# PRELIMINARY ANALYSIS OF THE SKIN MICROBIOTA COMPOSITION OF GIANT MOTTLED EEL (*ANGUILLA MAMORATA* QUOY AND GAIMARD, 1824) CULTURED IN HO CHI MINH USING METAGENOMIC SEQUENCING

**Nguyen Van Thong[1,2], Nguyen Phu Hoa[1], Nguyen Bao Quoc[1*], Nguyen Thi My Dung[2], Nguyen Ngoc Tai[2]**
*[1] Nong Lam University of Ho Chi Minh city, [2]Hung Vuong University of Ho Chi Minh city*

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## ABSTRACT

This study investigates the bacterial community structure present on the skin of Giant mottled eels (*Anguilla marmorata*) cultured in Ho Chi Minh City, Vietnam. Samples were collected from individuals exhibiting abnormal swimming behavior and signs of illness, including pale patches and mild hemorrhaging on the skin, from an indoor aquaculture facility located in Ho Chi Minh City. Metagenomic DNA was extracted and analyzed using Illumina paired-end sequencing technology. The phyla Bacteroidetes (9.92%), Firmicutes (23.89%), Proteobacteria (63.5%), Cyanobacteria (0.33%), unclassified (0.15%) and other phyta groups (0.20%) were found. The predominating class in samples were Gammaproteobacteria (61.71%), Bacilli (13.35%), Clostridia (12.55%), Bacteroidia (9.92%). The predominating families were Aeromonadaceae (43.10%), Enterobacteriaceae (12.06%), Streptococcaceae (11.24%), Clostridiaceae (10.36%), Weeksellaceae (3.33%). The predominating genus in sampes were *Aeromonas* (43.08%), *Lactococcoccus* (11.24%), *Enterobacter* (5.03%), *Macellibacteroides* (4.46%), *Plesiomonas* (4.01%), *Acidovorax* (1.33%), and *Edwardsiella* (0.79%). The identified species included *Aeromonas caviae* (29.72%), *Lactococcus lactis* (11.23%), Plesiomonas shigelloides (3.91%), *Aeromonas encheleia* (2.58%), *Enterobacter roggenkampii* (1.72%), *Acidovorax delafieldii* (1.25%), *Edwardsiella hoshinae* (0.79%), and *Acinetobacter junii* (0.06%).

# NGHIÊN CỨU HỆ VI SINH VẬT TRÊN DA CÁ CHÌNH BÔNG (*ANGUILLA MAMORATA* QUOY AND GAIMARD, 1824) NUÔI TẠI THÀNH PHỐ HỒ CHÍ MINH BẰNG METAGENOMIC

**Nguyễn Văn Thống[1,2], Nguyễn Phú Hòa[1], Nguyễn Bảo Quốc[1*], Nguyễn Thị Mỹ Dung[2], Nguyễn Ngọc Tài[2]**
*[1]Trường Đại học Nông Lâm Thành phố Hồ Chí Minh, [2]Trường Đại học Hùng Vương Thành phố Hồ Chí Minh*

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**TỪ KHÓA**

*Cá chình Bông*
Metagenomic
Hệ vi sinh vật cá chình
Vi khuẩn gây bệnh cá
Bệnh trên da cá chình

## TÓM TẮT

Đây là nghiên cứu bước đầu về cấu trúc và thành phần hệ vi sinh trên da của cá chình Bông (*Anguilla marmorata*) nuôi tại Thành phố Hồ Chí Minh, Việt Nam. Mẫu da cá nghiên cứu được thu thập từ những cá chình Bông có dấu hiệu bất thường như bơi lội không bình thường, lớp da bên ngoài có các đốm (vết màu trắng) và xuất huyết nhẹ từ trang trại nuôi cá chình ở Thành phố Hồ Chí Minh. Mẫu da cá được thu thập và trích ly DNA sau đó giải trình tự metagenomic bằng công nghệ giải trình tự bằng Illumina. Kết quả phân tích cho thấy, ở mức độ phân loại ngành, các ngành vi khuẩn được xác định bao gồm Bacteroidetes (9,92%), Firmicutes (23,89%), Proteobacteria (63,5%), Cyanobacteria (0,33%), nhóm chưa định danh được (0,15%) và các nhóm ngành khác (0,20%). Ở mức độ phân loại lớp, các lớp chiếm ưu thế trong mẫu là Gammaproteobacteria (61,71%), Bacilli (13,35%), Clostridia (12,55%), và Bacteroidia (9,92%). Các họ vi khuẩn chủ đạo gồm Aeromonadaceae (43,10%), Enterobacteriaceae (12,06%), Streptococcaceae (11,24%), Clostridiaceae (10,36%), và Weeksellaceae (3,33%). Các chi vi khuẩn phổ biến trong mẫu gồm *Aeromonas* (43,08%), *Lactococcoccus* (11,24%), *Enterobacter* (5,03%), *Macellibacteroides* (4,46%), *Plesiomonas* (4,01%), *Acidovorax* (1,33%), và *Edwardsiella* (0,79%). Các loài được ghi nhận gồm *Acidovorax delafieldii* (1,25%), *Acinetobacter junii* (0,06%), *Lactococcus lactis* (11,23%), *Plesiomonas shigelloides* (3,91%), *Aeromonas caviae* (29,72%), *Aeromonas encheleia* (2,58%), *Enterobacter roggenkampii* (1,72%) và *Edwardsiella hoshinae* (0,79%).

