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应用 PCR-DGGE 和 Q-PCR 分析不同体重斑点叉尾鮰皮肤、鳃和胃肠道菌群结构

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摘要: 采用聚合酶链式反应−变性梯度凝胶电泳(PCR-DGGE)和荧光定量−聚合酶链式反应(Q-PCR)技术分析高体重(high weight, HW)和低体重(low weight, LW)斑点叉尾鮰(*Ietalurus punetaus*)皮肤、鳃和胃肠道菌群多样性, 为斑点叉尾鮰微生态研究及筛选斑点叉尾鮰源益生菌提供理论依据。结果显示, 斑点叉尾鮰菌群丰富度由低到高依次为鳃、皮肤、前肠、后肠和胃。肠杆菌科(Enterobacteriaceae)和气单胞菌属(*Aeromonas*)是皮肤的优势菌群; 肠杆菌科(Enterobacteriaceae)、气单胞菌属(*Aeromonas*)和肠球菌属(*Enterococcus*)是水体、鳃和胃的优势菌群; 肠杆菌科(Enterobacteriaceae)、拟杆菌属(*Bacteroidetes*)、气单胞菌属(*Aeromonas*)和酵母菌属(*Saccharomyces*)是肠道的优势菌群。HW 斑点叉尾鮰鳃菌群的香农多样性指数、均匀度和丰富度及前肠菌群的丰富度显著高于 LW 斑点叉尾鮰(P<0.05)。皮肤的黄杆菌属(*Flavobacterium*), 胃的肠杆菌科(Enterobacteriaceae), 前肠的拟杆菌属(*Bacteroidetes*)和双歧杆菌属(*Bifidobacterium*)及后肠的拟杆菌属(*Bacteroidetes*)、双歧杆菌属(*Bifidobacterium*)和酵母菌属(*Saccharomyces*)的拷贝数分别是 $10^{1.97}$、$10^{7.69}$、$10^{6.19}$、$10^{3.83}$、$10^{6.13}$、$10^{3.92}$ 和 $10^{4.26}$, 均显著高于 LW 斑点叉尾鮰(P<0.05)。结果表明, 斑点叉尾鮰皮肤、鳃、胃肠道均形成独特的菌群结构, LW 和 HW 斑点叉尾鮰菌群结构存在明显差异, HW 斑点叉尾鮰菌群多样性增加。

关键词: 斑点叉尾鮰; PCR-DGGE; Q-PCR; 皮肤菌群; 鳃菌群; 肠道菌群
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斑点叉尾鮰(*Ietalurus punetaus*)原产于美洲, 是全世界公认的适合淡水养殖的优良品种。1984 年湖北省水产研究所首次从美国引进该物种, 通过多年的试养与推广, 已成为中国普遍养殖的一种重要经济鱼类, 但在斑点叉尾鮰规模化养殖中, 暴发性死亡时有发生, 严重影响其产量和经济效益[1]。目前, 对鱼类疾病的防治主要是用抗生素类药物[2], 而抗生素的弊端凸显, 寻求安全有效的替代物成为研究热点。益生菌能分泌细菌抑制物, 提供营养成分, 促进消化吸收, 增强免疫力, 改善水质和维持肠道平衡[3−4]等, 更利于水产养殖业的可持续发展。宿主正常菌群是益生菌的主要来源。目前, 渔业动物正常微生物的研究多在胃肠道, 且大多采用活菌培养法, Luo 等[5]用平板培养法筛选出斑点叉尾鮰源枯草芽孢杆菌 BHl344, 但未对其菌群结构进行研究; 而对鱼类鳃和皮肤的微生态, 以及比较分析不同体重鱼类的微生态的研究较少。此外, 现代分子技术已成为研究微生态的主要技术手段, Fang 等[6]采用 PCR- DGGE 技术较全面揭示了益生菌对匙吻鲟肠道菌群结构的影响。因此, 本研究采用 PCR-DGGE 和 Q-PCR 技术, 对比分析低体重(low weight, LW)和高体重

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(high weight, HW)斑点叉尾鮰鳃、皮肤和胃肠道细菌的结构, 及其与水体菌群的相关性, 为今后斑点叉尾鮰皮肤、鳃和胃肠道微生态的研究和斑点叉尾鮰源益生菌的筛选提供理论依据。

1　材料与方法

1.1　实验动物和样品采集

实验用鱼购于四川省成都市温江区某养殖场, 选择同一规格的鱼苗和养殖在同一池塘内的斑点叉尾鮰50 尾, 淘汰畸形和状态不好的个体, 根据体重分为低体重和高体重群体, 然后随机从低体重和高体重群体中分别选取 LW 和 HW 斑点叉尾鮰各 6 尾, 平均体重差异显著(P<0.05), 分别为(40.24±1.03) g 和(48.87±1.43) g。无菌生理盐水冲洗 3 次, 无菌棉签擦拭鱼体表面(30 cm^2 左右), 无菌取鳃、胃、肠道前段(前肠)和肠道后段(后肠)内容物于采样袋中, 肠道前段有纤细平滑和内容物少的特点, 肠道后段略粗, 内容物较多; 并从池塘的中央和四边各收集 1 L 水于无菌容器中, –20℃保存备用。

