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Research Article 研究报告

高通量测序分析小头裸裂尻鱼皮肤和肠道的微生物多样性

郝佳慧[1,2]，蔡伟杰[1,2]，李柯懋[3]，关弘弢[3]，杨朝杰[1,4]，高强[1]，刘丹[1]，聂苗苗[1]，祁得林[1]，张存芳[1*]

1 青海大学 省部共建三江源生态与高原农牧业国家重点实验室，青海 西宁 810016
2 青海大学生态环境工程学院，青海 西宁 810016
3 青海省渔业技术推广中心(青海省渔业环境监测中心)，青海 西宁 810012
4 青海大学农牧学院，青海 西宁 810016

郝佳慧, 蔡伟杰, 李柯懋, 关弘弢, 杨朝杰, 高强, 刘丹, 聂苗苗, 祁得林, 张存芳. 高通量测序分析小头裸裂尻鱼皮肤和肠道的微生物多样性[J]. 微生物学报, 2023, 63(1): 233-250.

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摘　要：【目的】解析小头裸裂尻鱼不同部位的微生物群落结构、物种组成、多样性特征以及菌群功能差异。【方法】通过 Illumina MiSeq 扩增子高通量测序，分析小头裸裂尻鱼皮肤黏膜、肠道黏膜和肠道内容物 3 个部位微生物菌群组成差异，并通过 Tax4Fun 预测菌群潜在功能。【结果】皮肤黏膜微生物 α 多样性最高，其 Shannon 指数显著高于肠道黏膜(P<0.05)和肠道内容物(P<0.001)。主坐标分析表明，皮肤黏膜微生物显著区别于其他 2 个部位。在门水平小头裸裂尻鱼 3 个部位相对丰度前五的微生物类群均为放线菌门(*Actinobacteria*)、变形菌门(*Proteobacteria*)、厚壁菌门(*Firmicutes*)、绿弯菌门(*Chloroflexi*)和蓝藻门(*Cyanobacteria*)，其中肠道内容物中放线菌门相对丰度(46.53%)显著高于肠道黏膜(29.23%，P<0.05)和皮肤黏膜(25.83%，P<0.01)；肠道黏膜中变形菌门的相对丰度(40.33%)显著高于肠道内容物(26.10%，P<0.05)。对各部位相对丰度前 10 的

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*Corresponding author. E-mail: zcf_1023@126.com
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菌群进行分析发现，小头裸裂尻鱼皮肤黏膜和肠道内容物菌群差异菌群更多，包括微杆菌科(*Microbacteriaceae*)、伯克氏菌科(*Burkholderiaceae*)和 JG30-KF-CM45 等 6 个差异菌科和冷杆菌属(*Cryobacterium*)、肉食杆菌属(*Carnobacterium*)、节杆菌属(*Arthrobacter*)等 9 个差异菌属，其中皮肤黏膜中相对丰度较高的菌属主要与有机化合物降解和抑菌作用相关。Tax4Fun 功能预测发现，3 个部位在第三级通路上表现出显著的差异特征，皮肤黏膜中的菌群在 ABC 转运蛋白代谢和组氨酸代谢通路富集，肠道黏膜在信号转导和甘油磷脂代谢通路显著富集，而肠道内容物则富集于脂肪酸合成、萜类化合物生物合成途径。【结论】小头裸裂尻鱼不同部位的菌群主要与环境相关，其中皮肤黏膜的微生物多样性最高，其微生物群主要与环境相关，而肠道黏膜和肠道内容物之间微生物多样性差异较小，除与环境相关菌群以外，还发现与自身生理特性、免疫以及营养物质的摄入与消化相关的菌群。阐明不同部位微生物分布特征，将为高原鱼类保护及生存环境改善提供基础数据和科学依据。

关键词：小头裸裂尻鱼；高通量测序；皮肤微生物；肠道微生物

Skin and intestinal microbial diversity of *Herzensteinia microcephalus* determined by high-throughput sequencing

HAO Jiahui[1,2], CAI Weijie[1,2], LI Kemao[3], GUAN Hongtao[3], YANG Chaojie[1,4], GAO Qiang[1], LIU Dan[1], NIE Miaomiao[1], QI Delin[1], ZHANG Cunfang[1*]

1 State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, Qinghai, China
2 College of Eco-Environmental Engineering, Qinghai University, Xining 810016, Qinghai, China
3 Qinghai Provincial Fishery Technology Promotion Center (Qinghai Provincial Fishery Environment Monitoring Center), Xining 810012, Qinghai, China
4 College of Agriculture and Animal Husbandry, Qinghai University, Xining 810016, Qinghai, China

Abstract: [Objective] To reveal the microbiota structure and diversity in different parts of *Herzensteinia microcephalus* and predict the functions of the microbiota. **[Methods]** High-throughput sequencing of 16S rRNA gene was performed to reveal the microbiota structure. Tax4Fun was employed to predict the functions of the microbiota. **[Results]** The microbiota in skin mucosa had the highest alpha diversity, with the Shannon index higher than that of the microbiota in intestinal mucosa (P<0.05) and intestinal content (P<0.001). Moreover, the microbiota in skin mucosa can be significantly distinguished from that in intestinal mucosa and intestinal content by principal co-ordinates analysis. *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Chloroflexi*, and *Cyanobacteria* were the top 5 phyla accounting for more than 75% of the total microbiota. Among them, *Actinobacteria* in intestinal content had higher relative abundance (46.53%) than that in intestinal mucosa (29.23%, P<0.05) and skin mucosa (25.83%, P<0.01). The relative abundance of *Proteobacteria* in intestinal mucosa (40.33%) was higher than in intestinal content (26.10%, P<0.05). The top 10 families and genera in each part were further analyzed, which showed that skin mucosa and intestinal

content had more different microorganisms, including 6 families (e.g., *Microbacteriaceae*, *Burkholderiaceae*, and JG30-KF-CM45) and 9 different genera (e.g., *Cryobacterium*, *Carnobacterium*, and *Arthrobacter*). The genera with higher abundance in skin mucosa were associated with organic material degradation and bacterial inhibition. The microbiota in the three parts showed different functional characteristics on pathway level 3 as predicted by Tax4Fun. Specifically, the microbiota in skin mucosa were mainly involved in ABC transporters and histidine metabolism pathway, that in intestinal mucosa in a two-component system and glycerophospholipid metabolism pathway, and that in intestinal content in fatty acid biosynthesis and terpenoid backbone biosynthesis pathway. **[Conclusion]** The microbiota structures in different parts of *H. microcephalus* were related to the environment. The skin mucosa had the highest microbial diversity among the three parts, and the intestinal mucosa and intestinal content had similar microbial diversity. In addition, the microorganisms related to the environment, those associated with the host's physiological characteristics, immunity, and digestion were identified. Revealing the characteristics of microbial distribution in different parts will provide basic data and a scientific basis for the protection and the living environment improvement of fish in the plateau regions.

