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北方吊笼养殖刺参肠道及其养殖环境菌群结构特征及其相关性分析*

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摘要 为探究北方吊笼养殖刺参(*Apostichopus japonicus*)肠道及其养殖环境菌群结构的关系，本研究基于高通量测序技术全面解析刺参肠道和养殖环境菌群结构和功能特征，并初步探讨刺参肠道及其养殖环境菌群相关性。结果显示，刺参肠道菌群丰度和多样性均显著高于养殖水体(P<0.05)。刺参肠道及养殖水体主要优势菌门均隶属于变形菌门(Proteobacteria)和拟杆菌门(Bacteroidetes)，二者存在 13 个相对丰度大于 0.1%的共有核心菌属。此外，肠道菌群具有一定的独立性，其特异性菌群主要隶属于厚壁菌门(Firmicutes)和绿弯菌门(Chloroflexi)，以芽孢杆菌属(*Bacillus*)、乳酸杆菌属(*Lactobacillus*)、海泥海球菌属(*Halioglobus*)、*Lutimonas* 和 *Woeseia* 为代表。基于 KEGG 代谢通路数据库，共注释到 300 条三级代谢通路，其中 146 条存在极显著差异(P<0.001)。刺参肠道菌群差异代谢通路主要表现在代谢方面，具体表现为碳水化合物消化吸收、蛋白质消化吸收和鞘脂类代谢。研究表明，刺参肠道菌群种类与其养殖水体呈高度相似性，但相对丰度存在显著性差异。本研究结果可为北方刺参吊笼健康养殖提供一定的理论依据。

关键词 刺参；肠道菌群；吊笼养殖；高通量测序；菌群结构

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刺参(*Apostichopus japonicus*)是我国北方重要的海水养殖品种之一，具有较高的经济和营养价值(Roggatz *et al*, 2018; 曹荣等, 2020; 陈士国, 2010)。其营底栖生活并以沉积物为食，细菌在肠道中占有较大比例，刺参所需 70%以上的能量均与细菌有关(Zhou *et al*, 2009; 廖玉麟, 1997)。肠道菌群与宿主健康生长息息相关，在机体消化代谢(Wu *et al*, 2012; 陈贞年等, 2021)、防御病原(Moran *et al*, 2005)和免疫功能(Chi *et al*, 2014)等方面均起着重要的作用。孙奕等(1989)研究表明，刺参肠道中的假单胞菌属(*Pseudomonas*)对多糖有较好的降解能力，可以为其提供能量促进生长。陆振等(2017)研究发现，刺参肠道中的芽孢杆菌属(*Bacillus*)具有降解蛋白质和琼脂的能力，有助于刺参对营养物质的分解利用，促进其

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健康生长。王伟等(2018)研究发现，刺参肠道中的乳酸片球菌属(*Pediococcus*)能够产生多肽类抗菌活性物质，这类活性物质对病原菌具有显著的抑制作用。宫魁等(2013)研究发现，刺参肠道中的乳酸菌(*Lactobacillus*)能够与有害菌竞争营养和空间，同时通过其产生的小分子代谢物，降低肠道内的 pH 值和氧化还原电位，从而拮抗病原性细菌。周慧慧等(2010)研究证实，刺参肠道菌群中肠球菌(*Enterococcus* sp.)通过提高肠道中的酶活力，进而增强刺参的免疫水平。

