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Isolation and Identification of Airborne Bacteria Inside Swiftlet Houses in Sarawak, Malaysia

Leong Sui Sien[1*], Chia Hwa Chuan[1], Samuel Lihan[1], and Ling Teck Yee[2]

1. Department of Molecular Microbiology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
2. Department of Chemistry, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

[*]*e-mail: leongsuisien87@gmail.com*

Abstract

Air consists of such microorganisms as bacteria, fungi, and viruses. Exposure to these airborne bacteria indoors may cause infectious and noninfectious adverse health effects. However, the sources and origins of bacteria are not fully understood. The aim of this study was to isolate and identify the bacteria present in the air inside swiftlet houses located in Kota Samarahan, Saratok, Betong, Maludam, Miri, Kuching, Semarang, Sepinang, Sarikei, and Sibu in Sarawak, Malaysia. A total of 100 bacterial isolates from 20 samples were collected from swiftlet houses. The bacteria present in the air were collected using Plate Count Agar. Two plates were exposed at the front and back inside swiftlet houses for 15 sec and then incubated at 37 ± 1 ^{o}C for 24 h. The 16S rRNA analysis method was used to identify the isolates from the samples. The air inside the swiftlet houses had a total mean airborne bacteria colony count of 2.02 ± 0.72 $\log_{10}$ cfu/m^2/sec; the highest was in Miri (3.08 ± 0.29 $\log_{10}$ cfu/m^2/sec), and the lowest was in Sibu (1.05 ± 0.85 $\log_{10}$ cfu/m^2/sec). Twenty-seven bacteria species were identified, and *Lysinibacillus* sp. B4 (16%) was most frequently isolated.

Abstrak

Isolasi dan Identifikasi Bakteri yang terdapat di Udara dalam Rumah Walet di Sarawak, Malaysia. Udara mengandung mikroorganisme seperti bakteri, jamur , dan virus. Paparan bakteri di udara dapat menyebabkan efek kesehatan yang merugikan. Namun, sumber dan asal bakteri tidak sepenuhnya dipahami. Tujuan dari penelitian adalah mengisolasi dan mengidentifikasi bakteri yang terdapat di udara di dalam rumah-rumah walet yang terletak di Kota Samarahan, Saratok, Betong, Maludam, Miri, Kuching, Semarang, Sepinang, Sarikei dan Sibu di Sarawak, Malaysia. Sebanyak 100 isolat bakteri dari 20 sampel diperoleh dari rumah-rumah walet. Bakteri diisolasi menggunakan medium Plate Count Agar. Sebanyak dua cawan petri dibuka di bagian depan dan belakang dalam rumah walet selama 15 detik, dan diinkubasi pada suhu 37±1 ^{o}C selama 24 jam. Sequencing DNA gen 16S rRNA dilakukan untuk mengidentifikasi bakteri dari sampel. Udara di dalam rumah walet memiliki total rata-rata jumlah koloni 2.02±0.72 $\log_{10}$ cfu/m^2/detik dan CFU tertinggi diperoleh dari Miri (3.08 ± 0.29 $\log_{10}$ cfu/m^2/detik) dan terendah di Sibu (1.05 ± 0.85 $\log_{10}$ cfu/m^2/detik). Terdapat 27 species bakteri dari udara dalam ruangan di rumah walet, dan *Lysinibacillus* sp. (16%) paling banyak ditemukan.