1.2　主要试剂和仪器

Dcode^{TM} Universal Mutation Detction System、PCR 仪和荧光定量 PCR 仪购自 Bio-Rad 公司; 核酸蛋白浓度测定仪购自 Wilmington 公司。DNA 提取试剂盒、胶回收试剂盒和质粒提取试剂盒购自 Omega 公司; SYBR® Premix Ex Taq^{TM} II、pMD® 19-T 载体和 2 × Tap MasterMix(含染料)购自宝生物工程(大连)有限公司; 大肠杆菌(*Escherichia coli*) DH5α 购自天根生化科技(北京)有限公司。

1.3　总 DNA 的提取、16S rDNA V3 区的扩增和 PCR-DGGE 电泳

按照 DNA 提取试剂盒操作手册提取 DNA, 测定总 DNA 浓度后置–20℃保存备用。细菌 16S rDNA V3 区通用引物[7]正反向序列: 314f-GC, 5′–CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T–3′和 518r, 5′–GTA TTA CCG CGG CTG CTG GCA C–3′。PCR 扩增体系(25 μL): 2×Tap Master Mix 12.5 μL, 正反向引物各 0.01 nmol, 模板 DNA 20 ng, ddH_2O 补足 25 μL; 扩增条件为 95℃ 4 min; 94℃ 30 s, 58℃ 30 s, 72℃ 1 min, 35 个循环; 72℃延伸 8 min。PCR-DGGE 采用 Dcode^{TM} Universal Mutation Detection System 进行, 凝胶浓度梯度为 35%~57.5%, 变性方向与电泳方向一致。电泳缓冲液为 1×TAE, 200 V, 5 min, 100 V, 60℃电泳 16 h。凝胶银染后用 Bio-Rad® GS800 Calibrated Densitometer 成像。

1.4　标准曲线的构建

以总 DNA 为模板, 各菌特异性引物(表 1) 进行 PCR 扩增, 2% 琼脂糖凝胶电泳后, 对目的条带进行回收和克隆测序。提取阳性克隆质粒, 检测浓度和 OD_{260nm}/OD_{280nm} 值, 以 10 倍梯度稀释的重组质粒为模板, 特异性引物进行 Q-PCR, 构建目的细菌的标准曲线。

1.5　Q-PCR 测定目的细菌

以总 DNA 为模板, 细菌特异性引物进行 Q-PCR。扩增体系(25 μL): 2×SYBR Premix Ex Taq II 12.5 μL, 50×ROX reference dye II 0.5 μL, 正反向引物各 0.01 nmol, 模板 DNA 20 ng, ddH_2O 补足 25 μL。反应程序: 95℃, 3 min; 95℃, 15 s, 适宜退火温度(表 1), 15 s, 40 个循环; 95℃, 15 s; 60℃, 60 s; 95℃, 15 s。将 C_t 值代入对应标准曲线中获得拷贝数。

1.6　数据分析

实验数据以平均值±标准差($\bar{x}$±SD)表示。用 Quantity one、NTSYS2.10 和 SPSS 19.0 软件对 PCR-DGGE 图谱进行处理、菌群聚类分析和 PCA。并根据文献[8]计算菌群多样性指数, 采用独立样本 t 检验进行差异性分析。

2　结果与分析

2.1　菌群 16s rDNA V3 区域 PCR-DGGE 图谱分析

斑点叉尾鮰皮肤、鳃、胃、前肠、后肠和水体的 PCR-DGGE 指纹图谱分别如图 1 (A–E 和 K)所示, 图谱中条带的数量及位置表示细菌丰富度和种类, 而条带颜色的深浅与其含量成正相关。由图 1 可知, 所有样本均展现出丰富的条带, 但条带位置、数目及颜色则表现出较大差异。菌群丰富度由低到高分别是鳃、皮肤、前肠、后肠和胃(表 2)。与 LW 斑点叉尾鮰比较, HW 斑点叉尾鮰