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[*] Corresponding author. *Email: baoquoc@hcmuaf.edu.vn*

## 1. Introduction

*Anguilla marmorata* (giant mottled eel) is widely cultured in aquaculture farms and is considered one of the economically important fish species in Vietnam's aquaculture sector [1]. Vietnam has over 1,000 eel farming establishments, which annually supply approximately 2,000–5,000 tons of commercial eel to the market. Presently, around 70% of this yield is consumed domestically, while the remaining 30% is exported to neighboring countries such as China, Japan, and Taiwan [2]. Enhancing productivity and mitigating disease outbreaks are essential for the sustainable development of eel aquaculture. Initial studies utilizing metagenomic approaches have begun to elucidate the microbial communities in eels, such as investigations into gut microbiota composition and its relationship with growth rates in European eels. These studies revealed significant differences in microbial community structure between fast-growing and slow-growing eel groups. The presence of the bacterial genus *Cetobacterium* in the gut of European eels has been identified as a distinguishing feature between these growth rate groups [3]. Further research on the gut microbiota of the white-spotted eel (*Anguilla bicolor bicolor*) demonstrated differences in both the composition and relative abundance of bacterial taxa, which may serve as a foundation for developing probiotic-supplemented diets suitable for this eel species [4]. A study on the intestinal microbiota of three eel species in Taiwan-*Anguilla japonica*, *Anguilla marmorata*, and *Anguilla bicolor pacifica*—identified species-specific microbial signatures and highlighted similarities in the gut microbiota among conspecifics from different rivers and among heterospecifics from the same river [5]. A study conducted in China on the gut microbiota of *Anguilla marmorata* revealed minor variations in the microbial communities between fast- and medium-growing individuals [6]. Current research on the skin microbiota of fish and eels such as European eels (*Anguilla anguilla*), particularly giant mottled eels (*Anguilla mamorata*), remains limited [7] – [9]. Therefore, a comprehensive understanding of the microbial communities inhabiting both the skin and gastrointestinal tract of eels is vital for enhancing host immunity, optimizing growth performance, and improving survival rates. These insights are critical to advancing sustainable eel aquaculture practices in Vietnam and worldwide. However, research on the microbiome of eels in general, and *Anguilla marmorata* in particular, remains limited, especially with regard to metagenomic applications in Vietnam**.** Understanding the microbial community present on the skin of eels is essential for improving fish health, increasing survival rates, and promoting growth—factors that are crucial to the sustainability and development of eel aquaculture in Vietnam and globally.

This study serves as a preliminary effort to characterize the skin microbiota of *A. marmorata* cultured in Ho Chi Minh City, Vietnam. The data presented herein aim to provide insight into the bacterial diversity inhabiting the mucosal layer of this species. Such information is particularly valuable for predicting and assessing the microbial balance during the eel's development and may contribute to disease management strategies in aquaculture systems.

## 2. Materials and Methods

### *2.1. Sample collection and preparation*

Fish samples were collected from two commercial eel farms located in Nha Be district, Ho Chi Minh City, Vietnam. Individuals selected for sampling exhibited abnormal swimming behavior and visible white patches on the skin accompanied by mild hemorrhaging.

The skin sampling procedure was adapted from [10] with the following steps:

- Eels were euthanized using mild sedation. The skin surface was disinfected with 70% ethanol for 90 seconds and rinsed three times with sterile deionized water.
- The skin was then swabbed using sterile cotton swabs and placed into 50 mL sterile tubes containing phosphate-buffered saline (PBS).

- Samples were obtained from three individuals (average body weight ~20 g/fish), and approximately 0.5 g of skin mucus was collected for each pooled sample.

Metagenomic DNA was extracted and analyzed using the Illumina paired-end sequencing platform, targeting the V3–V4 hypervariable regions of the bacterial 16S rRNA gene.