水产动物肠道中复杂的细菌群落主要来自于养殖环境，环境菌群与肠道菌群之间存在着密切的共生关系(陈孝煊等, 2005; 李靖等, 2019; 王轶南等, 2011; 宫晗等, 2023)。理清肠道菌群结构和功能特征及其养殖环境错综复杂的关系，阐明肠道菌群在机体生长中的重要作用，有助于丰富肠道菌群机理研究，探索提高养殖产量的生态调控途径，促进刺参养殖产业绿色健康发展。迄今为止，关于不同养殖模式下刺参肠道菌群结构特征相关方面的研究工作较为普遍。李彬等(2016)基于 PCR-DGGE 技术解析了池塘养殖刺参肠道内容物、附着基和底泥中菌群结构特征及其相关性。陆振等(2017)采用 16S rDNA 测序技术进行了池塘养殖和福建吊笼养殖刺参肠道菌群结构特征的对比分析。杨求华等(2016)利用 16S rDNA 技术研究了南移池塘养殖刺参肠道菌群结构特征。然而，目前关于综合效益最好和管理问题最少的健康高效北方吊笼养殖刺参肠道菌群特征及其与养殖环境菌群相关性方面的报道则较为匮乏。

因此，本研究采用同家系刺参作为研究对象，基于不投饵的吊笼养殖模式使养殖水体成为影响刺参肠道菌群结构的主要影响因素，全面解析北方吊笼养殖刺参肠道菌群结构和功能特征，并初步探讨刺参肠道及其养殖环境菌群相关性，以期为北方刺参吊笼健康养殖提供一定的理论依据。

1　材料与方法

1.1　样品采集

实验用刺参取自辽宁大连(39°9′28″N；121°33′5″E)，规格为 25 g/头，放养密度约为 10 头/m^3，养殖周期为 150 d。在无菌环境下，用灭菌解剖剪剪开刺参体壁，取出肠道，用无菌水冲洗干净肠壁组织，将采集到的肠壁组织置于无菌离心管中(每 5 只刺参肠壁样品混为 1 管)，分别标记为 GDL-1、GDL-2、GDL-3、GDL-4 和 GDL-5。

利用玻璃采水器采集刺参养殖海域水体样品 4 L，经 0.22 μm 无菌醋酸纤维素滤膜抽滤，用于细菌总 DNA 的提取。样品分别标记为 WDL-1、WDL-2、WDL-3、WDL-4 和 WDL-5。所有样品均于–80 ℃超低温冰箱冷冻保存。

1.2　DNA 提取

采用 HiPure Soil DNA 试剂盒，分别提取刺参肠道和养殖水体细菌总 DNA。使用超微量分光光度计检测 DNA 浓度和纯度，浓度≥50 ng/μL，纯度 $OD_{260\ nm}/OD_{280\ nm}$=1.8~2.0。利用 1%琼脂糖凝胶电泳检测 DNA，条带清晰、带型完整、无尾迹，表明所提 DNA 质量合格。

1.3　PCR 扩增与测序

将上述所提取的样本总DNA，以细菌16S rRNA基因V3~V4片段的扩增引物343F (5′-TACGGRAGGCA GCAG-3′)(5′-CCTACGGGNGGCWGCAG-3′)和798R (5′-AGGGTATCTAATCCT-3′)进行PCR扩增。将扩增产物送至欧易生物股份有限公司，基于Illumina Novaseq测序平台和PE250测序策略进行高通量测序。

1.4　数据处理

运用 Trimmomatic (Version 0.35)软件，对原始序列进行扫描去杂，截掉质量低于 20 的序列，并去除长度小于 50 bp 的序列；使用 Flash (Version 1.2.11)软件，对合格序列的 reads 进行拼接、过滤，得到完整序列；利用 QIIME 中的 Split (Version 1.8.0)软件，去除单碱基重复大于 8 与长度小于 200 bp 的序列，质控后用 UCHIME (Version 2.4.2)软件检验，去除嵌合体序列得到有效数据。采用 Usearch 软件将所得到的全部有效序列进行距离矩阵，默认在 97%水平下进行 OTUs 操作分类单元聚类。使用 PICRUSt 软件，预测已知微生物基因功能。

1.5　数据分析

使用 VennDiagram 软件(Roux *et al*, 2016)，绘制 Venn 图。使用 Mothur 软件包(Schloss *et al*, 2009)，分析菌群多样性。基于加权的 Unifrac 距离算法，计算样本间的相似性。采用 RDPclassifier 软件(Wang *et al*, 2007)，统计菌群相对丰度。基于 *T*-test 统计方法，检验样本差异显著性(P<0.05)。