Keywords: *Herzensteinia microcephalus*; high-throughput sequencing; skin microorganisms; intestinal microorganisms

青藏高原被称为"世界屋脊"，平均海拔超过 4 500 m，气候环境极端恶劣，其低温、严寒、强紫外线辐射等环境是制约高寒区生物生存的关键因素，同时也是迫使该区域生物特有种分化形成的主要原因[1-2]。青藏高原及其周边地区是世界上生物群落结构最复杂的地区之一，除了植物和动物外，还有极为丰富的微生物，高寒地区微生物的研究主要集中在土壤[3]和冰川[4]以及藏野驴、藏羊、牦牛等动物[5-6]，而对高寒地区土著鱼类微生物的研究相对缺乏，如高度特化等级裂腹鱼，全身几乎无鳞多黏液，更易于适应高海拔环境。

皮肤黏膜是生物与外界环境之间的第一道物理屏障，含有非特异性的体液因子，有研究表明，皮肤菌群可以促进对皮肤病原体的先天免疫和适应性免疫[7-8]，此外，由于鱼的皮肤长期暴露于水环境中，表面形成了黏液与外界隔离[9]，其表面的微环境给一些有害细菌提供了繁衍生息的温床，为了抵御相关有害菌的侵害，同时会形成与其存在竞争关系的益生菌[10]。宿主肠道中的微生物包括肠道内容物和肠道黏膜中的微生物，配合肠道发挥免疫、消化和吸收营养物质的作用[11-13]。鱼类的肠道内容物源于鱼类生存的外部环境，通过营养摄入进入肠道内与肠道黏膜相接触，部分微生物随着食物进入肠道系统，大部分微生物群在肠道中不会持续很长时间，被附着在肠道黏膜上的常驻微生物所包围[14]，这些微生物除了小部分寄居在宿主肠道中外，其余随宿主自身的代谢作用而排出体外，寄生在宿主体内外的微生物中，多数为有益菌。

小头裸裂尻鱼是青藏高原特有的鱼类，也是我国特有鱼类，属于鲤形目(*Cypriniformes*)、鲤科(*Cyprinidae*)、裂腹鱼亚科(*Schizothoracinae*)、裸裂尻鱼属(*Schizopygopsis*)[15]。分布在我国西藏和青海，是世界上分布海拔最高的鱼类之一，平均海拔在 4 500 m 左右，其主要以着生硅藻和植物碎片为食，兼食水生昆虫。小头裸裂尻鱼适应于青藏高原特有环境，直到 2021 年才实现规模

化繁殖[16]。本研究以小头裸裂尻鱼为研究对象，采用Illumina MiSeq高通量测序和生物信息学方法比较其皮肤黏膜、肠道黏膜和肠道内容物微生物的多样性、菌群结构及潜在功能，从而研究微生物在高度特化等级裂腹鱼适应环境变化的过程中起到的关键作用。为揭示微生物—宿主—环境之间的相互作用奠定基础，为开发和利用有益微生物，改善高原鱼类的营养和健康，实现高原冷水鱼的规模化繁殖提供参考。

1 材料与方法

1.1 试验材料

从青海省格尔木市南域的唐古拉山镇的沱沱河支流(92°07′461″E，34°18′55″N，海拔4 588 m)，选择体重体长相近的9尾小头裸裂尻鱼进行微生物样品采集，分别取皮肤黏膜、肠道内容物和肠道黏膜微生物样本，具体方法如下：用无菌磷酸缓冲盐溶液(phosphate-buffered saline, PBS)冲洗鱼体表面3次，用2–3个拭子粘取皮肤上的微生物置于2 mL的冻存管中；取出鱼的肠管，挤出其中内容物置于2 mL的冻存管中；之后进行纵向解剖，经无菌PBS冲洗肠道内部3次后，用2–3个拭子粘黏膜定殖微生物置于2 mL的冻存管；冻存管投入液氮中保存带回实验室，用干冰送上海美吉生物医药科技有限公司进行扩增子高通量测序分析。

1.2 DNA抽提和PCR扩增

根据DNeasy® PowerSoil® Pro Kit Handbook说明书进行微生物群落总DNA抽提，使用上游引物338F (5′-ACTCCTACGGGAGGCAGCAG-3′)和下游引物806R (5′-GGACTACHVGGGTWTCTAAT-3′)对16S rRNA基因V3–V4可变区进行PCR扩增，扩增程序：95 ℃ 3 min；95 ℃ 30 s，55 ℃ 30 s，72 ℃ 30 s，27个循环；72 ℃ 10 min，最后在4 ℃进行保存。PCR反应体系：5×TransStart Fast*Pfu*缓冲液4 μL，2.5 mmol/L dNTPs 2 μL，上、下游引物(5 μmol/L)各0.8 μL，TransStart Fast*Pfu* DNA聚合酶0.4 μL，模板DNA 10 ng，ddH_2O补足至20 μL。

1.3 Illumina MiSeq测序

将同一样本的PCR产物混合后使用2%琼脂糖凝胶回收PCR产物，利用AxyPrep DNA Gel Extraction Kit (Axygen Biosciences试剂盒)进行产物回收纯化，2%琼脂糖凝胶电泳检测，并用Quantus™ Fluorometer (Promega)对回收产物进行检测定量。使用TruSeqIM DNA Sample Prep Kit进行建库，利用Illumina公司的MiSeq PE300平台进行测序，原始数据上传至国家微生物科学数据中心(NMDC)数据库(编号：NMDC40021815–NMDC40021832)。

1.4 数据处理及生物信息学分析

使用fastp (https://ICthub.com/OpenGene/fastp，version 0.19.6)软件对原始测序序列进行质控，使用FLASH (https://ccb.jhu.edu/software/FLASH/index.shIMl，version 1.2.11)软件进行拼接：

(1) 过滤reads尾部质量值20以下的碱基，设置50 bp的窗口，如果窗口内的平均质量值低于20，从窗口开始截去后端碱基，过滤质控后50 bp以下的reads，去除含N碱基的reads；

(2) 根据PE reads之间的重叠(overlap)关系，将成对reads拼接(merge)成一条序列，最小重叠长度为10 bp；

(3) 拼接序列的重叠区允许的最大错配比率为0.2，筛选不符合序列；

(4) 根据序列首尾两端的barcode和引物区分样品，并调整序列方向，barcode允许的错配数为0，最大引物错配数为2。

使用Uparse软件，根据97%的相似度对序列进行分类操作单元(operational taxonomic unit,

OTU)聚类并剔除嵌合体。利用 RDP classifier 对每条序列进行物种分类注释，比对 Silva 16S rRNA 数据库，设置比对阈值为 70%。

按最小样本序列数抽平后，采用 Venn 图比较不同部位间的物种分布差异，α 多样性指数组间差异显著性检验采用 Student's *t* 检验；利用 QIIME (1.9.1)软件计算 β 多样性 Bray-Curtis 距离矩阵，使用非加权组平均法(unweighted pair-group method with arithmetic means, UPGMA)构建样本等级聚类树，相同距离矩阵下进行主坐标分析(principal co-ordinates analysis, PCoA)并进行相似性组分检验，比较不同样品细菌群落结构的相似性和差异程度；利用 LEfSe 软件，寻找不同样品中的差异物种，并结合 LDA 线性判别分析评估这些差异物种对组间差异影响的大小；采用 R 语言统计和绘图，分析不同样品门、科、属水平上的优势物种组成情况，在门水平上采用 Kruskal-Wallis 秩和检验的方法进行多组比较，在科和属水平上采用 Wilcoxon 秩和检验的方法两两进行比较。最后基于 Tax4Fun 数据库进行不同样本菌群功能预测。以上分析在美吉生物云平台(https://cloud.majorbio.com)上完成。

2 结果与分析

共采集 27 个样品，其中 DNA 提取及每条鱼 3 个部位测序成功的样品共 18 个，测序所得的序列用 fastp 和 FLASH 软件进行预处理，18 个样本总共获得 958 254 条合格的 16S rRNA 基因序列，每个样本产生的有效序列数目为 11 327– 81 125，有效序列平均长度为 416 bp。对所有序列进行 97%相似水平 OTU 划分并物种注释，可划分为 4 587 个 OTU，分类为 45 个门、104 个纲、275 个目、527 个科、1 124 个属、2 056 个种。