### *2.2. Genomic DNA Extraction*

Extracted DNA was diluted to a final concentration of 1 ng/µL in sterile distilled water. After extraction, the DNA was eluted in 50 µL of elution buffer C6 provided in the kit and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) for nucleic acid and protein quantification. DNA samples were stored at –80°C until further analysis.

### *2.3. Amplification of the V3–V4 Region of the 16S rRNA Gene*

The sequencing library was constructed using the MetaVX Library Preparation Kit. Briefly, 20–50 ng of genomic DNA was used to generate amplicons targeting the V3–V4 hypervariable regions of the bacterial 16S rRNA gene. DNA concentration was measured using a microplate reader (Tecan, Infinite 200 Pro), and amplicon fragment size (~600 bp) was confirmed by 1.5% agarose gel electrophoresis.

### *2.4. Sequencing and bioinformatics analysis*

#### *2.4.1. Illumina sequencing*

Next generation sequencing was conducted on an Illumina Miseq/Novaseq Platform (Illumina, San Diego, USA) at the company. Automated cluster generation and 250/300-bp paired-end sequencing with dual reads were performed according to the manufacturer’s instructions.

#### *2.4.2. Data analysis*

Double-end sequencing of positive and negative reads the first of the two joining together to filter joining together the results contained in the sequence of N, retains the sequence length is larger than 200 bp sequence. After quality filter, purify chimeric sequences, the resulting sequence for OTU (Operational Taxonomic Units) clustering, use VSEARCH clustering (1.9.6) sequence (sequence similarity is set to 97%) [11], than the 16 s rRNA reference database is Silva [12]. Then use RDP classifier (Ribosomal Database Program) bayesian algorithm of OTU species taxonomy analysis representative sequences, and under different species classification level statistics community composition of each sample. Based on OTU analysis results are obtained, using the method of random sampling sample sequences is flat, calculate Shannon, Chao1 alpha diversity index, community species Abundance and diversity of Rarefaction curves and Rank - Abundance graph can also reflect the species richness and evenness [13]. Through the weighted clustering hierarchy and the group average method to construct UPGMA (Unweighted pair group method with arithmetic mean) clustering tree.

## 3. Results and Discussion

**Table 1.** *DNA Quality Assessment Results Prior to Sequencing*

| Sample Name | Mass (gram) | Dissolution Volume (µL) | Nucleic Acid Concentration (ng/µL) | A260 (Abs) | A280 (Abs) | A260/A280 * |
|---|---|---|---|---|---|---|
| S1 | 0.2593 | 50 | 284.7 | 5.693 | 2.945 | 1.93 |
| S2 | 0.2512 | 50 | 227.3 | 4.546 | 2.391 | 1.9 |
| S3 | 0.2525 | 50 | 259.9 | 5.197 | 2.717 | 1.91 |
| S4 | 0.2543 | 50 | 184.5 | 3.69 | 1.934 | 1.91 |

According to the results (Table 1), samples S1, S2, and S4 met the DNA quality criteria for sequencing, as their A260/A280 ratios were within the acceptable range of 1.8–2.0, indicating sufficient purity for sequencing ([14], [15]).

Preliminary results on the skin bacterial community of *Anguilla marmorata* identified at the phylum level are presented in Table 2 and Figure 1. The most dominant phylum was Proteobacteria, accounting for 63.50% of the total community. This was followed by Firmicutes (23.89%), Bacteroidota (9.92%), Patescibacteria (0.96%), Campylobacteria (0.88%), Actinobacteriota (0.17%), unclassified (0.15%) and other groups (0.20%).

**Table 2.** *Relative abundances of phylum*

| Phylum | Relative abundance |
|---|---|
| Actinobacteriota | 0.17% |
| Bacteroidota | 9.92% |
| Campylobacterota | 0.88% |
| Firmicutes | 23.89% |
| Proteobacteria | 63.50% |
| Patescibacteria | 0.96% |
| unclassified | 0.15% |
| Other groups | 0.20% |