表 1 细菌引物序列

Tab. 1 The sequences of bacterial primers

目标细菌 target species	引物序列(5′−3′) primer sequence (5′−3′)	退火温度/℃ Tm	片段大小/bp size	参考文献 reference
总菌 total bacteria	F: ACTCCTACGGGAGGCAGCAG	60	200	[9]
	R: ATTACCGCGGCTGCTGG			
拟杆菌属 *Bacteroidetes*	F: GGARCATGTGGTTTAATTCGATGAT	58	151	
	R: AGCTGACGACAACCATGCAG			
气单孢菌属 *Aeromonas*	F: GCCTTCGGGTTGTAAAGCAC	59	141	KT363960.1
	R: CGATTAACGCTTGCACCCTC			
弧菌属 *Vibrio*	F: GGGGCTCAACCTCGGAATAG	60	117	KT369808.1
	R: CTTCGCCACCGGTATTCCTT			
黄杆菌属 *Flavobacterium*	F: CGGCTTACCAAGGCTACGAT	60	509	KP277508.1
	R: TACCCACGCTTTCGTCCATC			
肠球菌属 *Enterococcus*	F: CCCTTATTGTTAGTTGCCATCATT	52	144	[10]
	R: ACTCGTTGTACTTCCCATTGT			
双歧杆菌属 *Bifidobacterium*	F: TCGCGTCYGGTGTGAAAG	62	243	
	R: CCACATCCAGCRTCCAC			
乳酸菌属 *Lactobacillus*	F:AGCAGTAGGGAATCTTCCA	55	341	[11]
	R:CACCGCTACACATGGAG			
肠杆菌科 Enterobacteriaceae	F: CATTGACGTTACCCGCAGAAGAAGC	52	230	[12]
	R: CTCTACGAGACTCAAGCTTGC			
梭菌属 *Fusobacterium*	F: CWAACGCGATAAGTAATC	60	158	[13]
	R: TGGTAACATACGAWAGGG			
酵母菌属 *Saccharomyces*	F: GTGAGCAGCGAAGGATTTGG	60	95	M35588.1
	R: TGCGACCGGCTATTCAACAA			

注: KT363960.1、KT369808.1、KP277508.1 和 M35588.1 分别表示气单胞菌属、弧菌属和黄杆菌属的 16S 核糖体基因序列登录号和酵母菌属的 18S 核糖体基因序列登录号.

Note: The accession number of 16S ribosomal gene sequences of *Aeromonas*, *Vibrio* and *Flavobacterium* are KT363960.1, KT369808.1 and KP277508.1, respectively. The accession number of 18S ribosomal gene sequence of *Saccharomyces* is M35588.1.

的鳃、皮肤、胃和前肠菌群丰富度更高, 表明 HW 斑点叉尾鮰菌群结构更复杂。聚类图(图 1)显示 LW 和 HW 斑点叉尾鮰皮肤样本(图 1A)分别聚为一簇, 相似性系数为 82%; 鳃(图 1B)、胃(图 1C)和后肠(图 1E)部分样本能够分开聚为一簇, 前肠(图 1D)个别样本差异较大, 但其余相似性较高(85%左右); 图 1K 显示水体菌群相似性达 90%以上。PCA (图 1)与聚类图的结果相似, 图 1F 中 PCA1 将 LW 和 HW 斑点叉尾鮰皮肤样本区分为左右两部分, 图 1G、图 1H、图 1J 中 PCA1 分别将 LW 和 HW 斑点叉尾鮰鳃、胃和后肠部分样本区分开; 然而, LW 和 HW 斑点叉尾鮰前肠(图 1I)样本相互交织在一起, 表明 LW 和 HW 斑点叉尾鮰皮肤、鳃、胃和后肠菌群存在较大差异性。此外, HW 斑点叉尾鮰鳃、皮肤、胃和前肠菌群的香农指数、均匀度和丰富度均高于 LW 斑点叉尾鮰, 其中鳃菌群的香农指数、均匀度和丰富度及前肠菌群丰富度均显著提高(P<0.05) (表 2)。因此, 菌群结构和数量在 LW 和 HW 斑点叉尾鮰间存在差异, 在 HW 斑点叉尾鮰中菌群多样性增加。

2.2 Q-PCR 结果

由表 3 和表 4 可知, 肠杆菌科和气单胞菌属是皮肤的优势菌群; 肠杆菌科、气单胞菌属和肠球菌属是水、鳃和胃的优势菌群; 肠杆菌科、拟杆菌属、气单胞菌属和酵母菌属是肠道的优势菌群。与 LW 斑点叉尾鮰比较, HW 斑点叉尾鮰皮肤的黄杆菌属显著增加(P<0.05), 鳃的肠球菌属显著减少(P<0.05), 胃的微生物总量和肠杆菌科显著增加(P<0.05), 肠球菌属显著减少(P<0.05), 前肠的微生物总量、拟杆菌属和双歧杆菌属(P<0.05)