Keywords: Swiftlet house, Air samples, Bacteria, Identification, 16S rRNA gene

1. Introduction

Air consists of tiny organisms such as bacteria, fungi, mycotoxins, and viruses. These small groups of organisms clump and survive in the air under high humidity [1]. Microorganisms are ubiquitous and can be transferred from the environment to everyday objects especially humans. Pathogenic microbes can be transmitted through air, skin, food, water, and other interpersonal contact [2]. Researchers have reported that exposure to pathogenic microbes may cause respiratory disorders, infections, hypersensitivity pneumonitis, and toxic reaction in infected humans [3]. Bacterial infection has been receiving increased attention in recent years

due to the increase in mortality and morbidity [4]. Swiftlet farming is defined as a modern and commercialized method of building birds' nests differently from the traditional cave-harvesting method. Malaysia is the third largest producer of edible birds' nests in the world, after Indonesia and Thailand [5]. An estimated 30,000 shop houses and commercial premises had been converted into swiftlet farms by June 2005 throughout Malaysia, especially northern Malaysia [6]. Researchers have reported that indoor air is mostly full of bacteria [4]. No research related to airborne bacteria inside swiftlet houses has been conducted. There are many methods for collecting airborne bacteria such as collection plates, electrostatic collectors and impactors, and mass spectrometers, which are suitable for research purposes [7]. More research should be done on airborne bacteria that may pose health risks to humans. Therefore, this study was conducted to isolate and identify the bacteria present in the air inside swiftlet houses.

2. Methods

Description of the study area. The sampling site areas were swiftlet houses located in Kota Samarahan, Saratok, Betong, Maludam, Miri, Kuching, Semarang, Sepinang, Sarikei, and Sibu in Sarawak, Malaysia.

Sample collection. Sampling and sample processing procedures for isolating airborne bacteria were carried out according to Malaysia Veterinary Health Air quality sampling [8]. Two Plate Count Agar plates (Oxoid, England) used to collect the airborne bacteria were placed at the front and back of each sampling site. The medium of the Plate Count Agar was exposed in the air for 15 sec. Then the cap of the agar plate was re-joined, sealed, and labeled. The agar plates were incubated at 37 ± 1 ^{o}C for 24 h. The airborne bacteria colony was counted according to Nyakundi and Mwangi (2011) and Tsai *et al*. (2002) [9-10]. The bacterial colonies formed on the medium were reported as the number of colony-forming units ($\log_{10}$ cfu/m^2/sec) [11]. Colonies were randomly picked for further identification.

Conventional biochemical tests. The bacteria isolated from the air samples were identified phenotypically using conventional biochemical tests according to Bergey's manual [12], involving Gram staining, the urease test, hydrogen sulfide test, motility test, indole (tryptone broth) test, lactose fermentation test, Simmons citrate test, starch hydrolysis test, carbohydrates fermentation test, methyl red-Voges-Proskauer test, bile-esculin test, oxidase test, catalase test, hemolysis test, and lysine decarboxylase test.

DNA extraction. After the bacterial genera were detected, bacterial DNA was extracted using the boiling method as described by Maria *et al.* (2008) [13] with minor modifications. The bacterial cultures were prepared by growing the bacteria in Luria broth (Scharlau, Spain) at 37 ± 1 °C for 24 h. One thousand and five hundred microliters of overnight culture was transferred into a 1.5 ml microcentrifuge tube and centrifuged at 10,000 rpm for 5 min. Then the supernatant was discarded. Five hundred microliters of sterile distilled water was added and vortexed to resuspend the cell pellet. The content of the microcentrifuge tubes was then boiled for 10 min together with the tubes, and then the tubes were immediately placed on ice for 5 min. After 5 min, the microcentrifuge tubes were centrifuged at 10,000 rpm for 10 min, and the supernatant was collected.

Polymerase chain reaction of the 16S rRNA gene. The genotypes of the bacterial isolates were characterized using gene 16S rRNA identification method with minor modification [14]. Primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG-3') were used to amplify the 16S rRNA gene [15-16]. The PCR reactions were performed in reaction mixtures containing 20 µl DNA, 1.0 µl each 20 pmol primers (First Base, Malaysia), 3 µl of 10 mM deoxynucleoside triphosphates mix (Promega, USA), 6.0 µl of 25 mM $MgCl_2$ (Promega, USA), 10.0 µl of 5X Buffer solution (Promega, USA), 8.0 µl of distilled water, and 1 µl of Taq polymerase (Promega, USA). PCR was performed as follows: initial denaturation at 95 °C for 10 min, denaturation at 94 °C for 30 sec, annealing at 55 °C for 1 min, primer extension 72 °C for 1.5 min with 35 cycles and final extension at 72 °C for 10 min. The PCR products were visualized with ethidium bromide staining after 1% agarose gel electrophoresis for 30 min at 90 V.