2.1 小头裸裂尻鱼不同部位微生物物种组成分析

小头裸裂尻鱼不同部位属水平微生物的 Venn 图分析显示(图 1A)，3 个部位共有的菌属有 432 个，皮肤黏膜、肠道黏膜和肠道内容物

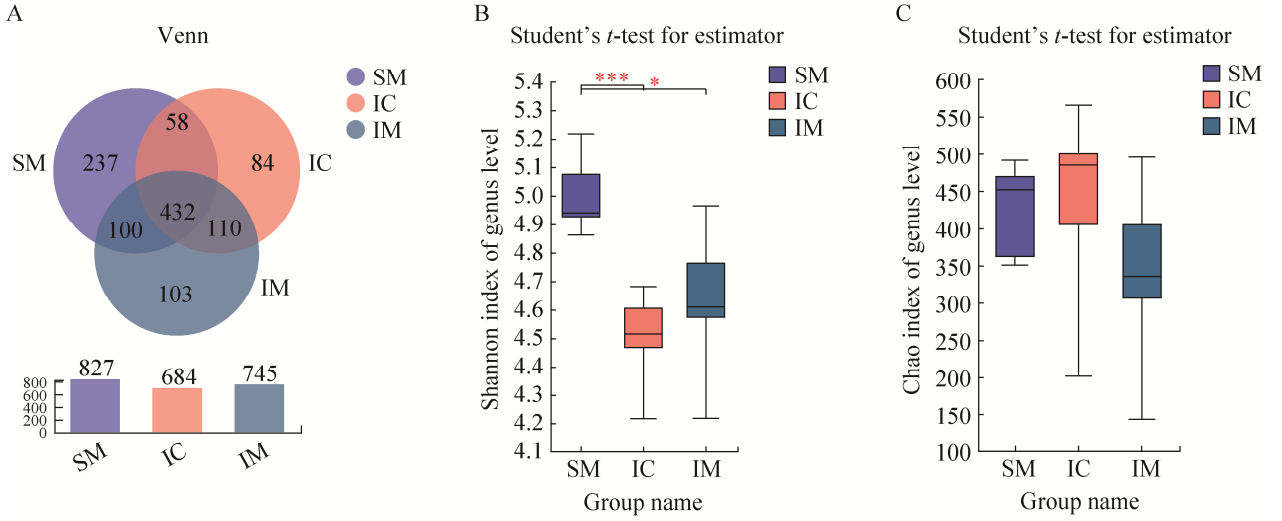

图 1 小头裸裂尻鱼皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)微生物属水平的 Venn 图(A)和 α 多样性指数组间差异检验(B、C)

Figure 1 Venn diagram in genus level (A) and alpha diversity difference test between index groups (B, C) of skin mucosa (SM), intestinal mucosa (IM) and intestinal contents (IC) of *Herzensteinia microcephalus*. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

两两共有的菌属分别为 100、110 和 58 个。皮肤黏膜部位特有菌属数量最多，为 237 个，肠道黏膜和肠道内容物特有属相对较少，分别为 103 和 84 个，该结果表明小头裸裂尻鱼皮肤黏膜微生物多样性较高。通过 Shannon 指数(图 1B)和 Chao 指数(图 1C)对 3 个部位的微生物的多样性和丰富度进行比较，结果显示肠道黏膜微生物多样性显著低于皮肤黏膜(P<0.05)，肠道内容物微生物多样性极显著低于后者(P<0.001)，3 个部位的微生物丰富度并未显示出差异性。

2.2 小头裸裂尻鱼不同部位菌群差异分析

对小头裸裂尻鱼皮肤黏膜、肠道黏膜和肠道内容物 3 个部位的微生物群进行 β 多样性分析，探索微生物组成的相似性与差异性。基于 Bray Curtic 的 PCoA 分析(图 2A)显示，肠道黏膜和肠道内容物样本之间有重叠，说明二者菌

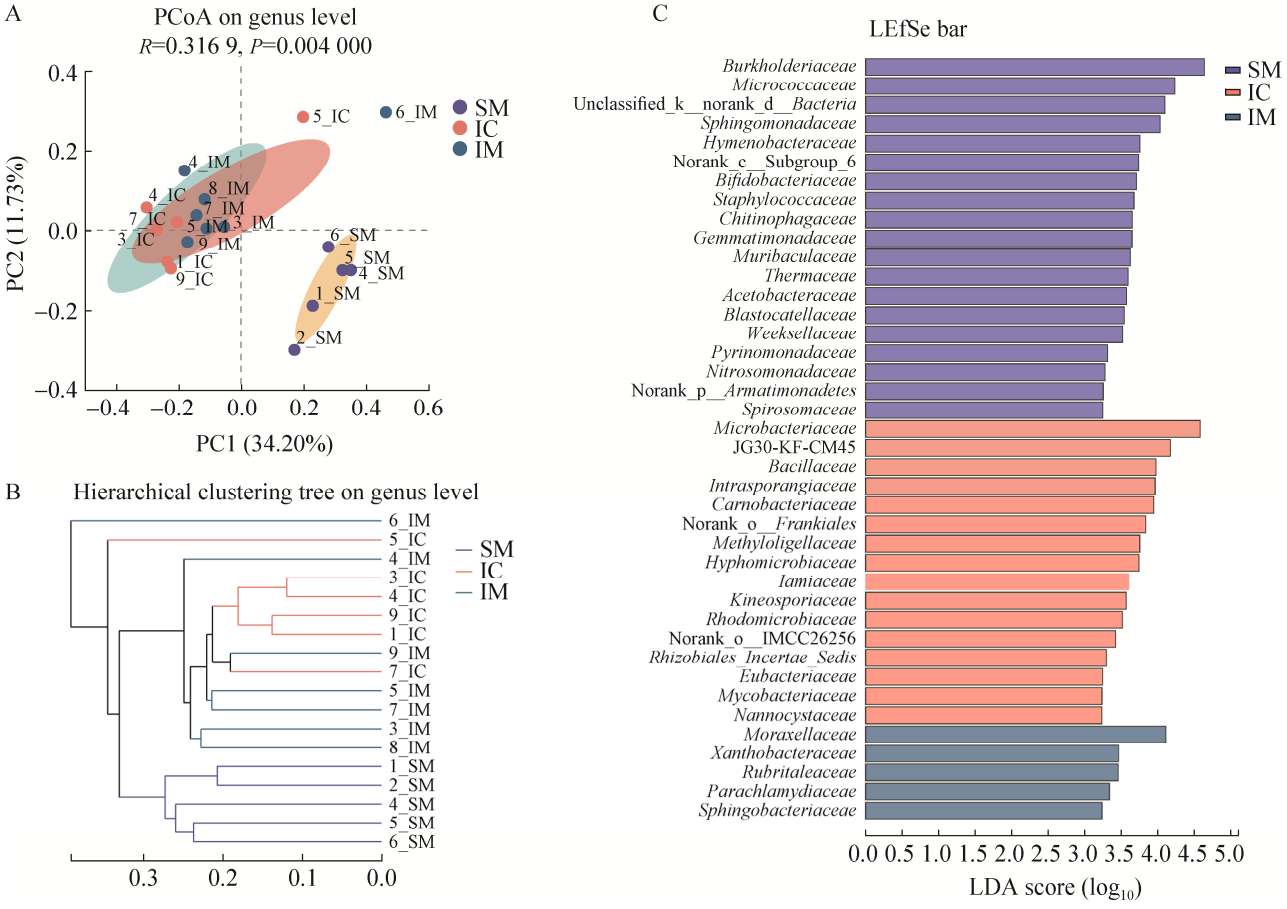

图 2　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)中微生物的 β 多样性(A、B)和物种差异(C)

Figure 2　Beta diversity (A, B) and species difference (C) of bacteria at skin mucus (SM), gut mucus (IM) and intestinal contents (IC). A: X-axis and Y-axis represent the two selected spindles, and percentage represents the value of the explanation degree of the spindles to the difference in the composition of bacteria in different parts. The scale of X axis and Y axis is relative distance, which has no practical significance. Each point represents a sample, and points of the same color and shape come from the same group. B: The length between branches represents the distance between samples, and different colors represent different parts. C: The LDA discriminant histogram statistics the microbial groups with significant effects in multiple groups. The LDA score obtained through LDA analysis (linear regression analysis), the larger the LDA score, the greater the impact of species abundance on the difference effect (LDA score>3.2).

