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IN-SILICO ANALYSIS OF SYMBIONT BACTERIA DIVERSITY IN THE MIDGUT OF *Aedes aegypti* USING 16S rDNA MOLECULAR MARKERS DATABASE

Analisis In-Silico Diversitas Bakteri Simbion Asal *Midgut Aedes aegypti* Berdasarkan Database Marka Molekuler 16S rDNA

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is caused by the dengue virus, which is transmitted through Aedes aegypti *mosquitoes when they feed on human blood. To effectively control the DHF vector, it is crucial to accurately characterize the symbiont bacteria associated with Ae. aegypti through an in-silico approach to identify potential targets. This study utilized in- silico analysis based on the 16S rDNA molecular marker to explore the diversity of symbiont bacteria obtained from bioinformatics databases. The analysis and visualization of bacterial diversity were conducted using the Pathosystem Resource Integration Center (PATRIC). The analysis results revealed that bacterial diversity in the midgut of Ae. aegypti, categorizedas culturable and non-culturable bacteria, exhibited similar abundance patterns at the family level, albeit with varying detection rates. The most dominant taxa included the phylum Proteobacteria, class Gammaproteobacteria, order* Enterobacterales*, and family Enterobacteriaceae. Within the culturable bacteria category, the dominant taxa were the genus* Salmonella *and species* Salmonella enterica*, whereas the non-culturable bacteria category indicated the prevalence of the genus* Escherichia *and species* Escherichia coli*.*

Keywords: *bacteria, bioinformatics, database, dengue, symbiont*

ABSTRAK

Demam Berdarah Deng ue (DBD) merup akan p enyakit infeksi virus d eng ue yang ditransmisikan melalui vektor *Aedes aegypti* ketika *blood feeding* kepad a manusia. Karakterisasi bakteri simbio n dari *midgut Ae. aegypti* untuk menspesifikkan target potensial seb agai agen pengendalian vektor DBD dap at dilakukan melalui pend ekatan *in-silico*. Analisis *in-silico* dilakukan b erd asarkan d ata marka molekuler 16S rDNA untuk memahami diversitas bakteri simbio n yang dikoleksi dari database bioinformatika. Analisis d an visualisasi diversitas bakteri menggunakan *software Pathosystem Resource Integration Center* (*PATRIC*). Hasil analisis *in-silico* diversitas bakteri dari *midgut Ae. aegypti* kategori kultur dan tid ak dap at dikulturkan menunjukkan kesamaan kelimpahan *Taxa* yang dominan hingg a pada tingkat Famili namun deng an persentase yang berbed a. Data *Taxa* paling dominan melip uti Filum Proteobacteria, Kelas Gammaproteobacteria, Ordo *Enterobacterales*, dan Famili Enterobacteriaceae. Kategori bakteri kultur menunjukkan *Taxa* dominan Genus *Salmonella* dan Sp esies *Salmonella enterica*, sedangkan kategori b akteri yang tidak dap at dikulturkan menunjukkan Taxa dominan genus *Escherichia* dan spesies *Escherichia coli*.

Kata Kunci: bakteri, bioinformatika, database, dengue, simbion

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains a global annual issue in tropical and subtropical regions. Approximately two-thirds of the world's population is highly vulnerable to DHF, with reported cases ranging from 100 to 390 million each year (Sun et al. 2020). DHF has been confirmed as endemic in 128 countries, including Indonesia (Koh et al. 2018). In 2021, Indonesia reported a total of 73,518 DHFcases with 661 deaths (KemenKes RI 2022). The infection of DHF is caused by the dengue virus, transmitted to humans through the vector *Ae. aegypti* (Harapan et al. 2020).

The primary vector for dengue transmission is the female Ae. aegypti mosquito (Malassigné et al. 2020). Transmission of the dengue virus can occur when female *Ae. aegypti* mosquitoes feed on DHF patients, facilitating the transfer of the virus from the mosquito's body to humans (Mapder et al. 2020). The success of virus transmission to humans depends on its abilityto infect various mosquito organs involved in dengue virus transmission, including the midgut, hemocoel, and salivary glands.

The midgut serves as an incubation site for the dengue virus (Koh et al. 2018). Once the virus has successfully multiplied, it migrates to the salivary glands through the hemocoel, and can be transmitted to healthy individuals during mosquito feeding (Zhang and Wang, 2020). However, transmission may fail when the dengue virus reaches the midgut, as the mosquito's natural immune response inhibits virus replication (Nouzova et al. 2019).

Mosquitoes possess physical, physiological, and molecular defense mechanisms as part of their immune system (Kumar et al. 2018). The physical defense is the first layer of defense, inhibiting pathogen infection through the Peritrophic Matrix layer (Simões et al. 2018). Physiological and molecular defenses are mediated by physiological processes and gene expression (Lee et al. 2019). To complete the transmission cycle, the dengue virus must penetrate these layers in the midgut (Kumar et al. 2018). Symbiotic bacteria can activate several defense mechanisms in the midgut upon detecting pathogen infections, such as dengue virus (Scolari et al. 2019). Additionally, symbiotic bacteria can inhibit dengue infection by secreting secondary metabolites (Gao et al. 2020).

The isolation and exploration ofsymbiotic bacteria establish an interaction between mosquito symbiotic bacteria and pathogens. Some symbiotic bacteria associated with *Ae. aegypti* have beenfound to inhibit dengue virus infection (Wilke and Marrelli, 2015), shortenmosquito lifespan (Wu et al. 2019), and affect the mosquito's life cycle (Coon et al. 2017). The role of symbiotic bacteria in inhibiting dengue infection highlights their potential as biocontrol agents against *Ae. aegypti*. Mapping the diversity of symbiotic bacteria helps identify the dominant symbiotic bacteria present in the midgut of *Ae. aegypti* and control the dengue vector. This can be achieved through in-silico analysis of symbiotic bacteria data in bioinformatics databases. Isolation andcharacterization of symbiotic bacteria can provide more accurate information for their expected biocontrol function.

In-silico analysis allows the mapping of symbiotic bacteria diversity without the need for conventional bacterial isolation methods. Mapping the diversity of living organisms can be achieved through taxonomic classification approaches using DNA barcoding in bioinformatics databases (Bennett et al. 2019; Scolari et al. 2019).This study employed an in-silico analysis to map the diversity of symbiotic bacteria in the midgut of *Ae. aegypti*, using 16S rDNA molecular markers available in the NCBI database (Table 1 and 2), which are commonly used for bacterial characterization. The in-silico analysis of symbiotic bacteria from the midgut of *Ae. aegypti* supports the laboratory analysisresults regarding the exploration ofsymbiotic bacteria's potential as a new biocontrol agent against DHF vectors.

