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Short communication **OPEN ACCESS**

Identification of lactic acid bacteria in captive primate species at Bukittinggi Zoo, West Sumatera

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Abstract

Background Lactic acid bacteria (LAB) provide health benefits, particularly in their role as probiotics and antimicrobials for treating digestive diseases in primates.

Objective This study aimed to identify and quantify the total colony count of lactic acid bacteria in the feces of five primate species: Sumatran orangutan (*Pongo abelii*), brown-ungko gibbon (*Hylobates agilis*), black-handed gibbon (*Hylobates agilis*), siamang (*Symphalagus syndactylus*), and langur (*Trachypithecus auratus*), collected from the Bukittinggi Zoo.

Methods The samples were serially diluted from 10^{-1} to 10^{-7} and inoculated into de Man Rogosa Sharpe Agar (MRSA) selective media using the pour plate method. LAB colonies growing on the medium were obser-ved and counted. Selected colonies were isolated and subsequently identified using several biochemical tests, including catalase, oxidase, sulphide indole motility (SIM), methyl red-Voges Proskauer (MR-VP), and carbohydrate fermentation tests.

Results Orangutans' samples had the highest total LAB count 3.1 × 10^{8} CFU/ml**.** In this study, we successfully isolated bacteria from the genera *Lactobacillus* (orangutan, brown ungko gibbon, black-handed gibbon, and siamang)*, Leuconostoc* (black-handed gibbon and siamang)*,* and *Weissella* (langur).

Conclusion Three genera of lactic acid bacteria were identified in primate feces from Bukittinggi Zoo, and further research is needed to determine their potential application as probiotics.

Keywords bacteria identification | lactic acid bacteria (LAB) | primate feces | probiotic potential | total bacteria

Introduction

Indonesia is a tropical country with a high level of biodiversity in both fauna and flora. Geographical conditions, environment, and year-round food availability contribute to the high biodiversity in Indonesia. Primates are a key component of Indonesia's biodiversity. Supriatna and Rizki (2016) stated that there are at least 480 primate species worldwide, with 59 species found in Indonesia, and approximately 35 species (60%) are endemic and only found in Indonesia. The population of primates in their natural habitat has been declining annually because of factors such forest fragmentation, deforestation for settlements, illegal hunting for trade, and natural disasters such as forest fires (Ruskhanidar *et al.*, 2017). Mukhlisah *et al.* (2017) reported that the risk of disease from pathogens such as viruses, bacteria, fungi, and parasites also threatens primate populations.

Several primate species, especially arboreal species, such as orangutans, play a crucial role in maintaining the sustainability of forest ecosystems (Jefsykinov *et al.*, 2021). Their primary diet consists of fruits (frugivorous); however, during non-fruiting seasons, they consume leaves, shoots, bark, and

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insects. Primates help disperse seeds from the fruits they eat, which is vital for forest vegetation (Safika *et al.*, 2023a). Primates have well-developed ceca, which enhance their digestive system's ability to process food (Karyawati, 2012). Primate food undergoes a series of physiological processes, including ingestion, digestion, absorption, and defecation. Digestion in primates is aided by lactic acid bacteria, which produce cellulase enzymes to assist the digestive process (Safika *et al.*, 2019). Failure of the absorption process can lead to food accumulation in the digestive tract, increasing the risk of gastrointestinal infections in primates (Rémond *et al.*, 2003).

In ex situ environments in Indonesia, infectious bacteria implicated in digestive disorders in orangutans include *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., and *Eschrichia* spp. (Wibowo *et al.*, 2016; Afiff *et al.*, 2024). The use of probiotics may be an alternative approach for managing digestive disorders in primates. One such probiotic is Lactic Acid Bacteria (LAB), which produces lactic acid as a byproduct of carbohydrate metabolism, hydrogen peroxide, and bacteriocins.

LAB are naturally present on various mucosal surfaces, including the digestive, reproductive, and respiratory tracts (Wang *et al.*, 2018; Plaza-Diaz *et al.*, 2019). However, research on the role of LAB in non-human primates is limited. The primary focus of this study was on ex situ primates, particularly orangutans and other species of the Bukittinggi Zoo. Therefore, this study aimed to contribute foundational data to support the potential application of LAB-based probiotics in improving digestive health among ex situ primate populations.