群差异较小，而皮肤黏膜与二者之间的菌群存在极显著差异(P<0.01)。在相同距离算法下通过 Average 聚类方式对所有样本进行层级聚类分析(图 2B)结果显示，大体可分为 2 支，其中皮肤黏膜样本聚为一支，肠道黏膜和肠道内容物样本之间有个别样本出现交叉聚为一支，表明皮肤与肠道微生物之间差异显著，而肠道黏膜与肠道内容物微生物个别样品有交叉。在 LDA 分析(图 2C)中发现皮肤黏膜上有大量差异显著物种，有 19 个，而肠道黏膜有 16 个，肠道内容物有 5 个。

2.3 小头裸裂尻鱼不同部位的优势菌群分析

小头裸裂尻鱼皮肤黏膜、肠道黏膜和肠道内容物中丰度前五的优势菌门(图 3A)均为放线菌门(*Actinobacteria*)、变形菌门(*Proteobacteria*)、厚壁菌门(*Firmicutes*)、绿弯菌门(*Chloroflexi*)和蓝藻门(*Cyanobacteria*)，且 5 个优势菌门之和占比均大于 75%，但是在不同部位的占比存在差异。经 Kruskal-Wallis 秩和检验，放线菌门(*Actinobacteria*)在肠道内容物(46.53%)和肠道黏膜(29.23%)中差异显著，在肠道内容物和皮肤黏膜(25.83%)中的差异极显著(P<0.05，P<0.01，图 3B)；变形菌门(*Proteobacteria*)在不同部位占比大小依次为肠道黏膜(40.33%)>皮肤黏膜(34.14%)>肠道内容物(26.10%)，仅肠道黏膜和肠道内容物之间存在显著差异(P<0.05，图 3C)。

在科水平(图 4A)和属水平(图 4B)上分别选择各部位相对丰度前十的优势类群进行分析，结果显示，红杆菌科(*Rhodobacteraceae*)、*Clostridiaceae*_1、动球菌科(*Planococcaceae*)和 JG30-KF-CM45 在 3 个部位占比均较高，伯克氏菌科(*Burkholderiaceae*)、拜叶林克氏菌科(*Beijerinckiaceae*)和莫拉菌科(*Moraxellaceae*)在 2 个黏膜部位的丰度较高，*Microbacteriaceae*、*Nocardioidaceae* 和假单胞菌科(*Pseudomonadaceae*)在肠道中的丰度较高，仅有微球菌科(*Micrococcaceae*)在皮肤黏膜和肠道内容物中占比均较高。

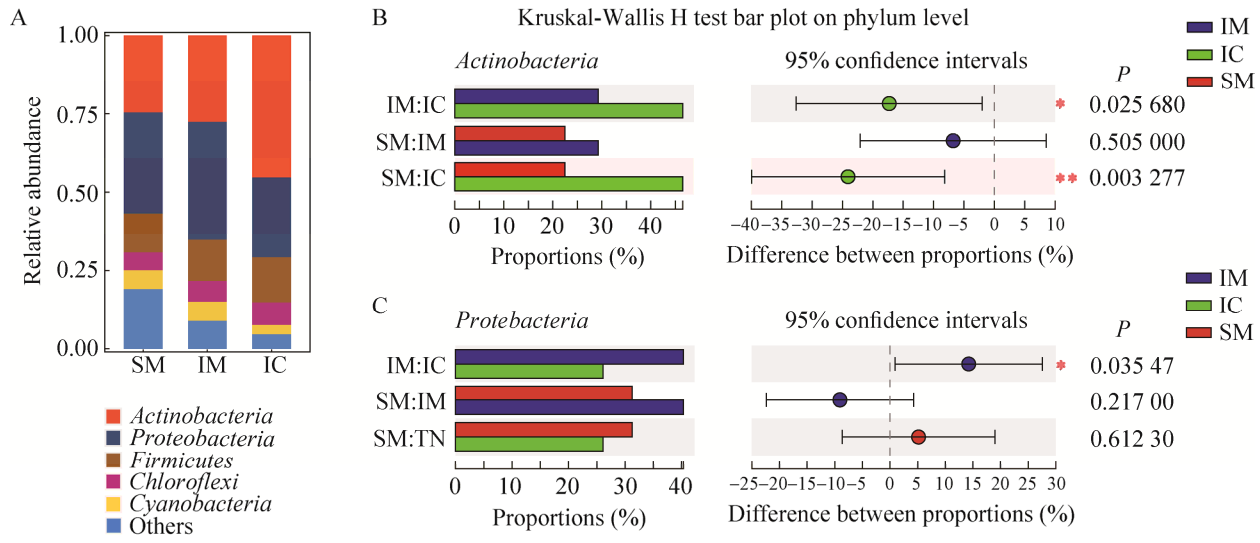

图 3 皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)微生物在门水平的优势类群(A)和差异显著菌的 K-W 检验(B、C)

Figure 3 The dominant species of bacteria in skin mucus (SM), gut mucus (IM) and intestinal contents (IC) at phylum (A) level and Kruskal-Wallis test of bacteria with significant difference (B, C). *: P<0.05; **P<0.01.

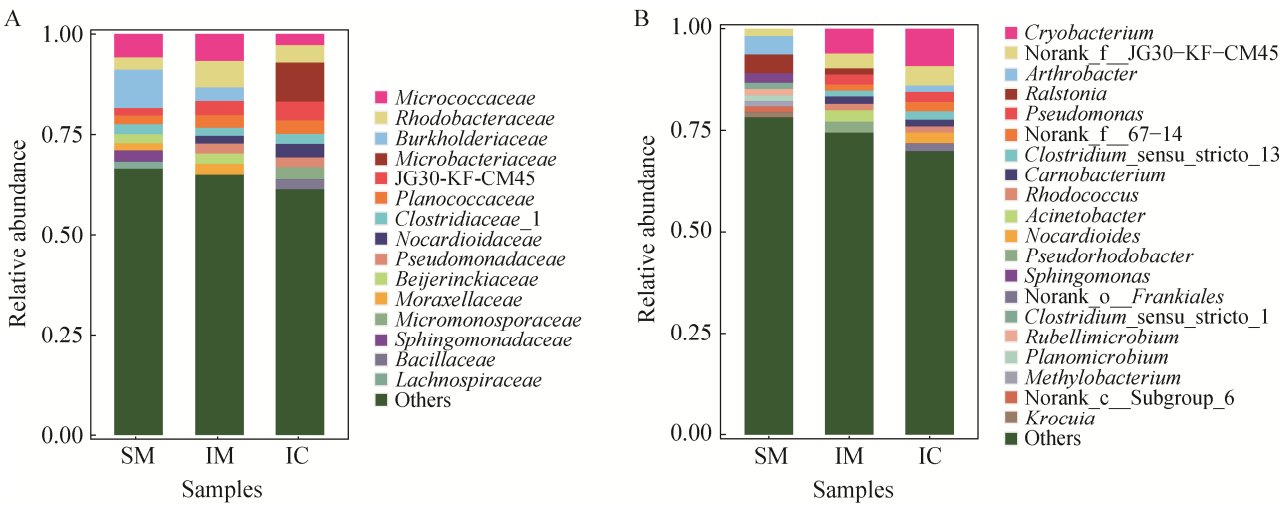

图 4　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)微生物在科水平(A)、属水平(B)的优势类群

Figure 4　The dominant species of bacteria in skin mucus (SM), gut mucus (IM) and intestinal contents (IC) at family (A) and genus (B) level.