MATERIALS AND METHODS

Location and time

This research was conducted from August to December 2022 at theBiotechnology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember.

Table 1. 16S rDNA Sequences Collection of Culturable Bacteria from NCBI Database

Acc. Number	Spesies	Sequence Size (bp)	References
JN201949.1	*Enterococcus faecalis* strain Kiv	1520	Terenius et al. 2012
MT279442.1	*Microbacterium* sp. strain CEL3	1508	Rodrigo et al. 2020
JN201946.1	*Bacillus* sp. JL6	1507	Lindh et al. 2011
JN201947.1	*Serratia marcescens* strain Ki	1502	Terenius et al. 2012
JN201948.1	*Klebsiella pneumoniae* strain Kiii	1499	Terenius et al. 2012
MT279473.1	*Terribacillus* sp. strain LE11	1494	Rodrigo et al. 2020
MT279351.1	*Lysinibacillus sphaericus* strain CEA2	1492	Rodrigo et al. 2020
MT279353.1	*Lysinibacillus sphaericus* strain CEA3	1492	Rodrigo et al. 2020
JN201945.1	*Burkholderiaceae bacterium* JL4 16S	1491	Terenius et al. 2012
MT279459.1	*Priestia flexa* strain LE1	1490	Rodrigo et al. 2020
MT279460.1	*Bacillus* sp. (in: firmicutes) strain LE2 16S	1488	Rodrigo et al. 2020
MT279468.1	*Priestia flexa* strain LE9	1488	Rodrigo et al. 2020
MT279354.1	*Lysinibacillus sphaericus* strain CEA4	1487	Rodrigo et al. 2020
MT279464.1	*Bacillus cereus* strain LE6	1485	Rodrigo et al. 2020
MT279461.1	*Niallia nealsonii* strain LE3	1484	Rodrigo et al. 2020
MT279463.1	*Bacillus* sp. (in: firmicutes) strain LE5	1484	Rodrigo et al. 2020
MT279443.1	*Bacillus cereus* strain CEL4	1483	Rodrigo et al. 2020
MT279462.1	*Lysinibacillus sphaericus* strain LE4	1483	Rodrigo et al. 2020
MT279445.1	*Lysinibacillus sphaericus* strain CEL5	1482	Rodrigo et al. 2020
MT277413.1	*Staphylococcus warneri* strain AE2	1478	Rodrigo et al. 2020
JN201943.1	*Elizabethkingia meningoseptica* strain JL1	1476	Terenius et al. 2012
MT279350.1	*Serratia liquefaciens* strain CEA1	1475	Rodrigo et al. 2020
MT052362.1	*Enterococcus gallinarum* strain L2002	1474	Vivero et al. 2020
MT277424.1	*Priestia endophytica* strain AE4	1474	Rodrigo et al. 2020
MT279467.1	*Acinetobacter* sp. strain LE8	1473	Rodrigo et al. 2020
MT277091.1	*Enterobacter sp. strain AE1*	1472	Rodrigo et al. 2020
MT279465.1	*Acinetobacter sp. strain LE7*	1472	Rodrigo et al. 2020
MT540255.1	*Acinetobacter nosocomialis strain AE6*	1472	Rodrigo et al. 2020
MT540024.1	*Pantoea dispersa strain AE5*	1469	Rodrigo et al. 2020
MT279472.1	*Acinetobacter sp. strain LE10*	1466	Rodrigo et al. 2020
MT277412.1	*Enterobacter sp. strain AE3*	1458	Rodrigo et al. 2020
MT279356.1	*Microbacterium paraoxydans strain CEL2*	1455	Rodrigo et al. 2020
JN201944.1	*Sphingomonas sp. JL3*	1449	Lindh et al. 2012
MT052365.1	*Bacillus aerius strain L2004*	1420	Vivero et al. 2020
MT052367.1	*Staphylococcus epidermidis strain L2007*	1420	Vivero et al. 2020
MT052358.1	*Bacillus safensis strain L4001*	1415	Vivero et al. 2020
MT052366.1	*Bacillus aerius strain L2005*	1415	Vivero et al. 2020
KP717398.1	*Bacillus cereus strain BAL34*	1411	Yadav et al. 2015
KP717414.1	*Lysinibacillus fusiformis strain BAE16*	1409	Yadav et al. 2015
KP717400.1	*Lysinibacillus fusiformis strain BAL 24*	1408	Yadav et al. 2015
KP717401.1	*Staphylococcus hominis strain BAL40*	1408	Yadav et al. 2015
KP717412.1	*Bacillus aryabhattai strain BAE14*	1408	Yadav et al. 2015
MT052361.1	*Enterobacter soli strain L2001*	1408	Vivero et al. 2020
KP717397.1	*Bacillus aryabhattai strain BAL 14*	1407	Yadav et al. 2015
FJ372766.1	*Serratia sp. I22*	1403	Gusmao et al. 2010
MT052363.1	*Serratia grimesii strain L4004*	1403	Vivero et al. 2020
KP717413.1	*Bacillus aerophilus strain BAE35*	1402	Yadav et al. 2015
FJ372767.1	*Serratia sp. I23*	1398	Gusmao et al. 2010
KP717403.1	*Enterobacter cloacae strain BAE1*	1397	Yadav et al. 2015
KP717403.1	*Enterobacter cloacae strain BAE1*	1397	Yadav et al. 2015

