), positive control (K+: chloramphenicol 30 µg), and negative control (K-: sterile distilled water) against L. acidophilus. The arrows explicitly indicate the dense growth stimulation zones (enhanced bacterial proliferation rings) surrounding the honey discs, contrasting sharply with the clear inhibition zone around the antibiotic control (K+).

Terakreditasi Nasional Sinta 4, berdasarkan Keputusan Direktur Jenderal
Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021