Methods

Sample collection

This study obtained a permit and a written recommendation letter from the Directorate General of Conservation of Natural Resources and Ecosystem, Ministry of Environment and Forestry (Decree No. 433/KSDAE/SET.3/KSA.2/8/2021). We did not seek ethical approval for the study because we collected only fecal samples and no individuals were affected or harmed.

The samples used in this study were fecal samples from five primate species: Sumatran orangutan (*Pongo abelii*) (n=1), brown ungko gibbon (*Hylobates agilis*) (n=1), black-handed gibbon (*Hylobates agilis*) (n=1), siamang (*Symphalagus syndactylus*) (n=1), and langur (*Trachypithecus auratus*) (n=1), collected from Bukittinggi Zoo and stored in the Medical Microbiology Laboratory, School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University.

Total plate count (TPC)

The culture process began by placing 1 gram of feces into a sterile test tube containing 9 ml of 0.9% NaCl solution, followed by homogenization. Serial dilutions were made from 10^{-1} to 10^{-7}, and 1 ml of the suspension was inoculated into Petri dishes using the pour plate method with de Man Rogosa Sharpe Agar (MRSA) at approximately 50°C. The plates were labeled according to the dilution factor and incubated at 37°C for 48 hours. After incubation, the colonies were counted using a colony counter. Each sample was plated in duplicates to ensure data consistency.

Isolation and identification of bacteria

Fecal samples were diluted at a 9:1 ratio using a 0.9% NaCl solution. The homogenized samples were then inoculated onto methicillin-resistant *Staphylococcus aureus* (MRSA) plates using the pour plate method. After 48 hours of incubation at 37°C, isolated colonies were purified and stored on slants for further analysis. Colonies were identified macroscopically and microscopically by Gram staining. Biochemical tests including catalase, oxidase, sulphide indole motility (SIM), methyl red-Voges Proskauer (MR-VP), and carbohydrate fermentation tests were conducted.

Results

Fecal samples from the five primate species were cultured on MRSA media and incubated at 37°C for 48 hours. The isolates were labeled A, B, C, D, and E, corresponding to Sumatran orangutan, brown ungko gibbon, black-handed gibbon, siamang, and langur, respectively. The total plate count (TPC) of lactic acid bacteria varied among primate species. Orangutans had the highest average LAB count at 3.1×10^{8} CFU/ml, while the brown-handed gibbon had the lowest at 1.1×10^{4} CFU/ml (**Table 1**).

Table 1 Total plate count of lactic acid bacteria (LAB) in primates

Primates	Isolate code	Total LAB (CFU/mL)
Orangutan	A	3.1×10^{8}
Brown ungko gibbon	B	1.1×10^{4}
Black-handed gibbon	C	3.5×10^{6}
Siamang	D	1.2×10^{7}
Langur	E	-

The purified isolates had round, convex, and smooth edges, ranging from clear to white or yellowish-white in color. Gram staining revealed that four isolates (A.1, B.1, C.1, and D.2) were bacilli, two isolates (C.2 and D.1) were cocci, and one isolate (E.1) was a coccobacillus (**Figure** 1). Biochemical tests identified *Lactobacillus* in orangutans, brown ungko gibbon, black-handed gibbon, and siamang (isolates A.1, B.1, C.1, and D.2, respectively). *Leuconostoc* was found in black-handed gibbon and siamang (isolates C.2 and D.1), and *Weissella* was identified in langur (isolate E.1) (**Table 2**).

Discussion

The gut microbiota of primates varied significantly. Factors such as diet, environment, stress, and medication can influence the composition of the gut microbiota. Gut microbiota, including Lactic Acid Bacteria (LAB), plays a fundamental role in digestion, immune modulation, and pathogens. Diet is considered to be the most important factor in primate evolution, affecting morphology, physiology, and ecology (Yildirim *et al.*, 2010; Clayton *et al.*, 2018; Moran

et al., 2019; Safika *et al*., 2023b). This study found that primate feces contain various types of lactic acid bacteria (LAB), with the total colony count varying among species. Orangutans had the highest LAB count (3.1×10^8 CFU/ml), likely due to their fiber-rich diet.