Sequencing analysis. The bacteria DNA was purified using the Qiagen sequencing purification kit (QIAquick Gel Extraction Kit, USA). The purified DNA product was sent to First Base Laboratories Sdn. Bhd. in Selangor, Malaysia, for the DNA sequencing process. All 16S rRNA sequences were compared using the BLAST program to determine the closest identity matches.

Statistical analysis. Statistical analysis of the data was conducted using the SPSS Statistics version 21.0 program. All data was analyzed for analysis of variance (ANOVA). All differences between the means were compared using the Tukey multiple range test after a significant F-test at $P < 0.05$.

3. Results and Discussion

Bacterial colony count. The mean total of the airborne viable bacteria counts inside the swiftlet houses is shown in Table 1. The table shows that the interior of

the swiftlet houses had a total mean airborne bacteria colony count of 2.02 ± 0.72 $\log_{10}$ cfu/m^2/sec. The mean airborne bacteria count was highest in Miri (3.08 ± 0.29 $\log_{10}$ cfu/m^2/sec) and lowest in Sibu (1.05 ± 0.85 $\log_{10}$ cfu/m^2/sec). The mean total bacterial colony counts collected from Miri were significantly ($P < 0.05$) higher than those collected from Sibu.

Occurrence of airborne bacteria inside swiftlet houses. The biochemical results showing the occurrence of the bacteria isolated and the 16S rRNA gene sequencing results of the bacteria isolated from the indoor air of the swiftlet houses are shown in Tables 2 and 3. The biochemical results showed 12 main bacterial genera among the indoor air bacteria isolated. The 16S rRNA gene sequencing results revealed that 27 bacterial species were isolated in the indoor air in the swiftlet houses: *Bacillus amyloliquefaciens* (8%), *Bacillus cereus* (10%), *Bacillus subtilis* (8%), *Bacillus* sp. 1 (5%), *Bacillus* sp. 2 (1%), *Corynebacterium* sp. (1%), *Dermacoccus* sp. (5%), *Enterococcus faecalis* (2%), *Escherichia coli* (3%), *Lysinibacillus* sp. (16%), *Micrococcus luteus* (2%), *Proteus penneri* (1%), *Roseomonas* sp. (2%), *Sphingobacterium* sp. (1%), *Sporosarcina aquimarina* (8%), *Sporosarcina* sp. (4%), *Staphylococcus kloosii* (2%), *Staphylococcus lentus* (5%), *Staphylococcus nepalensis* (7%), *Staphylococcus* sp. 1 (3%), *Staphylococcus* sp. 2 (3%), and *Staphylococcus* sp. 3 (3%) (Figure 1).

The types and number of airborne bacteria were investigated in ten areas in Sarawak. Airborne bacteria colonies were counted to measure the total number of microorganisms present in the air inside swiftlet houses. The total mean bacteria viable counts in the air inside swiftlet houses at the sampling sites was 2.02 ± 0.72 $\log_{10}$ cfu/m^2/sec (Table 1). The mean total airborne bacteria count was highest in Miri (3.08 ± 0.29 $\log_{10}$ cfu/m^2/sec) (Table 1). Miri is situated in northern Sarawak, which has higher temperatures than the southern and central Sarawak regions [17]. Temperature can affect the growth rate of various bacterial species [18]. According to Chan *et al.* (2005) [18], the growth rate of microorganisms doubled as the temperature increased. Airborne bacteria count could be a standard for determining the hygiene levels and housing regulations [19].