2　结果

2.1　高通量测序结果

通过高通量测序，刺参肠道和养殖水体 10 个样

品检测得到的原始序列为 69 933~87 168 条，通过序列优化去杂，过滤长度短、低质量及嵌合体后，得到的有效序列为 62 391~78 243 条，且有效序列百分比都达到了 80.66%以上，覆盖率为 98.71%以上(表 1)，表明测序结果可以真实反映样本信息。

2.2 菌群多样性

2.2.1 Alpha 多样性分析 菌群丰度常以 Chao1 和 ACE 指数来表示，多样性常以 Shannon 和 Simpson 指数来衡量。如图 1 所示，吊笼养殖刺参肠道与养殖水体菌群的丰度和多样性均呈现出显著性差异(P<0.05)。具体表现为刺参肠道菌群丰度和多样性均显著高于水体菌群。

等级分布曲线(rank abundance)用于同时解释样品中细菌多样性的两个方面，即样品所含物种的丰富

表 1 高通量测序结果

Tab.1 Analysis of the high-throughput sequencing results

样品 Sample	有效序列 Effective tag	有效序列百分比 Effective tag percent/%	覆盖率 Goods coverage/%	OTU数目 OTU number
WDL1	72 506	89.65	98.98	752
WDL2	62 391	89.22	99.14	630
WDL3	63 167	90.13	99.05	681
WDL4	72 408	90.77	99.04	679
WDL5	75 523	89.38	99.02	697
GDL1	76 609	88.04	98.80	1 476
GDL2	75 411	90.48	98.98	1 609
GDL3	78 243	89.76	98.71	1 591
GDL4	78 131	91.38	99.05	1 332
GDL5	68 113	80.66	99.12	998