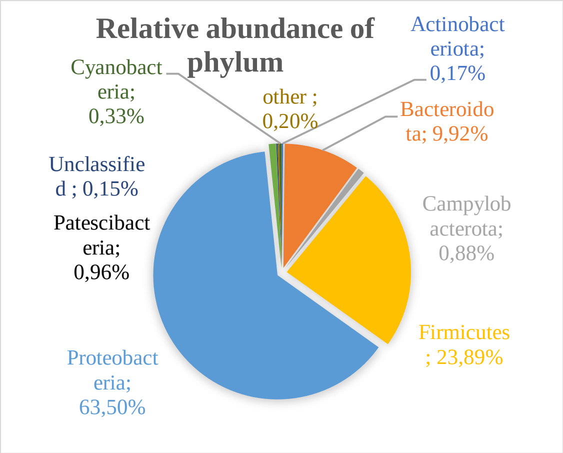

**Figure 1**. *The relative abundance of phylum*

At the class level, *Gammaproteobacteria* was the predominant class, comprising 61.71% in the skin. The second most abundant class was *Clostridia*, accounting for 12.55%, followed by *Bacilli* (11.35%) and *Alphaproteobacteria* (10.29%). Bacteroidia (9.92%), Gracilibacteria (0.96%), *Campylobacteria* (0.89%), *Sericytochromatia* (0.09%), and *Vampirivibrionia* (0.24%) were also detected. The remaining portion belongs to other class groups (10.28%).

At the order level, *Enterobacterales* was the most dominant order in sample, with 56.33% of the skin. *Burkholderiales* was in the skin at 4.22%, *Clostridiales* was prevalent in the skin (11.39%), *Lactobacillales* represented 11.25% and *Flavobacteriales* was found at only 3.36%. *Bacteroidales* (4.68%), *Campylobacterales* (0.88%), *Chitinophagales* (1.75%), *Deinococcales* (0.09%), *Tissierellalles* (2.16%), unclassified (1.07%) and other order group (2.82%).

At the family level, *Aeromonadaceae* was dominant in the skin of sample, accounting for 43.10%. *Weeksellaceae* followed, with 3.33% and *Enterobacteriaceae* was notably more abundant in the skin of 12.06%. *Clostridiaceae* showed a presence in the skin of 10.39%, *Peptostreptococcaceae*, *Obscuribacteraceae*, and *Magnetospirillaceae* were detected in the skin of sample, at 2.16%, 0.24%, and 0.37%, respectively. *Streptococcaceae* (11.24%), *Weeksellaceae* (3.33%), unclassified (0.43%) and other family groups (9.21%).

The genera with the high relative abundance were shown in Table 3 and Figure 2.

At the genus level (Table 3 and Figure 2), *Aeromonas* dominated with 43.08% in the skin of sample. *Cloacibacterium* was small abundant in the skin of sample (3.33%). *Plesiomonas* (4.01%) and *Lactococcus* accounted for 11.24% in the skin and other genus groups (8.12%).

The species richness of the samples is shown in Figure 3, over the past decade, several members of the *Acinetobacter* complex and non-*Acinetobacter* complex have been reported as causative agents of fatal sepsis in aquatic animals such as fish, including mandarin fish (*Siniperca chuatsi*), channel catfish (*Ietalurus punctatus*), hybrid Prussian carp (*Carassais auratus gibelio*), and loach (*Misgurnus anguillicaudatus*) [16].

**Table 3**. *Relative abundances of genus*

| Genus | Relative abundance |
|---|---|
| *Acidovorax* | 1.33% |
| *Aeromonas* | 43.08% |
| *Cloacibacterium* | 3.33% |
| *Enterobacter* | 5.03% |
| *Lactococcus* | 11.24% |
| *Macellibacteroides* | 4.46% |
| *Edwardsiella* | 0.79% |
| *Helicobacter* | 0.88% |
| *Legionella* | 0.74% |
| *Sediminibacterium* | 1.71% |
| *Plesiomonas* | 4.01% |
| *Paraclostridium* | 2.16% |
| *Klebsiella* | 0.65% |
| *Citrobacter* | 1.88% |
| *Clostridium sensu stricto 13* | 1.24% |
| *Clostridium sensu stricto 1* | 7.38% |

**Table 4**. *Relative abundances of species*

| Species | The highest relative abundance |
|---|---|
| *Acidovorax delafieldii* | 1.25% |
| *Acinetobacter junii* | 0.06% |
| *Aeromonas caviae* | 29.72% |
| *Aeromonas encheleia* | 2.58% |
| *Deinococcus misasensis* | 0.09% |
| *Edwardsiella hoshinae* | 0.79% |
| *Enterobacter roggenkampii* | 1.72% |
| *Exiguobacterium acetylicum* | 0.09% |
| *Helicobacter trogontum* | 0.87% |
| *Lactococcus lactis* | 11.23% |
| *Plesiomonas shigelloides* | 3.91% |
| Unclassified *Aeromonas* | 10.71% |
| Unclassfied *Cloacibacterium* | 3.33% |
| Unclassfied *Macellibacteroides* | 4.46% |
| Unclassfied *Clostridium sensu 1* | 7.01% |
| Other species groups | 22.18% |