A total of 100 airborne bacterial were isolated, and 27 bacteria species were identified using 16S rRNA gene sequence analysis. Results of this preliminary microbiological examination of the airborne bacteria inside the swiftlet houses revealed that most of the bacteria isolated were a mixture of Gram-positive and -negative bacteria. Figure 1 shows that *Lysinibacillus* sp. (16%) was the most frequently isolated, followed by *B. cereus* (8%), *B. subtilis* (8%), *B. amyloliquefaciens* (8%), *S. aquimarina* (8%), and others.

Table 1. Mean Total Airborne Bacteria Viable Counts Inside the Swiftlet Houses at the Sampling Sites

Sites	Mean total of bacterial colony count ($\log_{10}$ cfu/ m^2/sec)
Kota Samarahan	$2.47 \pm 0.22^{ab*}$
Saratok	2.00 ± 0.82^{ab}
Betong	2.36 ± 0.90^{ab}
Maludam	1.98 ± 1.49^{ab}
Miri	3.08 ± 0.29^{b}
Kuching	1.38 ± 0.98^{ab}
Semarang	1.84 ± 0.56^{ab}
Sepinang	1.96 ± 0.86^{ab}
Sarikei	2.05 ± 0.21^{ab}
Sibu	1.05 ± 0.85^{a}
Total	2.02 ± 0.72

* The means with the same letter superscript are not significantly different at the 5% level.

Table 2. The Occurrence of Bacterial Isolates in Indoor Air in the Swiftlet Houses at the Sampling Sites

Bacteria	Morphology	GRM	CAT	SPO	STA	VP	MR	SWO	MOT	BE	LAC	IDL	GLU	ARA	MAN	OXI	GAS	SUSPECTED ORGANISM
1	Big Rod	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	*Bacillus* sp.
2	Rod	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	*Lysinibacillus* sp.
3	Rod	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	+	*Proteus* sp.
4	Rod	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	*Shingobacterium* sp.
5	Rod	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	*Sporosarcina* sp.
6	Cocci	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	*Dermococcus* sp.
7	Cocci	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	*Staphylococcus* sp.
8	Cocci	+	+	-	-	+	-	-	-	+	+	-	+	-	-	-	-	*Enterococcus* sp.
9	Cocci	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus* sp.
10	Coccobacillus	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	*Roseomonas* sp.
11	Coccobacillus	-	-	-	-	+	-	-	+	-	+	+	-	-	-	+	+	*E. coli*
12	Rod	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	*Corynebacterium* sp.

Key: GRM, Gram stain; CAT, Catalase test; SPO, Spores formation; STA, Starch hydrolysis test; VP, Voges-Proskauer test; MR, Methyl red test; SWO, Swollen cell; MOT, Motility test; BE, Bile-esculin test; LAC, Lactose fermentation test; IDL, Indole test; GLU, Glucose fermentation test; ARA, Arabinose fermentation test; MAN, Mannitol fermentation test; OXI, Oxidase test; GAS, Gas formation

Table 3. The 16S rRNA Sequencing homology search (BLAST) Results of the Indoor Air Bacteria Isolated in Swiftlet Houses at the Sampling Sites