Acc. Number	Spesies	Sequence Size (bp)	References
KP717406.1	*Klebsiella pneumoniae strain BAE25*	1395	Yadav et al. 2015
KP717399.1	*Bacillus tequilensis strain BAL19*	1392	Yadav et al. 2015
KP717399.1	*Bacillus tequilensis strain BAL19*	1392	Yadav et al. 2015
KP717392.1	*Pseudomonas aeruginosa strain BAL13*	1387	Yadav et al. 2015
KP717411.1	*Stenotrophomonas maltophilia strain BAE29*	1386	Yadav et al. 2015
MT052364.1	*Chryseobacterium oncorhynchi strain L2003*	1386	Vivero et al. 2020
KP717396.1	*Alcaligenes faecalis strain BAL33*	1384	Yadav et al. 2015
KP717409.1	*Pseudomonas mosselii strain BAE27*	1384	Yadav et al. 2015
MT052359.1	*Chryseobacterium oncorhynchi strain L4002*	1383	Vivero et al. 2020
KP717393.1	*Pseudomonas monteilii strain BAL37*	1381	Yadav et al. 2015
KP717388.1	*Enterobacter hormaechei strain BAL26*	1374	Yadav et al. 2015
KP717408.1	*Pseudomonas monteilii strain BAE34*	1381	Yadav et al. 2015
KP717388.1	*Enterobacter hormaechei strain BAL26*	1374	Yadav et al. 2015
KP717404.1	*Enterobacter xiangfangensis strain BAE23*	1374	Yadav et al. 2015
KP717391.1	*Klebsiella michiganensis strain BAL29*	1373	Yadav et al. 2015
KP717394.1	*[Pseudomonas] geniculata strain BAL31*	1372	Yadav et al. 2015
KP717395.1	*Acinetobacter pittii strain BAL43*	1367	Yadav et al. 2015
KP717416.1	*Micrococcus yunnanensis strain BAE13*	1356	Yadav et al. 2015
KP717402.1	*Elizabethkingia anophelis strain BAL36*	1355	Yadav et al. 2015
KP717389.1	*Enterobacter asburiae strain BAL27*	1352	Yadav et al. 2015
KP717415.1	*Staphylococcus hominis strain BAE39*	1351	Yadav et al. 2015
FJ372764.1	*Serratia sp. I17*	1343	Gusmao et al. 2010
KP717407.1	*Pantoea dispersa strain BAE21*	1343	Yadav et al. 2015
KP717387.1	*Enterobacter cloacae strain BAL1*	1340	Yadav et al. 2015
KP717405.1	*Klebsiella michiganensis strain BAE24*	1310	Yadav et al. 2015
FJ372768.1	*Bacillus sp. I24*	1301	Gusmao et al. 2010
KP717390.1	*Klebsiella oxytoca strain BAL28*	1283	Yadav et al. 2015
FJ372771.1	*Bacillus sp. I28*	1280	Gusmao et al. 2010
KP717410.1	*Aeromonas veronii strain BAE28*	1274	Yadav et al. 2015
FJ372772.1	*Enterococcus sp. I34*	1271	Gusmao et al. 2010
FJ372763.1	*Klebsiella sp. I12*	1245	Gusmao et al. 2010
MT277459.1	*Acinetobacter baumannii strain AE7*	1214	Rodrigo et al. 2020
DQ855292.1	*Pantoea agglomerans strain AE10*	1023	Apte-Deshpande and Deobagkar 2006
KU096887.1	*Elizabethkingia sp. VV11*	1022	David et al. 2016
DQ855290.1	*Pseudomonas alcaligenes strain AE8*	1021	Apte-Deshpande and Deobagkar 2006
DQ855287.1	*Aeromonas salmonicida strain AE5*	1016	Apte-Deshpande and Deobagkar 2006
KU096883.1	*Elizabethkingia sp. VV3*	1012	David et al. 2016
KU096886.1	*Elizabethkingia sp. VV10*	1012	David et al. 2016
DQ855293.1	*Edwardsiella tarda strain AE11*	1011	Apte-Deshpande and Deobagkar 2006
DQ855291.1	*Burkholderia mallei strain AE9*	1004	Apte-Deshpande and Deobagkar 2006
FJ372760.1	*Klebsiella sp. I5*	1004	Gusmao et al. 2010

Acc. Number	Spesies	Sequence Size (bp)	References
KU096884.1	*Elizabethkingia sp. VV8*	999	David et al. 2016
KU096906.1	*Microbacterium sp. VV42*	994	David et al. 2016
KU096893.1	*Enterobacter sp. VV6*	993	David et al. 2016
DQ855289.1	*Aeromonas hydrophila strain AE7*	992	Apte-Deshpande and Deobagkar 2006
KU096894.1	*Enterobacter sp. VV*	992	David et al. 2016
DQ855294.1	*Brevibacillus agri strain AE12*	991	Apte-Deshpande and Deobagkar 2006
KU096892.1	*Enterobacter sp. VV5*	990	David et al. 2016
DQ855295.1	*Bacillus cereus strain AE13*	983	Apte-Deshpande and Deobagkar 2006
KU096888.1	*Elizabethkingia sp. VV59*	982	David et al. 2016
KU096889.1	*Elizabethkingia sp. VV60*	982	David et al. 2016

Table 2. 16S rDNA Sequences Collection of Non-Culturable Bacteria from NCBI Database

Acc. Number	Spesies	Sequence Size (bp)	References
KF672364.1	*Uncultured bacterium clone ss1*	1545	Hill et al. 2014
HQ873693.1	*Uncultured bacterium clone M97B*	1156	Charan et al. 2011
HQ873696.1	*Uncultured bacterium clone M03B*	1144	Charan et al. 2011
HQ873682.1	*Uncultured bacterium clone R48B*	1135	Charan et al. 2011
HQ873679.1	*Uncultured bacterium clone R101B*	1131	Charan et al. 2011
HQ873680.1	*Uncultured bacterium clone R03B*	1125	Charan et al. 2011
HQ873691.1	*Uncultured bacterium clone M78B*	1122	Charan et al. 2011
HQ873688.1	*Uncultured bacterium clone S56B*	1116	Charan et al. 2011
HQ873681.1	*Uncultured bacterium clone R90B*	1112	Charan et al. 2011
HQ873695.1	*Uncultured bacterium clone M40B*	1103	Charan et al. 2011
HQ873687.1	*Uncultured bacterium clone S37B*	1035	Charan et al. 2011
KY041048.1	*Uncultured bacterium clone 5BBF_4A_94*	1007	Suryavanshi and Charan, 2017
KY041104.1	*Uncultured bacterium clone 1BBF_3A_64*	1007	Suryavanshi and Charan, 2017
HQ873689.1	*Uncultured bacterium clone S48B*	1000	Charan et al. 2011
KY040891.1	*Uncultured bacterium clone HTC_0_29*	989	Suryavanshi and Charan, 2017
KY040889.1	*Uncultured bacterium clone HTC_0_19*	988	Suryavanshi and Charan, 2017
KY040895.1	*Uncultured bacterium clone HTC_0_56*	986	Suryavanshi and Charan, 2017
KY040886.1	*Uncultured bacterium clone HTC_0_11*	984	Suryavanshi and Charan, 2017
KY040887.1	*Uncultured bacterium clone HTC_0_15*	982	Suryavanshi and Charan, 2017
KY040890.1	*Uncultured bacterium clone HTC_0_22*	981	Suryavanshi and Charan, 2017
KY040898.1	*Uncultured bacterium clone HTC_0_81*	980	Suryavanshi and Charan, 2017
KY040888.1	*Uncultured bacterium clone HTC_0_18*	977	Suryavanshi and Charan, 2017
KY040894.1	*Uncultured bacterium clone HTC_0_43*	977	Suryavanshi and Charan, 2017