Differences in LAB counts among primates are influenced by multiple factors, including diet, habitat, and host physiology. Diets rich in fiber, such as those consumed by folivorous or frugivorous primates, promote the growth of lactic acid bacteria because of the increased availability of fermentable substrates (Amato *et al*., 2013). Moreover, gastrointestinal morphology and enzymatic activity also play a role in LAB colonization (Muegge *et al*., 2011; Bornbusch *et al*., 2023). Primates, with their high-fiber diet, have optimal conditions for lactic acid bacteria (LAB) fermentation in their digestive tracts, promoting the growth of genera, such as *Lactobacillus, Leuconostoc*, and *Weissella*.

The present study only identified LAB at the genus level. Previous studies have reported probiotic species within these groups, including *Lactobacillus plantarum, Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Leuconostoc pseudomesenteroides, Leuconostoc lactis* (Wang *et al*., 2018; Paritova *et al*., 2024), *Weissella cibaria* and *Weissella confusa* (Safika *et al*., 2019).

Lactic acid bacteria are fastidious organisms that require nutrient-rich and selective growth media for optimal proliferation. Their ability to produce lactic acid as a growth-associated product is directly correlated with their growth rates (Wang *et al*., 2018). Probiotics containing LAB have been ability to enhance digestion, improve feed conversion efficiency, and promote host health through the competitive exclusion of pathogenic bacteria (Plaza-Diaz *et al*., 2019). In the context of primate health and conservation, probiotics represent a promising strategy to stabilize the gut microbiota, especially in captive or rehab-ilitated individuals subjected to environmental and dietary changes.

In Indonesia, research on gastrointestinal diseases in primates is still limited. Factors such as environment and food quality contribute to enteritis in rehabilitated orangutans (Rahmi *et al*., 2014). A recent study by Afiff *et al*. (2024) reported that *Klebsiella pneumoniae* was identified in the gastrointestinal tract of Sumatran orangutans, highlighting its potential to become an opportunistic pathogen, particularly in immunocompromised individuals.

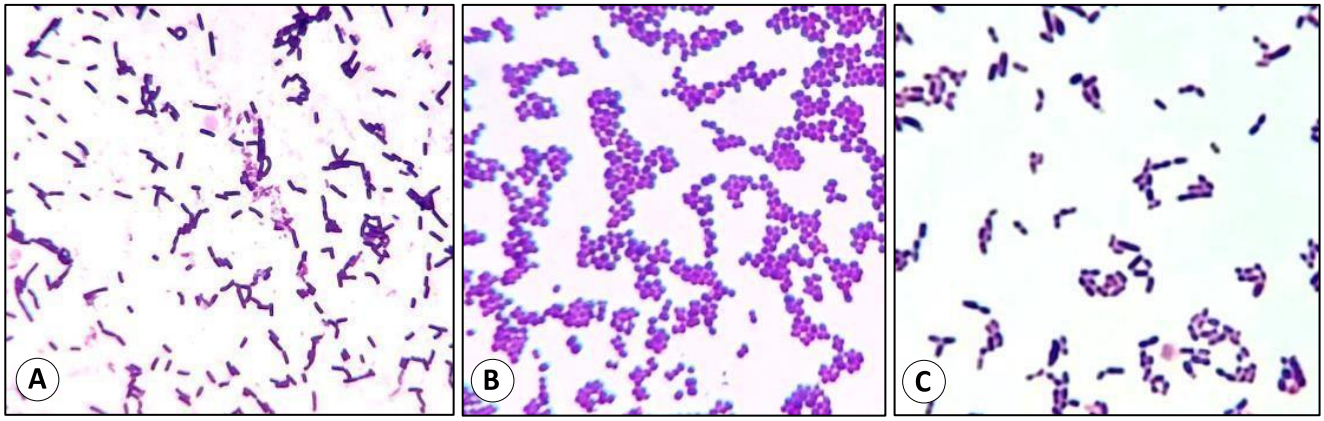

Figure 1 Gram staining of bacterial isolates from primates: (A) Bacil, (B) Coccus, and (C) Coccobacillus.