在属水平上，三者共有优势菌仅有一个JG30-KF-CM45 科中未知分类的属。此外，青枯菌属(*Ralstonia*)在皮肤黏膜和肠道黏膜中的相对丰度均较高，节杆菌属(*Arthrobacter*)在皮肤黏膜和肠道内容物中的相对丰度较高，*Cryobacterium*、红球菌属(*Rhodococcus*)、假单胞菌属(*Pseudomonas*)、*Clostridium*_sensu_stricto_13、肉杆菌属(*Carnobacterium*)和 67-14 科中的一个未知属主要分布于肠道中，而在皮肤黏膜上特有菌较多，如考克氏菌(*Kocuria*)和鞘氨醇单胞菌属(*Sphingomonas*)等。

进一步对不同部位优势菌科差异进行Wilcoxon秩和检验，结果显示，皮肤黏膜和肠道黏膜中有3个差异显著菌(图5A)，其中微球菌科(*Micrococcaceae*)差异极显著(P<0.01)；皮肤黏膜和肠道内容物中有6个差异显著菌(图5B)，其中伯克氏菌科(*Burkholderiaceae*)和芽胞杆菌科(*Bacillaceae*)差异极显著(P<0.001)；肠道黏膜和肠道内容物中仅有2个差异显著菌莫拉菌科(*Moraxellaceae*)和鞘脂单胞菌科(*Sphingomonadaceae*)（图5C，P<0.05)。

对 3 个部位优势菌属差异进一步进行Wilcoxon 秩和检验，结果显示，尽管部分菌在不同部位丰度均比较高，但彼此之间也显示出了差异显著性。在皮肤黏膜和肠道黏膜(图 6A)、皮肤黏膜和肠道内容物(图 6B)以及肠道黏膜和肠道内容物(图 6C)之间差异显著优势菌分别为 9 个、5 个和 2 个。其中冷杆菌属(*Cryobacterium*)、节杆菌属(*Arthrobacter*)、微红球菌属(*Rubellimicrobium*)和肉食杆菌属(*Carnobacterium*)在皮肤黏膜与另外两者之间均存在显著差异(P<0.05)；且节杆菌属(*Arthrobacter*)在肠道黏膜和皮肤黏膜之间，肉食杆菌属(*Carnobacterium*)在皮肤黏膜和肠道内容物之间皆差异极显著(P<0.01)。不动杆菌属(*Acinetobacter*)的丰度在肠道内容物中显著高于皮肤黏膜(P<0.05)，极显著高于肠道黏膜(P<0.01)。

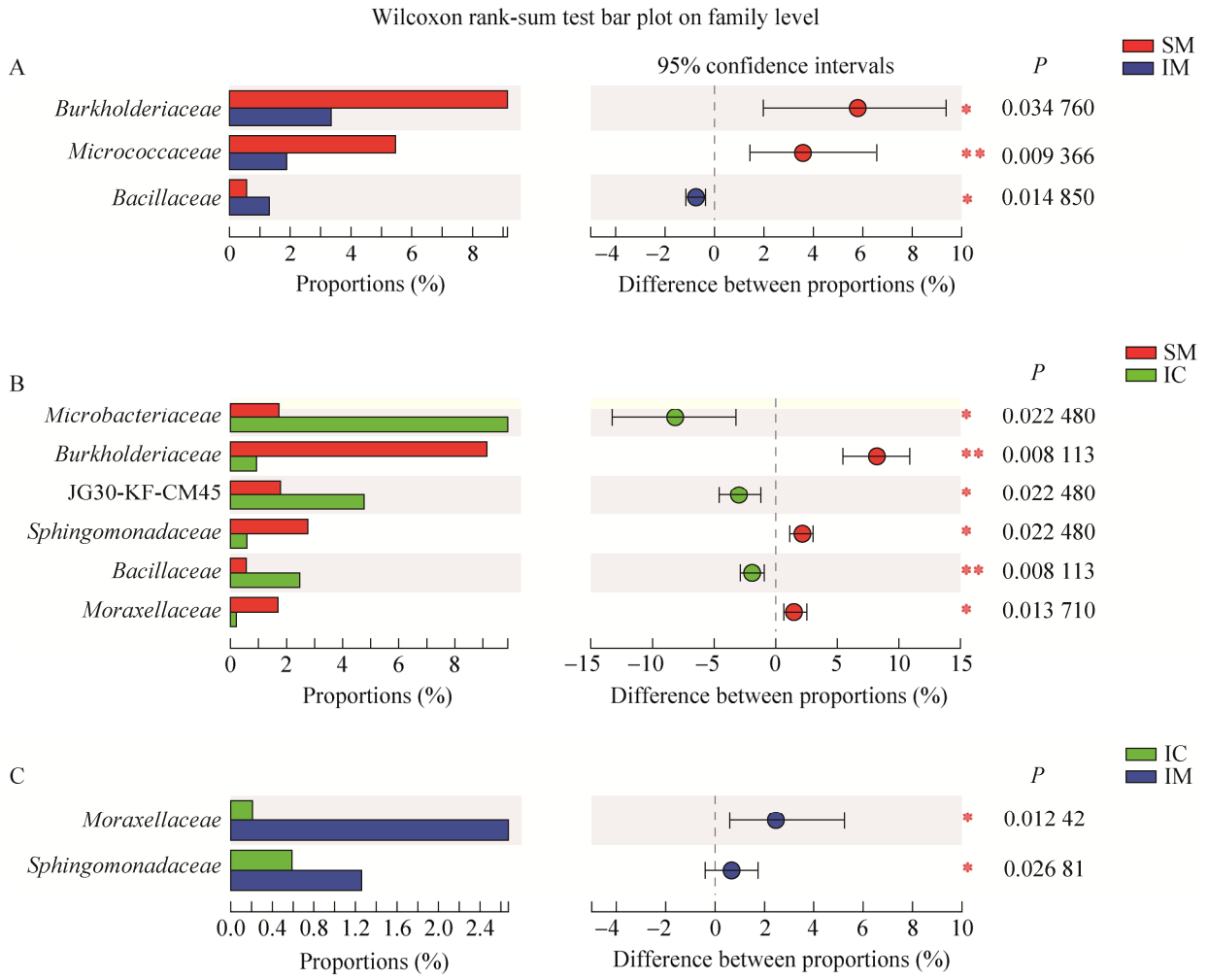

图5　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)科水平差异显著优势物种

Figure 5　The family level of skin mucosa (SM), intestinal mucosa (IM) and intestinal contents (IC) were significantly different in the dominant species. A: Skin mucosa and intestinal mucosa were significantly different dominant species. B: Skin mucosa and intestinal contents were significantly different dominant species. C: Intestinal mucosa and intestinal contents were significantly different dominant species. *: $P<0.05$; **: $P<0.01$.