Acc. Number	Spesies	Sequence Size (bp)	References
HQ873685.1	*Uncultured bacterium clone S69A*	976	Charan et al. 2011
HQ873686.1	*Uncultured bacterium clone S21A*	976	Charan et al. 2011
HQ873683.1	*Uncultured bacterium clone S47A*	974	Charan et al. 2011
KY040901.1	*Uncultured bacterium clone HTC_0_86*	970	Suryavanshi and Charan, 2017
KY041209.1	*Uncultured bacterium clone 3ABF_6_34*	970	Suryavanshi and Charan, 2017
HQ873684.1	*Uncultured bacterium clone S46A*	969	Charan et al. 2011
KY041254.1	*Uncultured bacterium clone 3ABF_6_84*	969	Charan et al. 2011
KY041269.1	*Uncultured bacterium clone 5ABF_7_8*	969	Suryavanshi and Charan, 2017
KY041369.1	*Uncultured bacterium clone 7ABF_8A_42*	969	Suryavanshi and Charan, 2017
KY041421.1	*Uncultured bacterium clone 7ABF_8B_42*	969	Suryavanshi and Charan, 2017
KY041517.1	*Uncultured bacterium clone 10ABF_A_96*	969	Suryavanshi and Charan, 2017
KY041524.1	*Uncultured bacterium clone 10ABF_B_12*	969	Suryavanshi and Charan, 2017
KY041527.1	*Uncultured bacterium clone 10ABF_B_18*	969	Suryavanshi and Charan, 2017
KY041530.1	*Uncultured bacterium clone 10ABF_B_25*	969	Suryavanshi and Charan, 2017
KY041197.1	*Uncultured bacterium clone 3ABF_6_20*	968	Suryavanshi and Charan, 2017
KY041353.1	*Uncultured bacterium clone 7ABF_8A_17*	968	Suryavanshi and Charan, 2017
KY041362.1	*Uncultured bacterium clone 7ABF_8A_33*	968	Suryavanshi and Charan, 2017
KY041392.1	*Uncultured bacterium clone 7ABF_8A_88*	968	Suryavanshi and Charan, 2017
KY041405.1	*Uncultured bacterium clone 7ABF_8B_15*	968	Suryavanshi and Charan, 2017
KY041414.1	*Uncultured bacterium clone 7ABF_8B_32*	968	Suryavanshi and Charan, 2017
KY041445.1	*Uncultured bacterium clone 7ABF_8B_86*	968	Suryavanshi and Charan, 2017
KY041325.1	*Uncultured bacterium clone 5ABF_7_71*	967	Suryavanshi and Charan, 2017
KY041063.1	*Uncultured bacterium clone 5BBF_4B_20*	966	Suryavanshi and Charan, 2017
KY041069.1	*Uncultured bacterium clone 5BBF_4B_28*	966	Suryavanshi and Charan, 2017
KY041114.1	*Uncultured bacterium clone 1BBF_3A_80*	966	Suryavanshi and Charan, 2017
KY041120.1	*Uncultured bacterium clone 1BBF_3A_88*	966	Suryavanshi and Charan, 2017
KY041232.1	*Uncultured bacterium clone 3ABF_6_58*	966	Suryavanshi and Charan, 2017
KY041382.1	*Uncultured bacterium clone 7ABF_8A_68*	966	Suryavanshi and Charan, 2017

Acc. Number	Spesies	Sequence Size (bp)	References
KY041395.1	*Uncultured bacterium clone 7ABF_8A_93*	966	Suryavanshi and Charan, 2017
KY041435.1	*Uncultured bacterium clone 7ABF_8B_66*	966	Suryavanshi and Charan, 2017
KY041448.1	*Uncultured bacterium clone 7ABF_8B_91*	966	Suryavanshi and Charan, 2017
KY041479.1	*Uncultured bacterium clone 10ABF_A_41*	966	Suryavanshi and Charan, 2017
KY040900.1	*Uncultured bacterium clone HTC_0_85*	965	Suryavanshi and Charan, 2017
KY040939.1	*Uncultured bacterium clone 7BBF_3A_40*	965	Suryavanshi and Charan, 2017
KY041057.1	*Uncultured bacterium clone 5BBF_4B_14*	965	Suryavanshi and Charan, 2017
KY041196.1	*Uncultured bacterium clone 3ABF_6_19*	965	Suryavanshi and Charan, 2017
KY041351.1	*Uncultured bacterium clone 7ABF_8A_13*	965	Suryavanshi and Charan, 2017
KY041360.1	*Uncultured bacterium clone 7ABF_8A_31*	953	Suryavanshi and Charan, 2017
KY041370.1	*Uncultured bacterium clone 7ABF_8A_44*	965	Suryavanshi and Charan, 2017
KY041379.1	*Uncultured bacterium clone 7ABF_8A_62*	965	Suryavanshi and Charan, 2017
KY041384.1	*Uncultured bacterium clone 7ABF_8A_73*	965	Suryavanshi and Charan, 2017
KY041388.1	*Uncultured bacterium clone 7ABF_8A_80*	965	Suryavanshi and Charan, 2017
KY041403.1	*Uncultured bacterium clone 7ABF_8B_10*	965	Suryavanshi and Charan, 2017
KY041409.1	*Uncultured bacterium clone 7ABF_8B_24*	965	Suryavanshi and Charan, 2017
KY041422.1	*Uncultured bacterium clone 7ABF_8B_44*	965	Suryavanshi and Charan, 2017
KY041432.1	*Uncultured bacterium clone 7ABF_8B_61*	965	Suryavanshi and Charan, 2017
KY041437.1	*Uncultured bacterium clone 7ABF_8B_70*	965	Suryavanshi and Charan, 2017
KY041441.1	*Uncultured bacterium clone 7ABF_8B_77*	965	Suryavanshi and Charan, 2017
KC484895.1	*Uncultured bacterium clone Sam7A45*	964	Charan et al. 2013
KY040918.1	*Uncultured bacterium clone 7BBF_3A_17*	964	Suryavanshi and Charan, 2017
KY041058.1	*Uncultured bacterium clone 5BBF_4B_15*	964	Suryavanshi and Charan, 2017
KY041304.1	*Uncultured bacterium clone 5ABF_7_45*	964	Suryavanshi and Charan, 2017
KY041337.1	*Uncultured bacterium clone 5ABF_7_88*	964	Suryavanshi and Charan, 2017
KY041339.1	*Uncultured bacterium clone 5ABF_7_90*	964	Suryavanshi and Charan, 2017