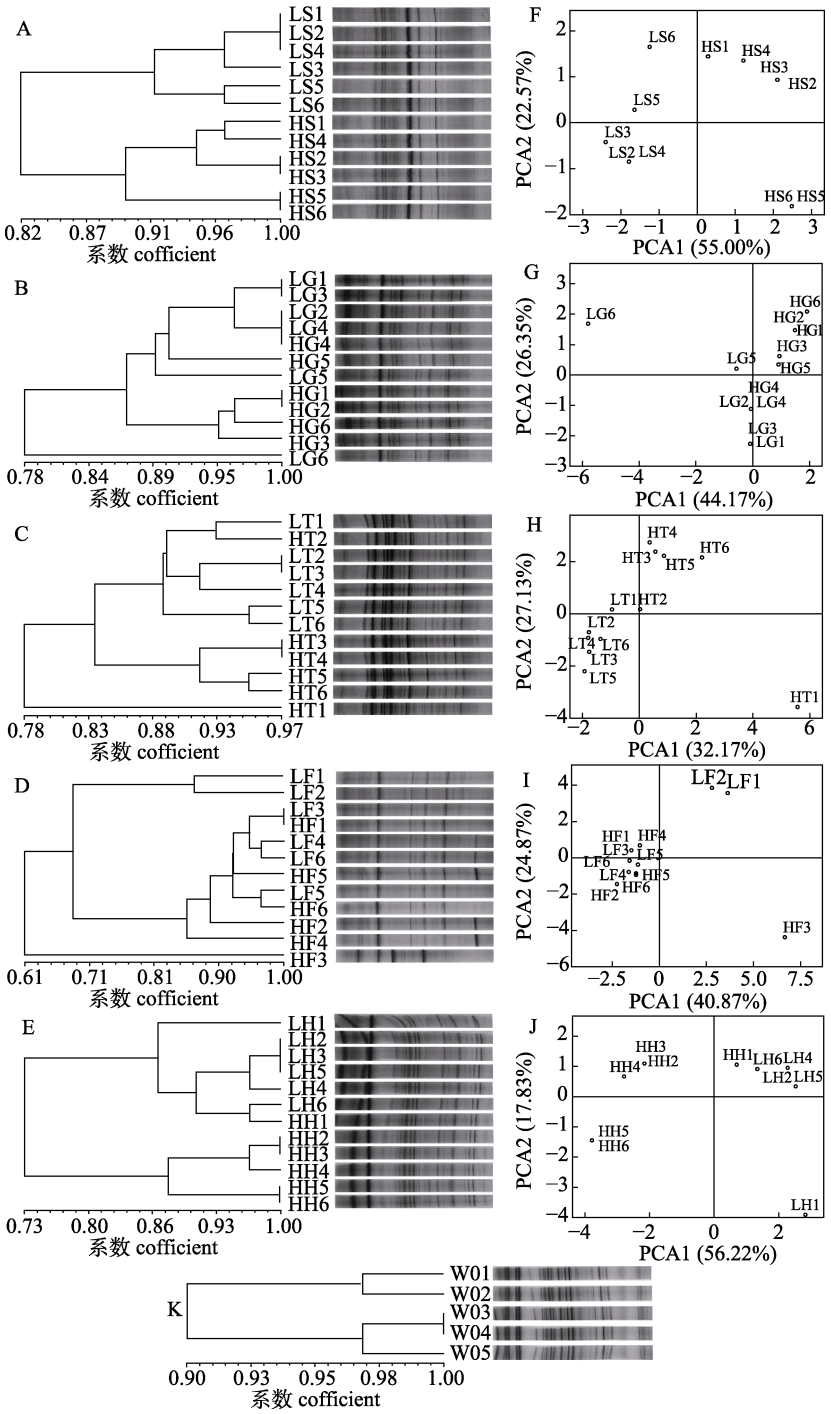

图 1　斑点叉尾鮰皮肤、鳃、胃、前肠、后肠和水体菌群 PCR-DGGE 图谱、聚类图(A−E 和 K)和主成分分析(F−J)

LS: 低体重斑点叉尾鮰皮肤; LG: 低体重斑点叉尾鮰鳃; LT: 低体重斑点叉尾鮰胃; LF: 低体重斑点叉尾鮰前肠; LH: 低体重斑点叉尾鮰后肠; HS: 高体重斑点叉尾鮰皮肤; HG: 高体重斑点叉尾鮰鳃; HT: 高体重斑点叉尾鮰胃; HF: 高体重斑点叉尾鮰前肠; HH: 高体重斑点叉尾鮰后肠. W: 水体. 样本(n=6), 水体样本(n=5).

Fig. 1　The PCR-DGGE profiles, cluster figures (A−E, and K) and principal component analysis (F−J) of the skin, gills, stomach, foregut, hindgut and water microorganisms from *Ietalurus punetaus*

LS: Skin of low weight *I. punetaus*; LG: Gills of low weight *I. punetaus*; LT: Stomach of low weight *I. punetaus*; LF: Foregut of low weight *Ietalurus punetaus*; LH: Hindgut of low weight *Ietalurus punetaus*; HS: Skin of high weight *Ietalurus punetaus*; HG: Gills of high weight *Ietalurus punetaus*; HT: Stomach of high weight *Ietalurus punetaus*; HF: Foregut of high weight *Ietalurus punetaus*; HH: Hindgut of high weight *Ietalurus punetaus* and W: Water. The skin, gills, stomach, foregut and hindgut species of low weight (LW) and high weight (HW) *Ietalurus punetaus* (n=6) and water species (n=5).