2.4　小头裸裂尻鱼不同部位菌群基因功能预测及优势菌功能分析

基于 Tax4Fun 对小头裸裂尻鱼不同部位菌群的潜在功能进行预测，结果表明，3 个部位 KEGG pathway 2 丰度基本相似(图 7)，功能基因主要参与碳水化合物代谢(carbohydrate metabolism)、氨基酸代谢(amino acid metabolism)和膜运输(membrane transport)相关通路。利用 heatmap 图展示 KEGG 第 3 层级上的基因功能差异(图 8)，选择丰度前四十的功能进行分析，发现肠道内容物菌群在脂肪酸合成(ko00061)、萜类化合物生物合成通路(ko00900)和非核糖体肽结构(ko01054)等中富集较高，皮肤黏膜菌群在 ABC 转运蛋白(ko02010)、核蛋白体(ko03010)

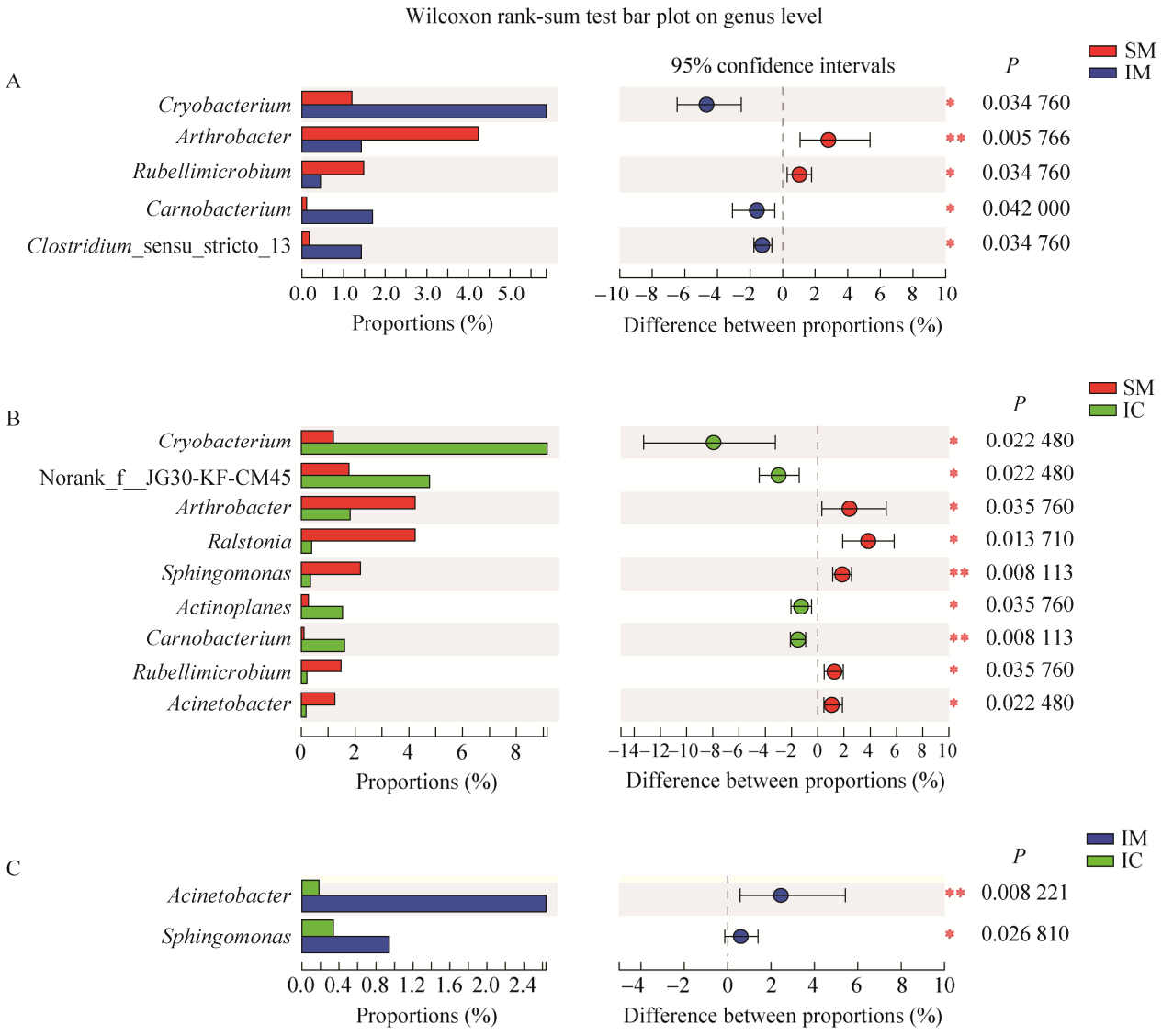

图6　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)属水平差异显著优势物种

Figure 6　The genus level of skin mucosa (SM), intestinal mucosa (IM) and intestinal contents (IC) were significantly different in the dominant species. A: Skin mucosa and intestinal mucosa were significantly different dominant species. B: Skin mucosa and intestinal contents were significantly different dominant species. C: Intestinal mucosa and intestinal contents were significantly different dominant species. *: $P<0.05$; **: $P<0.01$.

和组氨酸代谢(ko00340)等通路中富集较高，肠道黏膜菌群中则以信号转导(ko02020)和甘油磷脂代谢(ko00564)等通路富集较高。

对小头裸裂尻鱼不同部位优势菌的相关功能进行统计(表 1)，17 个菌属均表现出了对复杂生存环境的适应性，其中多数菌属表现出对有害物质的降解作用。肠道部分的优势菌中，已知菌功能体现出降解作用及抑菌作用；黏膜部位优势菌如青枯菌属(*Ralstonia*)存在感染性菌株，同时也可降解有毒物质、吸附重金属。