Table 2 Microscopic and bichemical test results of bacterial isolates from primates

Primate	Isolate Code	Gram staning	Ox	Cat	Mot	I	MR	VP	Glu	Lac	Suc	Mal	Raf	Tre	Bakteri
Orangutan	A.1	*Bacil*	-	-	-	-	+	-	+	+	+	+	+	+	*Lacto-bacillus*
Brown ungko gibbon	B.1	*Bacil*	-	-	-	-	+	-	+	+	+	+	-	-	*Lacto-bacillus*
Black-handed gibbon	C.1	*Bacil*	-	-	-	-	+	-	+	+	+	+	-	-	*Lacto-bacillus*
	C.2	*Coccus*	-	-	-	-	-	-	+	-	+	+	-	-	*Leuco-nostoc*
Siamang	D.1	*Coccus*	-	-	-	-	+	-	+	+	+	+	-	+	*Leuco-nostoc*
	D.2	*Bacil*	-	-	-	-	+	-	+	+	+	-	-	+	*Lacto-bacillus*
Langur	E.1	*Cocco-bacilli*	-	-	-	-	+	-	+	+	+	-	-	+	*Weissella*

Ox: oxidase, Cat: catalase, Mot: motility, Ind: indole, MR: methyl red, VP: Voges-Proskauer, G: glucose, Lac: lactose, Suc: sucrose, mal: maltose, Raf: raffinose, Tre: trehalose, (+): positive, (−): negative.

Overall, this study provides insights into the diversity of lactic acid bacteria in the primate gut microbiota, which can contribute to the development of primate-based probiotics for conservation and animal health. The use of probiotics in primates could be a conservation effort for endangered species in Indonesia. In addition to their probiotic benefits, LAB have antimicrobial properties that can suppress the growth of pathogenic bacteria in the primate digestive system.

Conclusion

The genera *Lactobacillus, Leuconostoc,* and *Weissella* are detedected in fecal samples of primate at Bukittinggi Zoo. These bacteria are known for their probiotic properties and their potential roles in maintaining gut health. Our findings provide a preliminary reference for the development of LAB-based probiotic strategies to support digestive health management in captive primate populations.

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Conflict of interest The authors declare that they have no competing interest.

Author contribution FNP: writing–original draft preparation, writing–review and editing, investigation; WRA: writing–review and editing, visualization, supervision, project administration; JR: Data curation, investigation; UA: validation; formal analysis, data curation, writing–original draft preparation; SS: conceptualization, methodology, validation, data curation, writing–review and editing, supervision, funding acquisition. All the authors have read and agreed to the published version of the manuscript.

Availability of data and materials The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

Afiff U, Indrawati A, Hidayat R, Sunartatie T, Hardiati A, Rotinsulu DA, Arifiantini RI, Naoremisa D, Mar'ah N, Safika S. 2024. Antibiotic resistance and virulence profile of Klebsiella pneumoniae isolated from wild Sumatran Orangutans (Pongo abelii). Journal of Advanced Veterinary and Animal Research, 11(4): 1066–1075. DOI: 10.5455/javar.2023.j673.

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR. 2013. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. The ISME Journal, 7(7): 1344–1353. DOI: 0.1038/ismej.2013.16.

Bornbusch SL, Muletz-Wolz CR, Lopez-Bondarchuk E, Maslanka MT, Kendrick EL. 2023. Gut microbiomes of captive primates show phylosymbiosis, respond to dietary sugar reduction, and select for host-specific dietary microbes. FEMS Microbiology Ecology, 99(8): fiad069. DOI: 10.1093/femsec/fiad069.

Clayton JB, Gomez A, Amato K, Knights D, Travis DA, Blekhman R, Knight R, Leigh S, Stumpf R, Wolf T, Glander KE, Cabana F, Johnson TJ. 2018. The gut microbiome of nonhuman primates: Lessons in ecology and evolution. American Journal of Primatology, 80(6): e22867. DOI: 10.1002/ajp.22867.

Jefsykinov E, Yumarni Y. Fauzan F. 2021. Jenis-jenis tumbuhan pakan primata di lembah anai kabupaten tanah datar provinsi Sumatera Barat. Sumatera Tropical Forest Research Journal, 5(2): 736–742.

Karyawati AT. 2012. Tinjauan umum tingkah laku makan pada hewan primata. Jurnal Penelitian Sains, 15(1): 44–47.

Moran NA, Ochman H, Hammer TJ. 2019. Evolutionary and ecological consequences of gut microbial communities. Annual Review of Ecology, Evolution, and Systematics, 50(1): 451–475. DOI: 10.1146/annurev-ecolsys-110617-062453.

Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, Gordon JI. 2011. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science, 332(6032): 970–974. DOI: 10.1126/science.1198719.

Mukhlisah N, Rell F, Muslimin L. 2017. Identifikasi nematoda gastrointestinal Macaca maura di Taman Nasional Bantimurung Bulusaraung, Sulawesi Selatan. Jurnal Primatologi Indonesia, 14(1): 22–27.

Paritova A, Nurgaliyev A, Nurgaliyeva G, Abekeshev N, Abuova A, Zakirova F, Zwierzchowski G, Kuanchaleyev Z, Issabekova S, Kizatova M, Sayakova Z, Zhanabayeva D, Kukhar Y, Stozhkov R, Aitkozhina B, Mayer Y, Bayantassova S, Satbek A, Andruchshak A, Kushaliyev K. 2024. The dietary effects of two strain probiotics (Leuconostoc mesenteroides, Lactococcus lactis) on growth performance, immune response and gut microbiota in Nile tilapia (Oreochromis niloticus). PloS One, 19(10): e0312580. DOI: 10.1371/journal.pone.0312580.

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. 2019. Mechanisms of action of probiotics. Advances in nutrition (Bethesda, Md.), 10(suppl_1): S49–S66. DOI: 10.1093/advances/nmy063.

Rahmi E, Agustina D, Jamin F. 2014. Isolasi dan identifikasi genus Salmonella dan Shigella dari faeses orangutan sumatera (Pongo abelii) di Pusat Reintroduksi Orangutan Jantho. Jurnal Medika Veterinaria, 8: 5–8.

Rémond D, Shahar DR, Gille D, Pinto P, Kachal J, Peyron MA, Dos Santos CN, Walther B, Bordoni A, Dupont D, Tomás-Cobos L, Vergères G. 2015. Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. Oncotarget, 6(16): 13858–13898. DOI: 10.18632/oncotarget.4030.

Ruskhanidar R, Maulana VS, Loe VR. 2017. Spesies dan sebaran satwa primata di Indonesia. Jurnal Primatologi Indonesia, 14(1): 3–8.

Safika, S. Wardinal, W. Ismail, Y.S. Nisa, K. Sari, W.N. 2019. Weissella, a novel lactic acid bacteria isolated from wild Sumatran

orangutans (Pongo abelii). Veterinary World, 12(7): 1060–1065. DOI: 10.14202/vetworld.2019.1060-1065.

Safika S, Indrawati A, Afiff U, Hastuti YT, Zureni Z, Jati AP. 2023a. First Study on profiling of gut microbiome in wild and captive Sumatran orangutans (Pongo abelii). Veterinary World, 16(4): 717–727. DOI: 10.14202/vetworld.2023.717-727.

Safika S, Indrawati A, Afif U, Hidayat R, Sunartatie T. 2023b. Metagenomic analysis of mycobiome in wild and captivity Sumatran orangutans (Pongo abelii). Journal of Advanced Veterinary and Animal Research, 10(2): 228–236. DOI: 10.5455/javar.2023.j673.

Supriatna J, Rizki R. 2016. Pariwisata Primata Indonesia. Jakarta (ID): Yayasan Pustaka Obor Indonesia.

Wang Y, Li A, Jiang X, Zhang H, Mehmood K, Zhang L, Jiang J, Waqas M, Iqbal M, Li J. 2018. Probiotic potential of leuconostoc pseudomesenteroides and lactobacillus strains isolated from yaks. Frontiers in Microbiology, 9: 2987. DOI: 10.3389/fmicb.2018.02987.

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. Frontiers in Bioengineering and Biotechnology, 9: 612285. DOI: 10.3389/fbioe.2021.612285.

Wibowo MH, Rosetyadewi AW, Wijayanti AD, Airin CM. 2016. Isolasi dan identifikasi bakteri dari tinja Orangutan penderita gangguan gastrointestinal. Jurnal Veteriner, 17(1): 7–15.

Yildirim S, Yeoman CJ, Sipos M, Torralba M, Wilson BA, Goldberg TL, Stumpf RM, Leigh SR, White BA, Nelson KE. 2010. Characterization of the fecal microbiome from non-human wild primates reveals species specific microbial communities. PloS One, 5(11): e13963. DOI: /10.1371/journal.pone.0013963.