Acc. Number	Spesies	Sequence Size (bp)	References
KY041356.1	*Uncultured bacterium clone 7ABF_8A_24*	964	Suryavanshi and Charan, 2017
KY041408.1	*Uncultured bacterium clone 7ABF_8B_22*	964	Suryavanshi and Charan, 2017
KY041497.1	*Uncultured bacterium clone 10ABF_A_64*	964	Suryavanshi and Charan, 2017
KY041507.1	*Uncultured bacterium clone 10ABF_A_76*	964	Suryavanshi and Charan, 2017
KY040905.1	*Uncultured bacterium clone 7BBF_3A_4*	963	Suryavanshi and Charan, 2017
KY040986.1	*Uncultured bacterium clone 5BBF_4A_3*	963	Suryavanshi and Charan, 2017
KY041013.1	*Uncultured bacterium clone 5BBF_4A_32*	963	Suryavanshi and Charan, 2017
KY041027.1	*Uncultured bacterium clone 5BBF_4A_53*	963	Suryavanshi and Charan, 2017
KY041035.1	*Uncultured bacterium clone 5BBF_4A_65*	963	Suryavanshi and Charan, 2017
KY041055.1	*Uncultured bacterium clone 5BBF_4B_11*	963	Suryavanshi and Charan, 2017
KY041060.1	*Uncultured bacterium clone 5BBF_4B_17*	963	Suryavanshi and Charan, 2017
KY041074.1	*Uncultured bacterium clone 1BBF_3A_3*	963	Suryavanshi and Charan, 2017
KY041098.1	*Uncultured bacterium clone 1BBF_3A_55*	963	Suryavanshi and Charan, 2017
KY041111.1	*Uncultured bacterium clone 1BBF_3A_77*	963	Suryavanshi and Charan, 2017
KY041180.1	*Uncultured bacterium clone 3ABF_6_3*	963	Suryavanshi and Charan, 2017
KY041224.1	*Uncultured bacterium clone 3ABF_6_49*	963	Suryavanshi and Charan, 2017
KY041229.1	*Uncultured bacterium clone 3ABF_6_54*	963	Suryavanshi and Charan, 2017
KY041249.1	*Uncultured bacterium clone 3ABF_6_78*	963	Suryavanshi and Charan, 2017
KY041330.1	*Uncultured bacterium clone 5ABF_7_77*	963	Suryavanshi and Charan, 2017
KY041371.1	*Uncultured bacterium clone 7ABF_8A_48*	963	Suryavanshi and Charan, 2017
KY041373.1	*Uncultured bacterium clone 7ABF_8A_50*	963	Suryavanshi and Charan, 2017
KY041375.1	*Uncultured bacterium clone 7ABF_8A_57*	963	Suryavanshi and Charan, 2017
KY041386.1	*Uncultured bacterium clone 7ABF_8A_75*	963	Suryavanshi and Charan, 2017

Materials and equipment

This research utilized both hardware and software. The hardware used was an ASUS FHD352 Laptop with an Intel Core i3-1005G1 3.4 GHz processor, 4GB DDR4 RAM, 512GB SSD, and NVIDIA GeForce MX330. The software used was Notepad for saving the sequence file. Bioin-

formatics databases provided by the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and Pathosystems Resource Integration Center (PATRIC) (https://patricbrc.org/) were also involved. The materials used in this study were bacterial 16S rDNA sequence data from *Ae. aegypti* midgut obtained from the NCBI database.

Research methods

The research method in this study can be seen in the Figure 1. The detailed research procedure is described as follow:

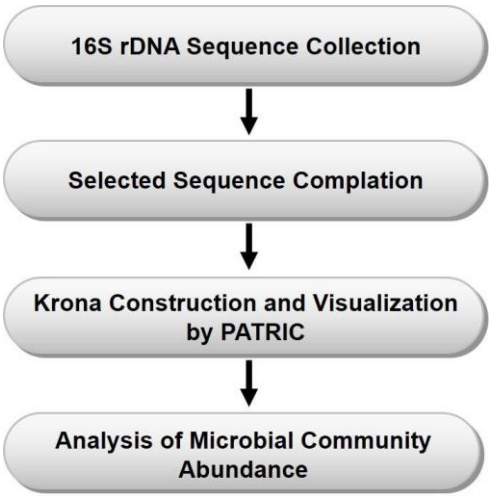

Figure 1. Research procedure for in-silico analysis of symbiont bacteria diversity from the midgut of *Aedes aegypti* based on 16S rDNA molecular markers database

16S rDNA sequence collection

Initially, 16S rDNA sequences for this study were obtained from the NCBI database. The data were collected using the keyword "bacteria midgut *Aedes aegypti* 16S rDNA." The collect-ed 16S rDNA sequences were saved in a notepad file.

Selected sequence compilation

The 16S rDNA sequences collected from the *Ae. aegypti* midgut in the NCBI database were compiled into a single notepad file with a .txt format (Garg et al. 2016). This data was then converted into. fasta format forfurther analysis.

Krona construction and visualization

Krona construction was performed using PATRIC, a bioinformatics information center for bacterial data, established by the National Institute of Allergy and Infectious Diseases (NIAID). PATRIC provided gene sequence data and analysis for studying pathogens. This website tool can be used to perform bioinformatics analysis, such as microbial community abundance based on an in-silico approach (Wattam et al. 2017). The analysis was performed on the 16S rDNA sequence data from the *Ae. aegypti* midgut accessed through PATRIC. The analysis was con-ducted using the Kraken 2 Algorithm. The results of the analysis were presented in a pie chart called Krona, which visualizes the metagenomic composition of the microbe (Gaio et al. 2021). Krona is an interactive metagenomic visualization platform that allows exploration of bacterial abundance data through metagenomic classification hierarchies. Additionally, Krona provides the composition of bacterial taxa based on sequence data in the bioinformatics database, reporting the percentage of bacterial taxa by referring to the NCBI database.

Analysis of microbial abundance

Krona displayed a pie chart of taxa in various regions with specific hierarchies. Thedata display consisted of circles with different color gradations to signify different hierarchies and microbial abundance (Ondov et al. 2011). The inner circle represented the highest taxonomic hierarchy, while the outer circle represented taxa at lower hierarchies. Different colors were used to indicate variousbacterial taxa, and

the abundance of each bacterium was represented by the percentage of metagenomic data obtainedfrom the bioinformatics database.