表 2 斑点叉尾鮰鳃、皮肤、胃、前肠和后肠菌群的多样性指数

Tab. 2 Diversity index of gills, skin, stomach, foregut and hindgut bacteria from *Ietalurus punetaus*

$\bar{x}$ ±SD

项目 item	香农指数 Shannon diversity index	均匀度 evenness	丰富度 richness
水体 water	3.45±0.06	0.99±0.02	31.40±1.82
低体重斑点叉尾鮰 low weight *Ietalurus punetaus*			
鳃 gill	2.97±0.10[a]	0.92±0.03[a]	19.67±1.86[a]
皮肤 skin	3.06±0.03[a]	0.94±0.01[a]	23.00±0.63[a]
胃 stomach	3.44±0.08[a]	0.93±0.02[a]	31.33±2.34[a]
前肠 foregut	3.16±0.03[a]	0.93±0.01[a]	23.50±0.84[a]
后肠 hindgut	3.30±0.07[a]	0.95±0.02[a]	26.00±1.67[a]
高体重斑点叉尾鮰 high weight *Ietalurus punetaus*			
鳃 gill	3.10±0.06[b]	0.96±0.02[b]	22.33±1.37[b]
皮肤 skin	3.11±0.04[a]	0.99±0.01[a]	23.83±0.98[a]
胃 stomach	3.45±0.04[a]	0.94±0.01[a]	31.50±1.38[a]
前肠 foregut	3.23±0.09[a]	0.94±0.03[a]	25.05±0.51[b]
后肠 hindgut	3.28±0.05[a]	0.95±0.02[a]	25.33±1.37[a]

注: 分析方法采用独立样本 *t* 检验, 水体样本量 *n*=5, 其余 *n*=6. 同列同部位之间字母相同表示差异不显著(*P*>0.05), 字母不同表示差异显著(*P*<0.05).

Note: Adopted independent sample *t* test for analysis, *n*=5 for water, *n*=6 for others. The same item and column with the same letters indicates no significant difference (*P*>0.05), and different letters indicate significant differences (*P*<0.05).

表 3 水体菌群荧光定量 PCR 结果

Tab. 3 The results of Q-PCR analysis of water flora

n=4; $\bar{x}$ ±SD

细菌种属 bacterial species	拷贝数/[lg(copy)·L^{-1})] copy	细菌种属 bacterial species	拷贝数/[lg(copy)·L^{-1})] copy
总菌 total bacteria	9.32±0.09	肠球菌属 *Enterococcus*	4.39±0.22
肠杆菌科 Enterobacteriaceae	6.89±0.08	弧菌属 *Vibrio*	3.38±0.08
气单胞菌属 *Aeromonas*	4.55±0.08	黄杆菌属 *Flavobacterium*	2.86±0.09

注: 分析方法采用独立样本 *t* 检验.

Note: Adopted *t*-test for independent samples for analysis.

上升, 后肠的拟杆菌属、双歧杆菌属和酵母菌增加(*P*<0.05), 微生物总量和气单胞菌属下降(*P*<0.05), 其余菌属变化不明显(*P*>0.05)。乳酸菌属和梭菌属在所有样品中均未检出, 而肠球菌属在皮肤、前肠和后肠样品中未检出。主成分分析显示, PCA1 均能将 LW 和 HW 斑点叉尾鮰区分开, 表明两者间存在差异(图 2A–E); 图 2F 显示水体与胃、鳃和皮肤样本距离较近, 表明水体与胃、鳃和皮肤菌群的相关性较大。因此, LW 和 HW 斑点叉尾鮰在皮肤、鳃、胃、前肠和后肠细菌数量上均存在差异, HW 斑点叉尾鮰微生物总量有增加趋势, 与 DGGE 图谱分析结果一致。

3 讨论

3.1 斑点叉尾鮰皮肤、鳃、胃和肠道菌群组成

研究表明鱼类皮肤细菌包括气单胞菌、肠杆菌、弧菌和黄杆菌属[14], 这与本研究结果类似, 肠杆菌科和气单胞菌属等需氧菌是斑点叉尾鮰皮肤的优势菌群。另有研究表明鳃是气单胞菌、肠杆菌、假单胞菌和弧菌等细菌的定居场所[15], 本研究中肠杆菌科、气单胞菌属和肠球菌属是斑点叉尾鮰鳃的优势菌群, 与其相似。鱼类定植菌群与生活水体有直接关系[16], 本研究显示水体中优势菌群为肠杆菌科、气单胞菌属和肠球菌属, 水体、鳃和胃中含大量的肠球菌属, 而肠道中并未检出该类细菌, 提示肠球菌属细菌的存在可能与鱼的种类及生存水体有关。

该研究中斑点叉尾鮰皮肤、鳃、胃、前肠和后肠中均形成了独特的菌群结构, 其中胃的菌群丰富度最高, 其细菌数量远高于 Ringø 经活菌技术法测得的幼鱼胃内细菌数量 10^4~10^5 copy/g[17], 且鱼类正常菌群结构及数量因鱼类不同而各异[18], 因此, 推测可能因动物种类或其生存环境及检测方法不同所致。

淡水鱼类肠道菌群数量为 10^8 copy/g, 厌氧菌为 10^5 copy/g 左右[15], 专性厌氧菌以拟杆菌属等为主, 兼性厌氧和需氧细菌则以气单胞菌属、肠杆菌科等为主[19]。这与本研究结果相似, 斑点叉尾鮰肠道总微生物量为 10^9 copy/g, 专性厌氧菌为拟杆菌属和双歧杆菌属, 兼性厌氧和需氧细菌则以肠杆菌科、气单胞菌属、酵母菌等为主, 而总微生物量高一个数量级则可能因检测方法不同所致。尹军霞等[20]发现鱼前肠菌群定植比后肠少; 同一肠段相比, 厌氧菌总数远大于需氧菌, 且需