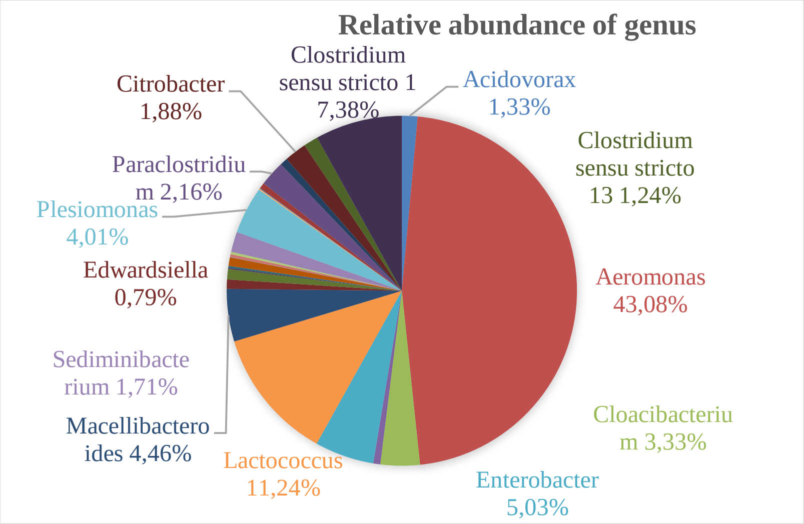

**Figure 2**. *The relative abundance of genus*

At the species level, *Acinetobacter junii* was found at 0.06% in the skin, *Lactococcus lactis* was dominant in the skin of sample. *Aeromonas caviae* is a known pathogen causing hemorrhagic disease in fish and is associated with hepatic and renal disease in tilapia [17], [18]. *Aeromonas encheleia* was detected in prevalences of 2.58%. However, according to a previous study by E. Consuelo et al.[19], this bacterial species is considered to have low pathogenicity in European eels. *Edwardsiella hoshinae*, a bacterium known to infect birds and reptiles and also previously identified in Japanese eels [20], was likewise found in both studied in the skin of fish groups. Additionally, members of the genus *Aeromonas*, particularly *Aeromonas hydrophila*, a Gram-negative bacterium are known to cause hemorrhagic diseases in fish. *A. hydrophila* has been implicated in infections affecting a wide range of warm-water fish species and is specifically associated with hemorrhagic disease in striped catfish (*Pangasianodon hypophthalmus*) [4], [21], was also present at a relative abundance of 1.05%. Specifically, this species is susceptible to outbreaks of diseases caused by pathogenic bacteria, especially *Aeromonas hydrophila* and *Flavobacterium columnare*, which have become a major problem contributing to the decline in pangasius production in Thailand [17].

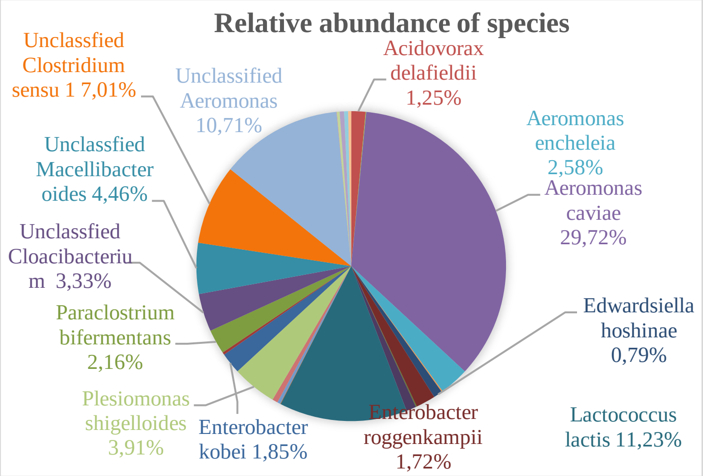

**Figure 3**. *The relative abundance of species*

## 4. Conclusion

This is the first metagenomic study of the skin microbiota of *Anguilla marmorata* conducted in Vietnam. This dataset establishes foundational knowledge of the skin-associated microbial community structure and bacterial diversity in *Anguilla marmorata* reared under controlled, roofed aquaculture environments. The results provide critical insights that can inform the formulation of species-specific diets and the strategic application of probiotics, with the objective of improving host health and supporting the sustainability of eel aquaculture systems. Furthermore, building upon the findings of this study, we intend to investigate the differences in microbial communities between healthy and diseased eels, and among eel populations across distinct ecological zones, as a foundation for future research.

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