RESULTS

The collection of 16S rDNA sequence

16S rDNA sequence data was gatheredfrom the NCBI, a comprehensive open-accessbioinformatics database known for its substantial collection of nucleotide sequences(Sayers et al. 2021). A collection of 16S rDNA sequences was obtained using the query "bacteria midgut *Aedes aegypti* 16S rDNA" with a lengthy filter to identify the longest sequence results. This approach aimed to obtain data that is close to full length in the 16S rDNA sequence region. The intact 16S rDNA sequence is approximately 1600 base pairs (bp) long, encompassing ninehypervariable regions (V1–V9). The 16S rDNA molecular marker is commonly used for bacterial characterization due to its conservation and universality among prokaryotic organisms, including bacteria andarchaea. The universality of this marker is observed in numerous prokaryotes. The analysis revealed a distinct and conserved hyper-variable region consisting of nine regions within the 16S rDNA sequence, whichremained intact across prokaryotic generations(Santos et al. 2020).

Clustering bacterial diversity

Bacterial diversity was clustered using PATRIC, and taxonomic classification was applied to group sequence data based on kinship. Taxa refer to metagenomic data. This analysis employed the Kraken 2 algorithm to identify k-mers as indicators of taxonomic units (Davis et al. 2020). The top 100 data sequences were retrieved from NCBI, prioritizing those with the most complete nucleotide count, considering the 16S rDNA molecular marker with a total length of 1600 bp (Santos et al. 2020). The research estimated that sequences closer to theoptimal size would yield higher-quality results. The clustering process led to the classificationof culturable and non-culturable bacteria.

Culturable bacteria

The collection of culturable bacteria documented the 100 longest sequences fromthe NCBI database. The classification of culturable bacteria revealed that the most dominant phylum was Protobacteria, accounting for 62% of the total Krona. This phylum is represented by red areas. The nextdominant phylum was the Terrabacteria clad group, comprising 29% of all Krona and appearing as green areas. The blue areas corresponded to FCB bacterial taxa or the phylum Sphingobacteria, with an abundance of 9% (Figure 2). Within the Protobacteria phylum, the Class Gammaproteobacteriashowed the highest abundance at 57% (Figure 2b). This class was further divided into orders, namely Enterobacterales and Pseudomonadales. Enterobacterales was themost abundant order identified, accounting for37% of the total class (Figure 3a). The order Enterobacterales consists of the family Enterobacteriaceae, known for its highest abundance at 35% (Figure 3b). The most dominant genus and species were *Salmonella* and *Salmonella enterica*, representing 17% of the entire class (Figure 4a and 4b).

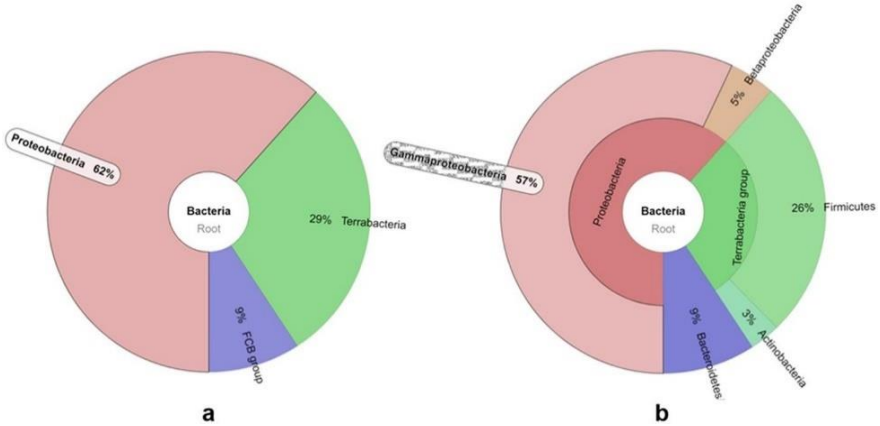

Figure 2. Krona of symbiont bacteria from *Ae. aegypti* midgut within culturable bacteria at the level of: a. Phylum; b. Class

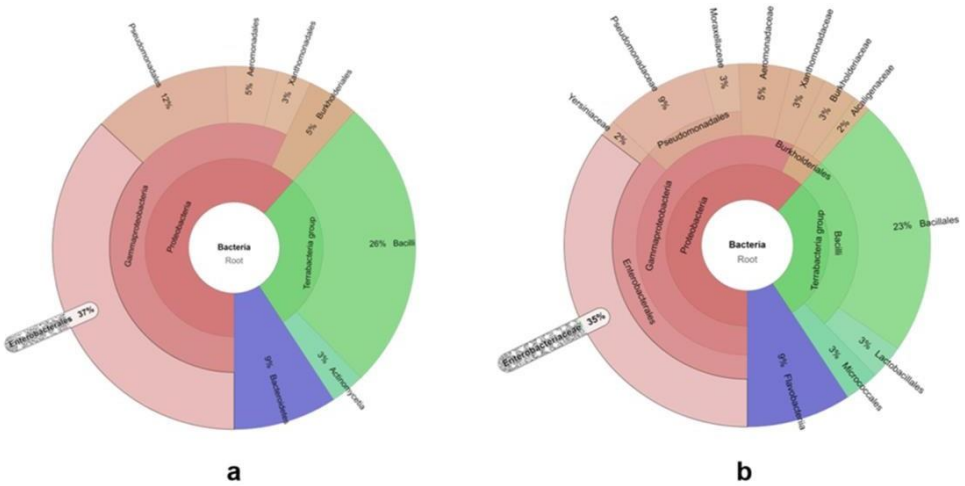

Figure 3. Krona of symbiont bacteria from *Ae. aegypti* midgut within culturable bacteria at the level of: a. Ordo; b.Famili

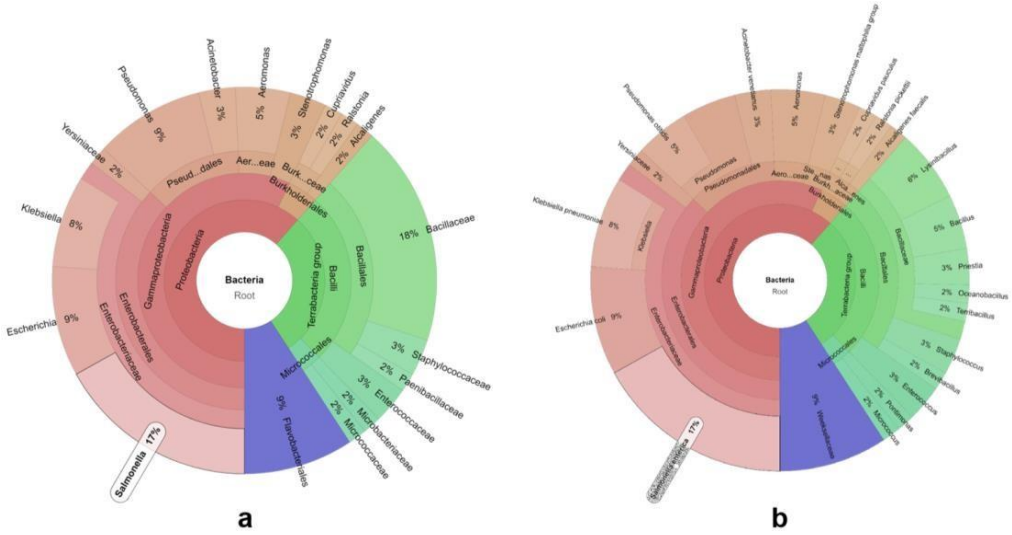

Figure 4. Krona of symbiont bacteria from *Ae. aegypti* midgut within culturable bacteria at the level of: a. Genus; b. Species