表 4　低体重(LW)和高体重(HW)斑点叉尾鮰皮肤、鳃、胃、前肠和后肠菌群荧光定量 PCR 结果

Tab. 4　The results of Q-PCR analysis of skin, gills, stomach, foregut and hindgut of different weight *Ietalurus punetaus*

n=4; $\overline{x}$ ±SD; lg(copy)·g^{-1}

细菌种属 bacterial species	LW	HW	细菌种属 bacterial species	LW	HW
皮肤 skin/[lg(copy)·cm^{-2}]			前肠 foregut		
总菌 total bacteria	4.61±0.03^a	4.63±0.13^a	总菌 total bacteria	9.08±0.04^a	9.27±0.01^b
气单胞菌属 *Aeromonas*	2.89±0.04^a	2.86±0.04^a	气单胞菌属 *Aeromonas*	4.50±0.06^a	4.57±0.08^a
肠杆菌科 Enterobacteriaceae	4.12±0.03^a	4.09±0.01^a	肠杆菌科 Enterobacteriaceae	6.81±0.15^a	6.97±0.57^a
弧菌属 *Vibrio*	2.35±0.01^a	2.31±0.07^a	弧菌属 *Vibrio*	3.67±0.13^a	3.78±0.06^a
黄杆菌属 *Flavobacterium*	1.93±0.01^a	1.97±0.01^b	拟杆菌属 *Bacteroides*	5.86±0.10^a	6.19±0.10^b
鳃 gill			双歧杆菌属 *Bifidobacterium*	3.72±0.03^a	3.83±0.07^b
总菌 total bacteria	6.62±0.04^a	6.67±0.09^a	酵母菌属 *Saccharomyces*	4.12±0.13^a	4.08±0.14^a
气单胞菌属 *Aeromonas*	4.33±0.15^a	4.25±0.11^a	后肠 hindgut		
肠杆菌科 Enterobacteriaceae	6.07±0.01^a	6.03±0.05^a	总菌 total bacteria	9.27±0.08^a	9.06±0.07^b
肠球菌属 *Enterococcus*	4.32±0.05^a	4.19±0.02^b	气单胞菌属 *Aeromonas*	4.68±0.06^a	4.55±0.07^b
弧菌属 *Vibrio*	3.34±0.06^a	3.35±0.11^a	肠杆菌科 Enterobacteriaceae	6.91±0.09^a	7.30±0.31^a
黄杆菌属 *Flavobacterium*	2.78±0.11^a	2.84±0.04^a	弧菌属 *Vibrio*	3.73±0.08^a	3.65±0.07^a
胃 stomach			拟杆菌属 *Bacteroides*	5.91±0.06^a	6.13±0.01^b
总菌 total bacteria	9.53±0.04^a	9.83±0.13^b	双歧杆菌属 *Bifidobacterium*	3.77±0.07^a	3.92±0.04^b
气单胞菌属 *Aeromonas*	4.51±0.09^a	4.46±0.14^a	酵母菌属 *Saccharomyces*	4.08±0.10^a	4.26±0.09^b
肠杆菌科 Enterobacteriaceae	7.15±0.05^a	7.69±0.20^b			
肠球菌属 *Enterococcus*	4.57±0.10^a	4.39±0.05^b			
弧菌属 *Vibrio*	3.72±0.03^a	3.87±0.13^a			

注: 分析方法采用独立样本 t 检验, 同行间字母相同表示差异不显著(P>0.05); 字母不同表示差异显著(P<0.05). 乳酸菌属和梭菌属在所有样品中均未检出, 而肠球菌属在皮肤、前肠和后肠样品中未检出.

Note: Adopted independent sample t test for analysis. The same letters in the same row indicate no significant difference (P>0.05), and different letters indicate significant differences (P<0.05). *Lactobacillus* and *Fusobacterium* were not detected in any of the samples, and *Enterococcus* was not detected in the skin, foregut and hindgut.