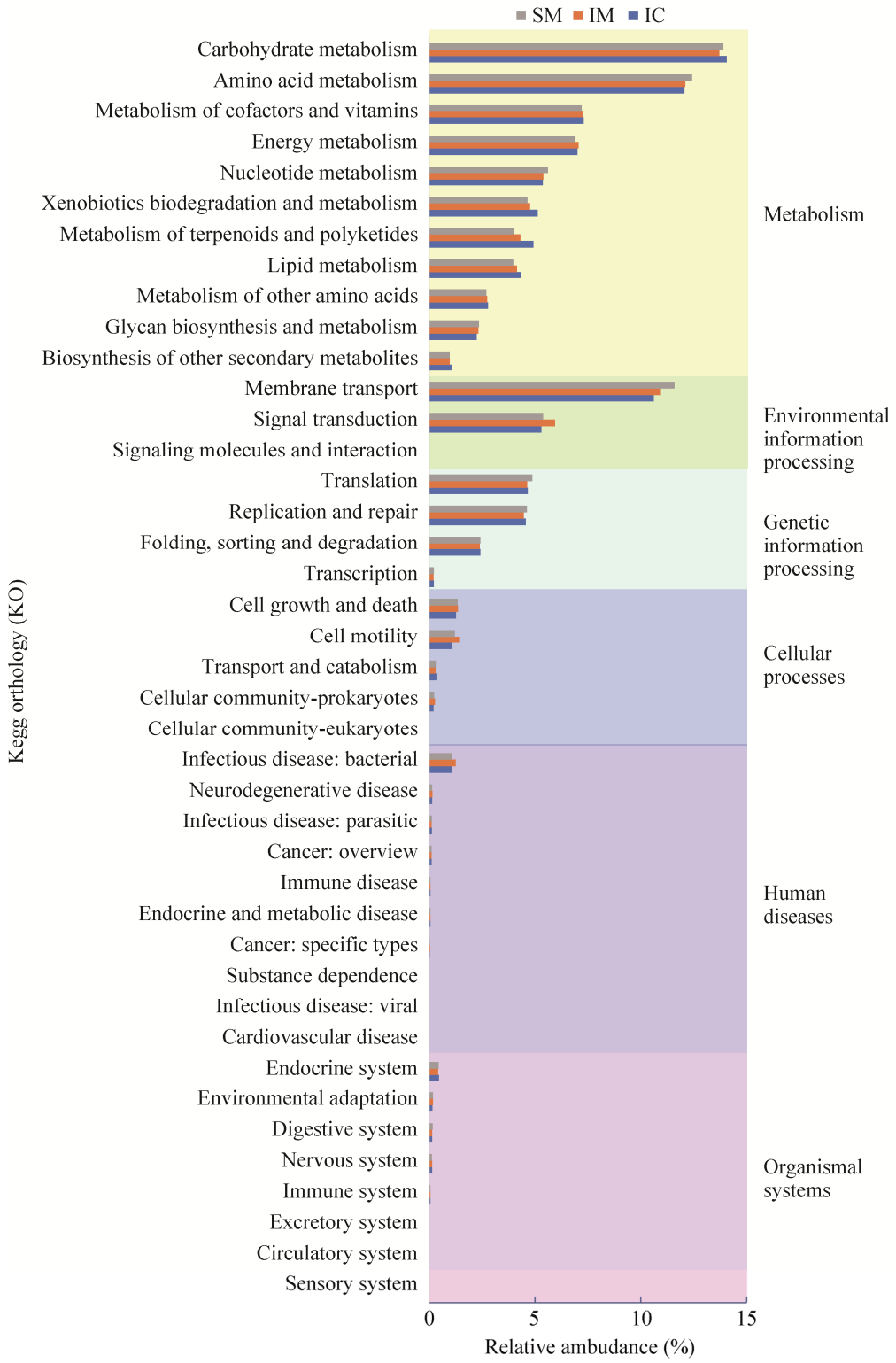

图 7　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)功能基因在 KEGG 第二层的注释结果

Figure 7　Annotation results of functional genes of skin mucosa (SM), intestinal mucosa (IM) and intestinal contents (IC) in the second layer of KEGG.

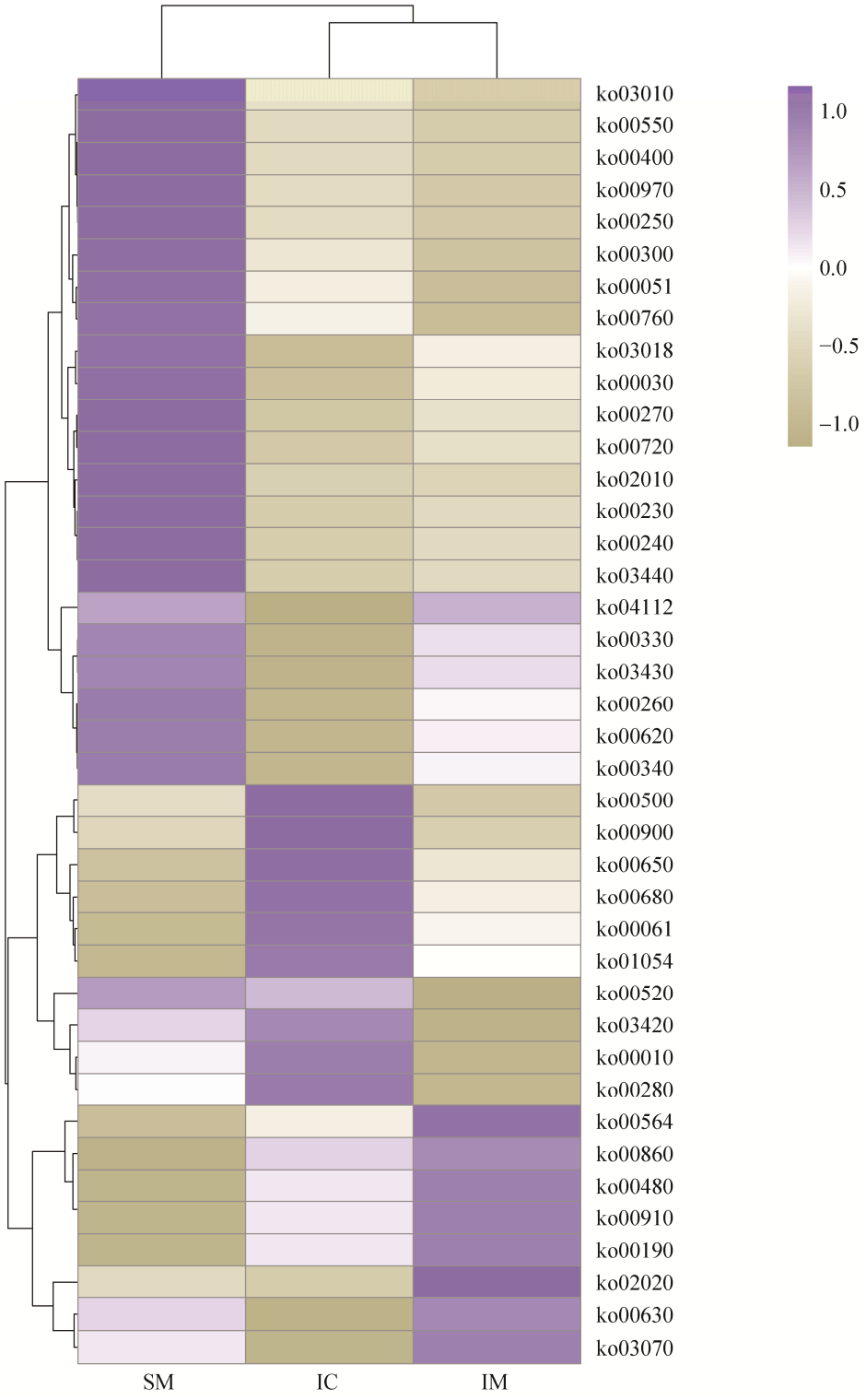

图 8　皮肤黏膜(SM)、肠道黏膜(IM)和肠道内容物(IC)功能基因在 KEGG 第三层的注释结果

Figure 8　Annotation results of functional genes of skin mucosa (SM), intestinal mucosa (IM) and intestinal contents (IC) in the third layer of KEGG.

表 1　小头裸裂尻鱼 3 个部位优势菌的功能分析

Table 1　Functional analysis of dominant bacteria from three parts of *Herzensteinia microcephalus*

Groups	Genus	Functional description
SM, IC	*Arthrobacter*	With strong environmental adaptability and stress resistance, it is widely distributed in various environments, especially in soil and water. It can effectively degrade some organic compounds by metabolizing some pollutants in the environment as its energy source[17-19].
SM, IM	*Ralstonia*	Strains of this genus are well adapted to low nutrient environments, can cause infection under certain conditions, and can effectively degrade toxic organic compounds, as well as adsorb heavy metals[20-21].
IM, IC	*Cryobacterium*	Strains of this genus are glacial cryogenic bacteria, mostly derived from soil, glacier, or base environment. Due to the complexity of its living environment, its physiological characteristics also show rich diversity[22].
IM, IC	*Rhodococcus*	The strain can effectively degrade petroleum, polycyclic aromatic hydrocarbons, and other environmental pollutants, and some strains showed a good degradation effect at low temperatures[23].
IM, IC	*Carnobacterium*	This genus is found in the intestines of hairtail and has obvious bacteriostasis[24].
IM, IC	*Pseudomonas*	Has the degradation effect, can prevent disease and promote growth, in the protection of the intestinal tract also plays a certain role[25-26].
IM, IC	*Clostridium*_sensu_stricto_13	Fermentation-related bacteria[27].
IC	*Nocardioides*	Strains of this genus were found in plants from the Qinghai-Tibet Plateau, which possess genes related to low-temperature adaptation, radiation tolerance, and the synthesis of secondary metabolites with physiological functions[28].
IC	*Actinoplanes*	The strain of this genus was found to be involved in the biosynthesis of acarbose deoxyaminosaccharide units, which can be used in the treatment of type ii diabetes[29-30].
IM	*Pseudorhodobacter*	This strain can degrade lignin and produce gambochlotin with broad-spectrum antibacterial activity, which can inhibit the growth of pathogenic bacteria[31-32].
IM	*Acinetobacter*	It has metabolic diversity and can degrade caprolactam, herbicides, organophosphorus pesticides, and a variety of petroleum hydrocarbon components, and other pathogenic bacteria[33].
SM	*Rubellimicrobium*	The genus can decompose cellulose, lignin, polycyclic aromatic hydrocarbons, and other macromolecular organic matter[34].
SM	*Sphingomonas*	The strain of this genus has a significant degradation effect on some environmental pollutants and has the characteristics of high efficiency, fast propagation, and no pollution[35].
SM	*Methylobacterium*	It can grow with single carbon or non-C-C bond low carbon compounds (such as methane, methanol, formaldehyde, etc.) as substrates, and can produce a variety of metabolites[36-37].
SM	*Kocuria*	Most of them are aerobic bacteria, widely distributed in the natural environment, there is a certain degree of infection on human skin, because of its radiation resistance and strong stability in different environmental conditions[38-39].
SM	*Clostridium*_sensu_stricto_1	It is related to the content of flavonoids[40].
SM	*Planomicrobium*	The strains can produce protease, which acts as metabolism and hydrolysis[41].