Non-culturable bacteria

The sequence data used to identify non-culturable bacteria consisted of the 100 longest sequences obtained from the NCBI database. All sequences were recorded in a notepad file and converted to. fasta format. The analysis results were visualized using Krona. Among the Krona categories, the most dominant phylum was Protobacteria, accounting for 88% of the total Krona and marked by red areas. The Terrabacteria clad group, marked by green areas, was the sec- ond most abundant bacteria, represent-ing 9%of the total Krona. The taxon FCB group or Sphin-gobacteria, indicated by the blue area, had an abundance of 2%. The phylum Verrucomicrobia, marked by the purple area, was the least abundant, com-prising 1% of the total Krona (Figure 5a). Within the Protobacteria phylum, the class Gammaproteobacteria exhibited the highest abundance at 71% (Figure 5b). This class was further divided into two orders: Entero-bacterales and Pseudomonadales. Among these, Enterobacterales was the mostcom-mon order, account-ing for 37% of the total (Figure 6a). The order Enterobacterales in-cluded the family Enterobacte-riaceae, which had the highest abundance of 35% (Figure 6b). The dominant taxa at the genus and species levels were Salmonella and Salmonella enterica, respectively, with abundances of 17% each (Figure 7a and 7b).

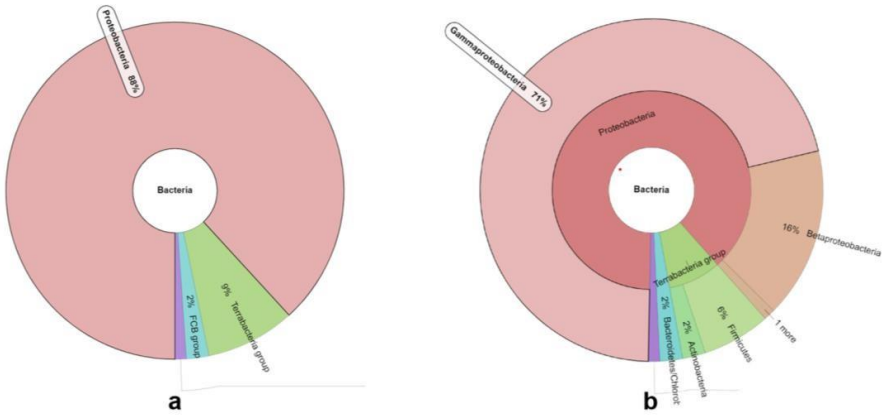

Figure 5. Krona of symbiont bacteria from *Ae. aegypti* midgut within non-culturable bacteria at the level of: a. Phylum; b. Clas

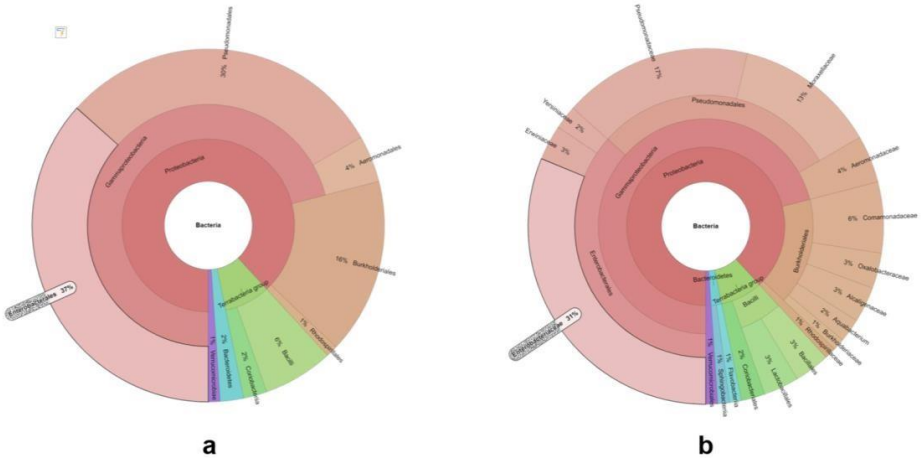

Figure 6. Krona of symbiont bacteria from *Ae. aegypti* midgut within non-culturable bacteria at the level of: a. Ordo;b. Famili

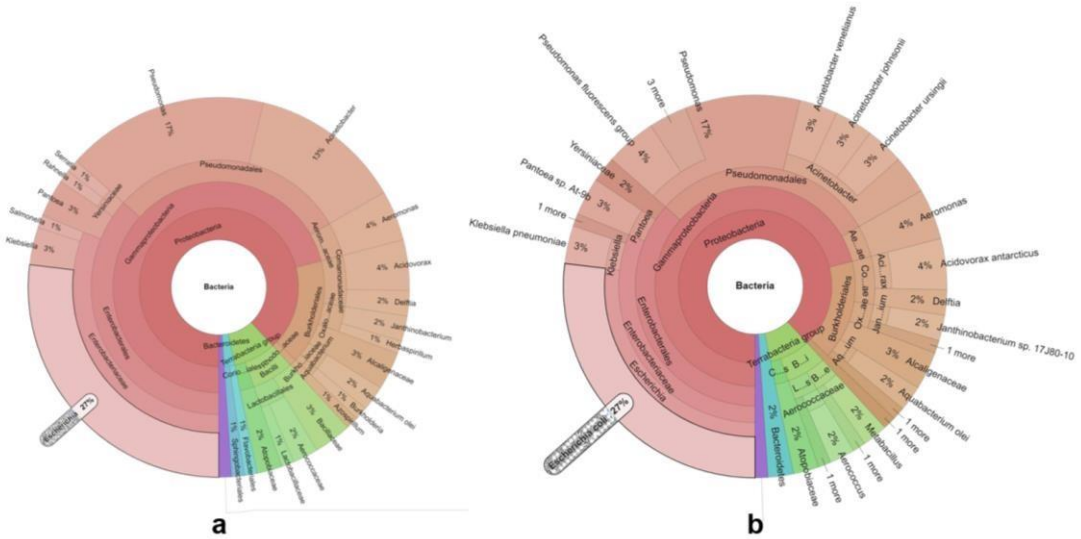

Figure 7. Krona of symbiont bacteria from *Ae. aegypti* midgut within non-culturable bacteria at the level of: a. Genus; b. Species