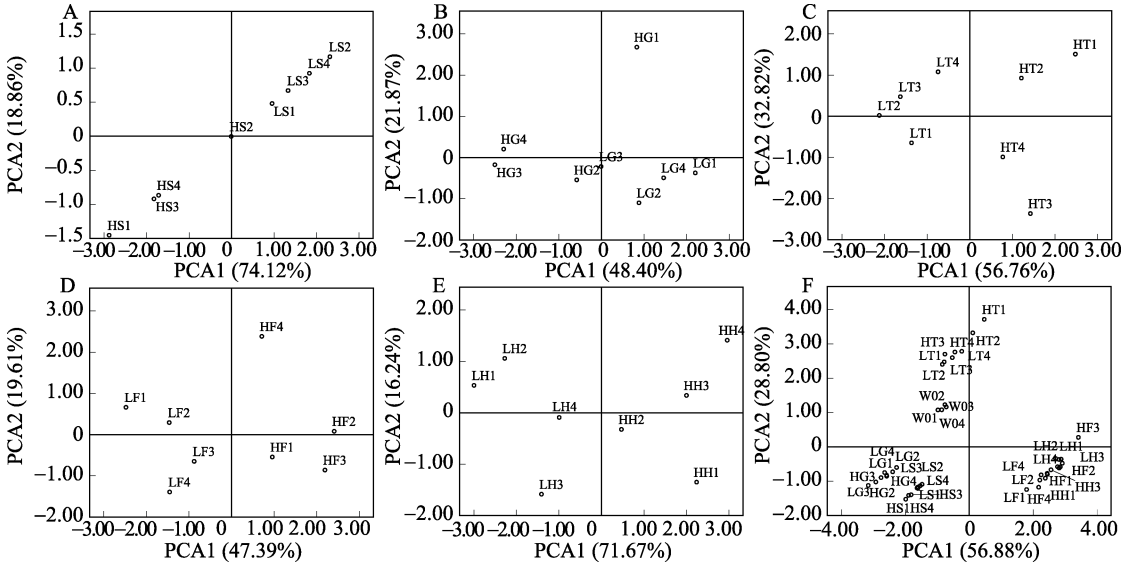

图 2　斑点叉尾鮰皮肤、鳃、胃、前肠和后肠菌群 Q-PCR 主成分分析(A–E)及斑点叉尾鮰皮肤、鳃、胃、前肠、后肠和水体菌群 Q-PCR 主成分分析(F)

Fig. 2　The PCA profiles of Q-PCR microorganisms on skin, gills, stomach, foregut and hindgut of *Ietalurus punetaus* (A−E), and the PCA profile of Q-PCR microorganisms in water and skin, gills, stomach, foregut and hindgut of *Ietalurus punetaus* (F)

氧菌总数较厌氧菌更易变化[21], 而本研究中需氧菌较厌氧菌多, 推测可能与鱼的年龄、种类、生存环境、检测方法及肠内需氧菌总数容易变化等因素有关。

3.2 不同体重斑点叉尾鮰皮肤、鳃、胃和肠道菌群差异分析

Zeng 等[22]发现不同体重獭兔的粪便菌群结构存在显著差异。本研究中聚类图和 PCA (图 1)均将 LW 和 HW 斑点叉尾鮰区分开, 表明 LW 和 HW 斑点叉尾鮰皮肤、鳃、胃肠道的菌群结构存在明显的差异; 此外, HW 斑点叉尾鮰较 LW 斑点叉尾鮰皮肤、鳃、胃和前肠菌群多样性指数高(表 2), 表明 HW 斑点叉尾鮰菌群结构更复杂和稳定。

宿主正常菌群与机体生长和发育密切相关, 尤其厌氧菌等益生菌数量与体重呈正相关[23]。Q-PCR 分析显示, LW 和 HW 斑点叉尾鮰皮肤、鳃、胃肠道优势菌群种类未变, 但数量有所变化(表 4), 说明其微生态系统在一定程度上有较好的稳定性, 这种稳定性是微生物发挥生理作用的重要基础[24]。低毒的黄杆菌属是鱼类皮肤和鳃的正常菌群, 通过碳水化合物结合受体与斑点叉尾鮰皮肤和鳃的黏液产生趋化反应[25], 诱发免疫反应, 促进机体生长[26], 本研究显示, HW 斑点叉尾鮰皮肤和鳃的总菌数高于 LW 斑点叉尾鮰, 且 HW 斑点叉尾鮰皮肤(P<0.05)和鳃定植的黄杆菌属数量明显高于 LW 斑点叉尾鮰, 表明 HW 斑点叉尾鮰皮肤和鳃菌群结构较复杂, 而定植的黄杆菌属是否与宿主体重有关, 有待进一步研究。此外, 本研究中 HW 斑点叉尾鮰胃(P<0.05)和肠道中肠杆菌科数量增加, 虽部分大肠杆菌能显著促进机体生长[27], 且鱼源大肠杆菌具有液化明胶、不产生吲哚等特殊的生理生化特征[28], 但本研究中大肠杆菌是否具有促生长作用, 还有待进一步研究。肠球菌属在皮肤及肠道中未检出, 且在 HW 斑点叉尾鮰鳃和胃中显著下降(P<0.05), 推测肠球菌属可能对斑点叉尾鮰的生长影响较小。另外, 拟杆菌和双歧杆菌等厌氧菌是维生素的主要产生菌[29], 拟杆菌属中的某些细菌可通过 TLRs 和 NF-κB 途径调节机体的免疫反应[30], 双歧杆菌下调炎性细胞因子的表达[31], 改善紊乱的免疫反应[32]。酵母菌可改善鱼类免疫反应及其对水质的耐受性, 并促进其生长[33]。该研究中 HW 斑点叉尾鮰肠道拟杆菌属和双歧杆菌属显著高于 LW 斑点叉尾鮰(P<0.05), 且酵母菌属在 HW 斑点叉尾鮰的后肠中显著增加(P<0.05), 说明 LW 和 HW 斑点叉尾鮰菌群结构存在差异, HW 斑点叉尾鮰益生菌数量增加。