3　讨论与结论

研究表明，微生物在不同部位表现出不同的菌群结构和功能，肠道微生物主要与生物的生理特性相关[42]，皮肤黏膜微生物主要与环境相关，且易受环境影响[43]。但与陆生动物皮肤外层凋亡的上皮细胞不同，鱼表皮细胞具有活性，可以分泌黏液，其中有许多微生物，生物结构和功能与肠道黏膜更为相似[44]。本研究对小头裸裂尻鱼 3 个部位的微生物种类进行鉴

定、分析结果显示，在鱼体不同部位之间菌群结构存在相似性和差异性。其中皮肤黏膜微生物多样性最大，且在微生物群落结构上与另外 2 个部位表现出显著差异，有研究发现肠道微生物易受环境影响，但在其进化过程中主要还与肠道结构和营养物质等相关[45-46]，而皮肤黏膜受环境影响程度大于肠道[46]，因此我们推测这一差异是由于其受外界环境影响较大导致的。

对小头裸裂尻鱼不同部位微生物在门、科、属水平的差异分析显示，其优势菌门与大多数鱼类研究相似，皮肤黏膜、肠道黏膜及肠道内容物三者之间共有或特有的菌群结构表现出与其所在部位发挥作用存在密切相关性。其他学者对鱼类肠道微生物优势菌的研究中发现，在门水平上，石首鱼为变形菌门(*Proteobacteria*)、拟杆菌门(*Bacteroidetes*)和厚壁菌门(*Firmicutes*)[47]；欧洲鳗鲡为变形菌门(*Proteobacteria*)和梭杆菌门(*Fusobacteria*)[48]，野生大西洋鲑为厚壁菌门(*Firmicutes*)和放线菌门(*Actinobacteria*)[49]，大多数鱼类的肠道中以变形菌门(*Proteobacteria*)和厚壁菌门(*Firmicutes*)为主[50]。在对皮肤黏膜微生物的研究中，人工饲养的虹鳟皮肤上发现了以变形菌门(*Proteobacteria*)和拟杆菌门(*Bacteroidetes*)为主的菌群[51]。而在小头裸裂尻鱼中，我们发现肠道及皮肤菌群中均是放线菌门(*Actinobacteria*)和变形菌门(*Proteobacteria*)为主要的优势菌门，拟杆菌门的占比极小，可能与小头裸裂尻鱼生存于高原低温、低氧以及食物相当匮乏的极端环境相关。因为变形菌门(*Proteobacteria*)具有多种生理功能，可以利用大量的碳源，并在宿主的能量积累中发挥重要作用[52]，与宿主的生理特性关系更为紧密；而放线菌是一种潜在的天然抗生物膜制剂[53]，对多种致病菌具有抗生物膜作用，且海洋放线菌具有抗菌、抗癌、抗病毒、杀虫和酶抑制等多种生物学特性[54]，我们推测，因野外生存环境较为复杂，此类菌群附着在鱼类皮肤上保护鱼类免受致病菌入侵。此外，放线菌还可以作为内生真菌在植物组织中存活，并促进营养物质的同化和生长[55]，肠道内容物中的放线菌门(*Actinobacteria*)占比达 45.40%，可能与其食性相关，在摄食过程中是否偏向于富含此类微生物的食物尚待研究。

经基因功能注释，在 KEGG pathway 2 上 3 个部位的功能基本一致，主要基因功能均与维持生物体正常的生命活动相关；而在第三层级上丰度较高基因功能的比较分析，发现部分肠道内容物菌群的基因功能与植物相关，推测可能是因为小头裸裂尻鱼以植物为食，此类微生物附着于食物进而转移至鱼类肠道；ABC 转运蛋白在生物体中发挥着重要的生理作用[56]，而该功能在皮肤黏膜中的丰度高于其他部位，这可能与微生物所处皮肤黏液的复杂环境有关。

在对 3 个部位微生物群基因功能预测的结果中显示，参与代谢相关功能的菌群占比最大，推测部分优势菌在黏膜定殖，主要参与降解有毒物质以及在黏膜上形成保护屏障。如皮肤黏膜中的考克氏菌属(*Kocuria*)有抗辐射作用，而青枯菌属(*Ralstonia*)有降解有毒物质和吸附重金属的特性，这可能与高原紫外线辐射较强和水体重金属超标，而高原鱼类能适应如此环境有关[57]。此外，有研究发现在哺乳动物的肠道中有 2 种不同的黏液层，外层富含微生物群，内层基本为无菌状态[58]，这一分层现象可能与宿主的免疫作用相关，外层黏液发挥保护作用，而内层发挥隔离作用，在鱼的 2 个黏膜部位也存在内外黏液层，可能也形成类似的保护机制。

此外，肠道黏膜菌群也可以起到帮助降解和吸收营养物质的作用，同时也与动物的免疫作用相关[59]。本研究在含量较为丰富的

优势菌中发现了抑菌微生物，如假红杆菌属(*Pseudorhodobacter*)、假单胞菌属(*Pseudomonas*)和肉杆菌属(*Carnobacterium*)，其中假单胞菌属(*Pseudomonas*)和肉食杆菌属(*Carnobacterium*)也是肠道内容物中的优势菌，此类微生物的存在可能与小头裸裂尻鱼的摄食相关。无论是皮肤黏膜还是肠道黏膜，黏液主成分为黏蛋白，在对肠道微生物的研究中发现，许多病原体使用黏液衍生的糖作为关键营养素，部分益生菌通过与其形成竞争关系而对宿主形成保护作用，如鼠杆菌科(*Muribaculaceae*)[10]。而本研究在皮肤中的差异显著物种(图 2C)中发现了鼠杆菌科(*Muribaculaceae*)，推测在小头裸裂尻鱼相关黏膜部位除优势菌外，非优势菌也起到一定保护作用，且此类菌群在鱼类的皮肤中可能形成了与哺乳动物肠道黏膜相似的保护机制。

小头裸裂尻鱼是一种国家保护动物，且难以进行人工繁殖，本研究对高原鱼类相关寄生菌群进行研究，分析其皮肤及肠道菌群结构，解析对于环境适应的菌群特征，对了解高寒地区生物对于极端环境的适应，特别是濒危物种的保护具有重要价值，对未来高度特化等级裂腹鱼的保护提供一定的参考。

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