DISCUSSION

The analysis revealed that the database primarily consisted of taxa from the phylum Proteobacteria. The majority of Proteobacteria taxa were found in the midgut of *Ae. aegypti* (Kozlova et al. 2021). Krona categorization of culturable bacteria identified differences in the taxa, including the presence of the order Flavobacteriales and genus *Elizabethkingia*, which were only found in culturable bacteria. These bacteria were predominantly present in laboratory-reared mosquitoes. Variationswere also observed in the families of taxa between culturable and non-culturable bacteria. For example, the Bacilli class accounted for 6% of the total taxa in non- culturable bacteria, while it comprised 26% of the taxa in culturable bacteria. Krona analysis further demonstrated differences in the diversity of symbiont bacteria in the mosquito midgut, influenced by factors such as species, sex, habitat, and food sources (Terenius et al. 2012). Food sources, particularly sugar or blood, playeda role in shaping the abundance and diversity of symbiont bacteria in the midgut. Sugar-rich diets, high in carbohydrates, and blood meals, rich in proteins, created different environmental conditions in the midgut of different mosquitoes, resulting in distinct taxa profiles (Wu et al. 2019). Additionally, variations in blood sources based on different blood types affected the abundanceand diversity of bacterial taxa, such as Pseudomonas and Serratia, which were more abundant in mosquitoes feeding on human blood compared to other mammalian blood sources (Sarma et al. 2022).

The composition of symbiont bacteria is influenced not only by the vector itself but also by the characteristics of the symbiont bacteria. Enterobacterales and Serratia were the most commonly found taxa in the mosquito midgut. The population of the Enterobacterales and Serratia genera tended to increase when mosquitoes fed on blood, possibly due to these bacteria'sability to withstand oxidative stress in theblood meal (Wang et al. 2011). Bacterial activity, particularly antagonisticinteractions, also played a role in shaping the bacterial communities. For example, Cedecea bacteria inhibited Serratia, while Serratia infections inhibited Asia infections in mosquito bodies (Kozlova et al. 2021).

The genera of symbiont bacteria knownto influence the mosquito's life cycle belong tothe phylum Proteobacteria, class Gammaproteobacteria, and order Enterobacterales. Based on Krona visualization, the most abundant bacterial taxain both culturable and non-culturable bacteriaincluded Serratia, Enterobacter, and Escherichia. However, bacterial taxa not belonging to the order Enterobacterales, suchas Wolbachia, Proteus, and Chromobacterium, were also identified. Some of these symbiontbacteria taxa were capable of influencing the transmission of pathogens in mosquitoes. Forinstance, *Serratia marcescens* inhibitedmosquito development by secretingSmEnhancin protein, rendering *Ae. aegypti* more susceptible to DENV infection. *Serratia odorifera* increased the suscepbility of *Ae.aegypti* to DENV-2 (Apte-Deshpande et al. 2012). Wolbachia strain Wmel blocked mosquito-borne viruses like DENV, Chikungunya, Zika, and yellow fever, and reduced mosquito lifespan (Gao et al. 2020). Wolbachia strain wAlbB increased ROS production, triggering one of the mosquito's immune response pathways and reducing DENV infection (Pan et al. 2012). Chromobacterium secreted AMP compounds, which degraded DENV protein and preventedDENV infection (Saraiva et al. 2018). Proteussp. was known to enhance resistance to DENVby regulating AMP (Wu et al. 2019). Several bacterial species played important roles in transmitting DHF by affecting *Ae. aegypti* as theDHF vector, as shown in Table 3.

The analysis results documented multiple symbiont bacteria in the mosquito midgut capable of influencing *Ae. aegypti*'s ability as a DENV vector. Wolbachia bacteria demonstrated the strongest potential for blocking arbovirus transmission, increasing susceptibility to DENV, increasing ROS and AMP secretion, and acting as biocontrol agents to control *Ae. aegypti* in Indonesia (Apte-Deshpande et al. 2012, Gao et al. 2020, Tantowijoyo et al. 2020). Another symbiotic bacterium in the midgut of *Ae. aegypti* with promising potential is the genus Serratia, known for its roles in vector control (Gao et al. 2020). Serratia belongs to the order Enterobacterales and although it may

not be the most abundant species, it has the highest abundance among other orders. The analysis acknowledged *Escherichia coli* and *Salmonella enterica* as the two most abundant species within this order. *Escherichia coli* and *Salmonella enterica* are also bacterial species that belong to the predominant taxa in the orderEnterobacterales.

Table 3. The Species, Potentials and Roles of Symbiont Bacteria

Spesies	Potentials and Roles	References
Serratia odorifera	enhancing mosquito vulnerability to DENV-2 infection	Wang et al. 2011
S. marcescens	producing SmEnhancin protein	Pan et al. 2012
Wolbachia wAlbB	triggering the production of ROS	Apte-Deshpande et al. 2012
Wolbachia Wmel	reducing the life span of a mosquito	Coon et al. 2017
Serratia spp. and *Enterobacter* sp.	maintaining hemolytic activity to facilitate blood feeding	Saraiva et al. 2018
Escherichia coli	Influencing the development of mosquitoes during the larval phase	Wu et al. 2019
Chromobacterium	secreting secondary metabolites that inhibit DENV Infection	Gao et al. 2020

CONCLUSION

The analysis of 16S rDNA data, incorporating both culturable and non- culturable bacteria, was facilitated by Krona for data presentation. The results reveal that the midgut of *Ae. aegypti* exhibits similar dominant taxa abundance at the phylum to family levels, albeit with varying percentage values. The dominant taxa identified in this study include Proteobacteria, Gammaproteobacteria, Enterobacterales, andEnterobacteriaceae, among others, at the levels of phylum, class, order, and family, respectively. Notably, within the category of culturable bacteria, the dominant taxa are observed at the genus level, specifically *Salmonella*, with *Salmonella enterica* being the most prevalent species. Conversely, in the category of non-culturable bacteria, the dominant taxa at the genus level are *Escherichia*, with *Escherichia coli* being the prevailing species. Serratia, belonging to the order Enterobacterales, demonstrates the most promising potential in controlling *Ae. aegypti*, despite not being the most dominantspecies.

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