4 结论

斑点叉尾鮰在皮肤、鳃、胃、前肠和后肠中均存在独特的菌群结构, 菌群丰富度由低到高依次是鳃、皮肤、前肠、后肠和胃。肠杆菌科和气单胞菌属是皮肤的优势菌群; 肠杆菌科、气单胞菌属和肠球菌属是水、鳃和胃的优势菌群; 肠杆菌科、拟杆菌属、气单胞菌属和酵母菌属是肠道的优势菌群。高体重斑点叉尾鮰菌群结构在一定程度上有复杂化的趋势。

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Application of PCR-DGGE and Q-PCR to analyze microflora of skin, gills, and the gastrointestinal tract of *Ictalurus punctatus* with different weights

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Abstract: *Ictalurus punctatus* is native to the Americas and a superior freshwater fish cultured worldwide. However, acute mortalities often occur during aquaculture. In addition, the disadvantages of antibiotics have been highlighted, and a safe and effective substitute has become a hot topic. Thus, many studies have been published on the beneficial effect of probiotics, and most have targeted the microflora of the gastrointestinal tract, but few studies have investigated microflora of the gills and skin. To provide a theoretical basis to study gill, skin, stomach, foregut, and hindgut microorganisms and to screen probiotics from *I. punctatus*, we compared the microbial structures of the gills, skin, stomach, foregut, and hindgut from *I. punctatus* of different weights and explored the correlation with aquatic flora using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and quantitative-polymerase chain reaction (Q-PCR). All samples possessed rich bands, but the location, number, and color of the bands were quite different among samples. The mean numbers of DGGE bands detected in the organs from low to high were gills, skin, foregut, hindgut, and stomach. The Shannon diversity index and evenness and richness of bacteria in the skin, gills, stomach, foregut, and hindgut were 3.06, 0.94, and 23.00; 2.97, 0.92, and 19.67; 3.44, 0.93, and 31.33; 3.16, 0.93, and 23.50; and 3.30, 0.95, and 26.00 in low weight (LW) *I. punctatus* and 3.11, 0.99, and 23.83; 3.10, 0.96, and 22.33; 3.45, 0.94, and 31.50; 3.23, 0.94, and 25.05; and 3.28, 0.95, and 25.33 in high weight (HW) *I. punctatus*, respectively. The Shannon diversity index, evenness, and richness of gills and richness of the foregut from HW *I. punctatus* were significantly higher than those of LW *I. punctatus* ($P<0.05$), indicating that microflora diversity was higher in HW *I. punctatus* than that in LW *I. punctatus*. Clustering and principal component analysis (PCA) distinguished LW from HW *I. punctatus*, and the similarity coefficients of LW and HW *I. punctatus* in the skin, gills, stomach, foregut, and hindgut were 0.82, 0.78, 0.78, 0.61, and 0.73, respectively, suggesting significant differences in the bacterial flora between LW and HW *I. punctatus*. In addition, the Shannon diversity index and evenness and richness of microflora in the aquatic environment were 3.45, 0.99, and 31.40, indicating slightly lower diversity than that of the stomach flora. The Q-PCR results showed that the dominant skin microflora were Enterobacteriaceae and *Aeromonas*, the dominant microflora in the water, gills, and stomach were Enterobacteriaceae, *Aeromonas,* and *Enterococcus*; and the dominant microflora in the foregut and hindgut were Enterobacteriaceae, *Bacteroidetes*, *Aeromonas,* and *Saccharomyces*. The numbers of *Flavobacterium* ($10^{1.97}$) on the skin, Enterobacteriaceae ($10^{7.69}$) in the stomach, *Bacteroidetes* ($10^{6.19}$) and *Bifidobacterium* ($10^{3.83}$) in the foregut, and *Bacteroidetes* ($10^{6.13}$), *Saccharomyces* ($10^{4.26}$), and *Bifidobacterium* ($10^{3.92}$) in the hindgut were significantly higher in HW than those in LW *I. punctatus* ($P<0.05$). Moreover, the total numbers of bacteria in the skin, gills, stomach, and foregut ($P<0.05$) were significantly higher in HW than those in LW *I. punctatus*. These results reveal an increasing trend for the numbers of microflora in HW *I. punctatus*. The Q-PCR results were analyzed by PCA to identify the correlations between the microflora in all samples. The results demonstrated that LW and HW *I. punctatus* were distinguished by PCA1; the skin microflora was associated with that on gills, the foregut microflora was associated with that in the hindgut, and the stomach microflora was associated with that in the water. These results indicate the unique flora of the skin, gills, and gastrointestinal tract in *I. punctatus* and show that the structure and abundance of microflora are complex in LW and HW *I. punctatus*.

Key words: *Ictalurus punctatus*; PCR-DGGE; Q-PCR; skin flora; gill flora; gastrointestinal flora
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