Sites	16S rRNA sequencing			
	Bacterial species (Number of isolates)	Size(s) bp	Data base	Homology (%)
Kota Samarahan	*B. cereus* (2)	1216	GenBank	98
Sibu, Sepinang, Sarikei	*B. cereus* (10)	1453	GenBank	99
Kota Samarahan	*Bacillus sp.* (1)	1509	GenBank	99
Kota Samarahan, Maludam, Miri, Sibu	*B. subtilis* (8)	943	GenBank	99
Betong	*B. amyloliquefaciens* (8)	1446	GenBank	98
Kuching	*Bacillus sp.* (5)	1456	GenBank	99
Kota Samarahan, Saratok, Miri, Kuching, Sibu, Sarikei	*Lysinibacillus sp.*(16)	1451	GenBank	99
Kota Samarahan	*P. penneri* (1)	290	GenBank	98
Kota Samarahan	*Sphingobacterium sp.* (1)	1377	GenBank	98
Semarang	*S. nepalensis* (4)	1456	GenBank	99
Kota Samarahan, Semarang	*S. nepalensis* (3)	1454	GenBank	99
Sibu	*S. nepalensis* (1)	1519	GenBank	99
Sarikei	*S. nepalensis* (1)	1519	GenBank	99
Saratok, Sarikei	*Staphylococcus sp.*(3)	1514	GenBank	99
Betong	*Staphylococcus* sp. (3)	1520	GenBank	99
Maludam	*Staphylococcus sp.* (3)	1516	GenBank	99
Kuching	*S. lentus* (2)	517	GenBank	99
Semarang	*S. lentus* (5)	859	GenBank	99
Kuching	*S. kloosii* (2)	1493	GenBank	99
Saratok, Sibu	*Sporosarcina sp.* (4)	1398	GenBank	99
Saratok, Maludam, Sibu, Semarang	*S. aquimarina (8)*	752	GenBank	99
Saratok, Sepinang	*Dermacoccus sp.*(5)	1484	GenBank	96
Maludam	*E. faecalis* (2)	1445	GenBank	100
Sepinang	*M. luteus* (2)	1373	GenBank	97
Sepinang	*Roseomonas sp.*(2)	1481	GenBank	96
Sarikei	*E. coli* (3)	5038386	GenBank	99
Sarikei	*Corynebacterium sp.* (1)	1492	GenBank	99

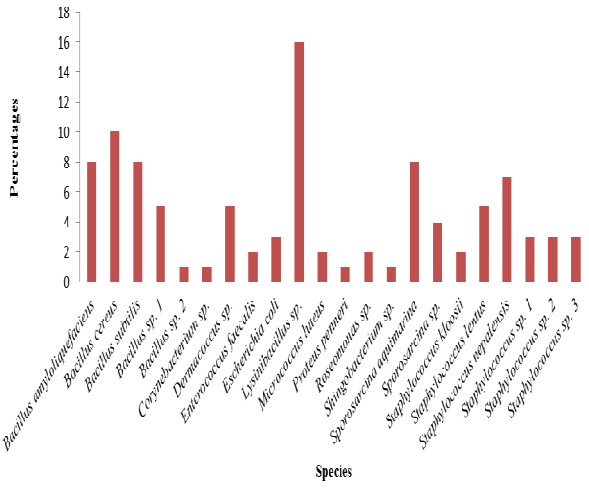

Figure 1. The Frequency Percentages of the Indoor Air Bacteria Isolated at the Sampling Sites

Lysinibacillus sp. is a member of the *Bacillaceae* family and produces endospores [20]. *Bacillus* sp. was the most frequently isolated airborne bacteria from all areas. *Bacillaceae* family bacteria are facultative anaerobe bacteria that can grow aerobically and anaerobically [21]. In addition, *Bacillus* sp. produces endospores that remain dormant under stressful environments for long periods [11] and thus can survive even in dry swiftlet droppings and spread further in the air. According to Kocijan 2009 [22], contamination of bird feed in the droppings sample could also be an explanation for the occurrence of various *Staphylococcus* sp. in the air. Researchers have isolated *Bacillus* sp. and *E. coli* from the air inside houses in Zaria [4]. Additional airborne bacteria are being investigated to determine their risk.

4. Conclusions

This preliminary study, using 16S rRNA gene sequence analysis, identified 27 types of airborne bacteria species: *B. amyloliquefaciens*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus* sp. 1, *Bacillus* sp. 2, *Corynebacterium* sp., *Dermacoccus* sp., *E. faecalis*, *E. coli*, *Lysinibacillus* sp., *M. luteus*, *P. penneri*, *Roseomonas* sp., *Sphingobacterium* sp., *S. aquimarina*, *Sporosarcina* sp., *S. kloosii*, *S. lentus*, *S. nepalensis*,

Staphylococcus sp. 1, *Staphylococcus* sp. 2, and *Staphylococcus* sp. 3 in the air inside the swiftlet houses.

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