人工繁育朱鹮肠道微生物宏基因组学分析

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摘　要：【目的】以德清县下渚湖人工繁育朱鹮种群为研究对象，采用宏基因组分析技术，系统分析朱鹮（*Nipponia nippon*）肠道微生物在不同季节的菌群结构和抗生素耐药基因（Antibiotic resistance genes，ARGs）携带状况，为朱鹮疾病防治提供了相关的基础数据。【方法】于 2020 年 7 月至 2022 年 7 月期间，采集湖州市下渚湖国家湿地公园朱鹮种群及其相关环境（泥鳅和排污口污泥）样本。共计采集了 270 份朱鹮新鲜粪便。提取样本总 DNA，进而用于宏基因组测序，并对测序数据进行了生物信息学和统计学分析。【结果】朱鹮粪便样本经 MetaPhlAn4 注释，结果发现存在 525 种细菌，分属 8 门，23 纲，53 目，112 科和 235 属。其中，61 种被定义为条件致病菌，可能成为朱鹮肠道中的潜在致病菌。不同样本间肠道菌群组成差异很大，经围绕中心点划分（Partitioning Around Medoids，PAM）聚类方法可以被聚类为两簇，并且它们的微生物组代谢和功能差异显著。基于 eggNOG 和 KEGG 数据库的注释，本研究对细菌的基因进行了功能分析。在代谢功能方面，从 eggNOG 数据库的注释结果看，除大量功能未知基因簇存在外，细菌的基因簇主要富集于复制，重组和修复、氨基酸的运输和代谢、转录、信号转导机制等相关功能基因。KEGG的注释结果表明，朱鹮肠道微生物功能基因主要分布在新陈代谢、全球和概述图、碳水化合物代谢通路和氨基酸代谢通路。这两部分研究都支持肠道对宿主生理代谢具有重要调节作用。朱鹮粪便样本中共检测到367 种 ARGs，这些 ARGs 主要与 fluoroquinolone、peptide、aminoglycoside 和 tetracycline 耐药相关。【结论】本研究初步概述了朱鹮肠道微生物群落结构和抗生素耐药基因携带情况，这对于有效评估朱鹮健康和控制抗生素耐药性具有重要意义。研究结果拓宽了我们对濒危鸟类朱鹮抗微生物耐药性（Anti-microbial Resistance，AMR）的理解，并对评估 ARGs 污染风险提供参考。

关键词：朱鹮；宏基因组；抗性基因；肠道微生物群；功能基因

Metagenomics of Intestinal Microorganisms in Captive Crested Ibis

Abstract:【Objective】This study focused on the captive crested ibis population in Xiazhu Lake, Deqing County, and used metagenomic sequencing technology to analyze the bacterial community structure and antibiotic resistance genes (ARGs) carrying status in the crested ibis gut during different seasons, providing relevant basic data for disease prevention and control of crested ibis. 【Method】In this study, samples of crested ibis populations and their associated environment (mud and muddy water mixture from the outfall) were collected from July 2020 to July 2022 in Haizhu Lake National Wetland Park, Huzhou City. A total of 270 fresh ibis feces were collected. Total DNA was then extracted from the samples, and used for macro-genome sequencing, and bioinformatics and statistical analyses were performed on the sequencing data. 【Result】After annotation by MetaPhlAn4, a total of 525 bacterial species belonging to 8 phyla, 23 classes, 53 orders, 112 families, and 235 genera were identified from the crested ibis. Of these 61 species were defined as potential opportunistic pathogens in the crested ibis gut. The dominant phyla in the crested ibis gut were Proteobacteria and Firmicutes. The composition of the gut microbiota was highly between the samples and could be grouped into two clusters, with significant differences in microbial metabolism and function. Functional analysis of bacterial genes was performed using the eggNOG and KEGG

databases. In terms of metabolic function, apart from a large number of gene clusters with unknown functions, bacterial gene clusters were mainly enriched in replication, recombination, and repair; amino acid transport and metabolism; transcription; and signal transduction mechanisms, as shown by the annotation results from the eggNOG database. Based on the annotation results from KEGG, the functional genes of the crested ibis gut microbiota were mainly distributed in metabolism, global and overview maps, carbohydrate metabolism pathways, and amino acid metabolism pathways. Both parts of this study support the important regulatory role of the gut in host physiological metabolism. A total of 367 ARGs are mainly associated with fluoroquinolone, peptide, aminoglycoside, and tetracycline resistance.【Conclusion】This study provides a preliminary overview of the gut microbial community structure and antibiotic resistance gene carriage in crested ibis, which is important for effective assessment of crested ibis health and control of antibiotic resistance. The findings broaden our understanding of anti-microbial resistance (AMR) in the endangered bird creasted ibis and provide a reference for assessing the risk of contamination by ARGs.

Key words: Crested ibis; Antibiotic resistance genes; Gut microbiome;Functional genes.