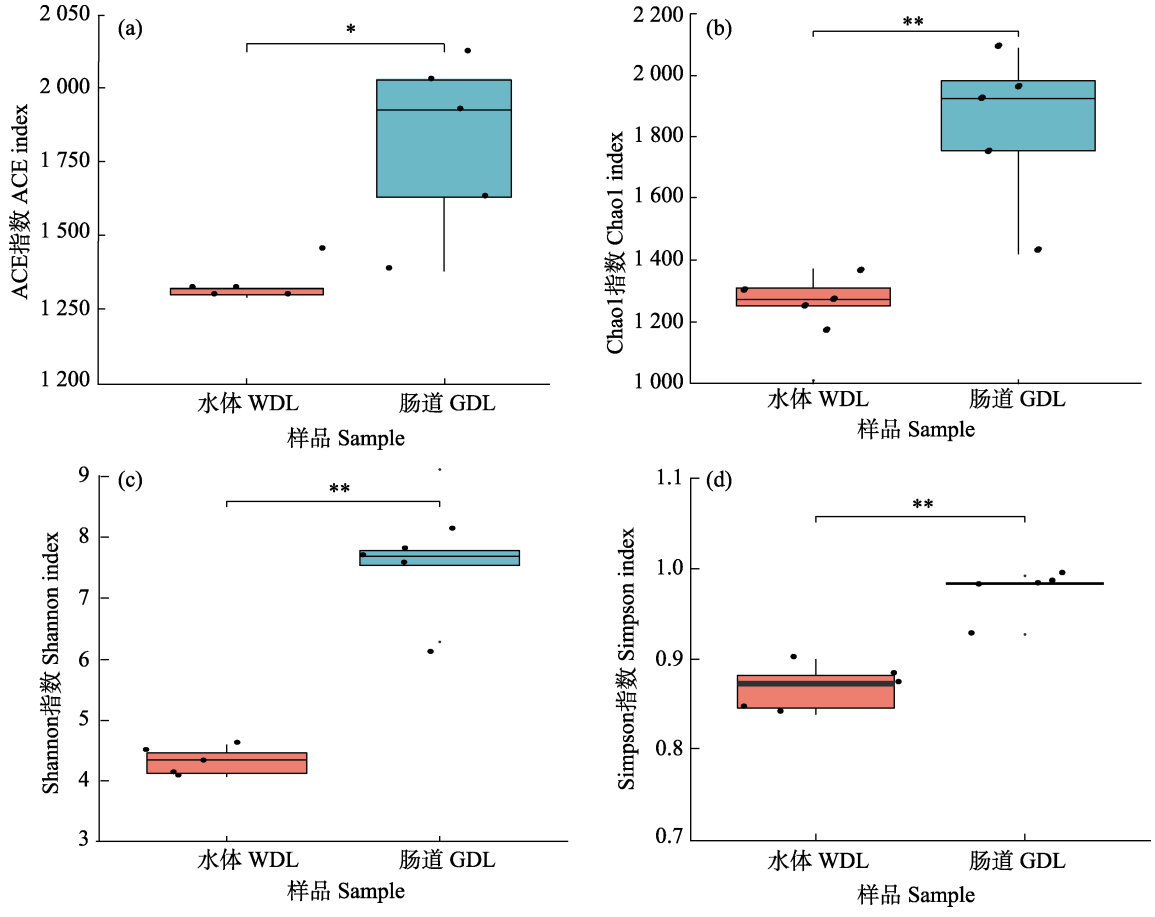

图 1 养殖水体和刺参肠道细菌群落丰富度和多样性

Fig.1 Richness and diversity of bacterial communities in the gut of *A. japonicus* and culture water

**表示差异极显著(P<0.01)；*表示差异显著(0.01<P<0.05)；无标注则为不显著(P>0.05)。

** indicates highly significant difference (P<0.01); * indicates significant difference (0.01<P<0.05); no marks indicates no significant difference (P>0.05).

程度和均匀程度。物种的丰富程度由曲线在横轴上的长度来反映，曲线越宽，表示物种的组成越丰富；物种组成的均匀程度由曲线的形状来反映，曲线越平坦，表示物种组成的均匀程度越高。由图 2 可知，刺参肠道细菌多样性高于水体样品，肠道中存在相对丰度明显占优的细菌种类。

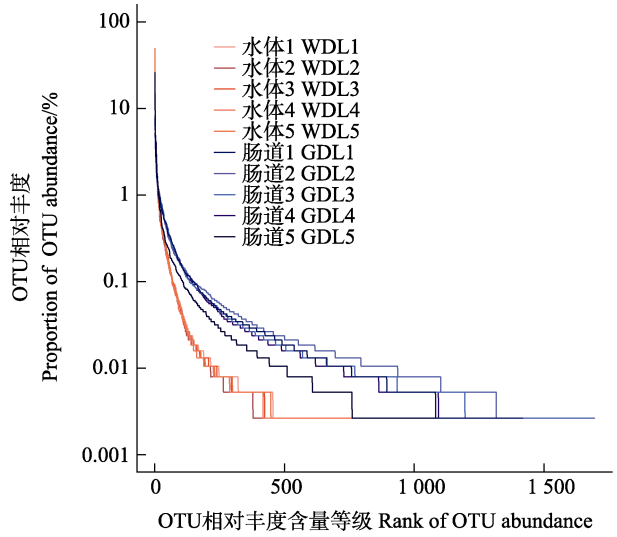

图 2　各样品中细菌多样性的等级分布曲线

Fig.2　Rank-abundance curves based on the OTU species number in each sample

2.2.2　Beta 多样性分析　　Beta 多样性可以反映刺参肠道和水体样品在不同分组上细菌群落的相似性和差异性。在 OTU 水平上，基于加权 Unifrac 距离对 10 个样本进行三维主坐标分析。通过三维 PCoA 分析评估样本间菌群结构相似性(图 3)。第一主坐标(PCoA1)的贡献率为 90.35%，第二主坐标(PCoA2)的贡献率为 6.39%，合计总贡献率为 96.74%。不同组别样品分散于不同象限，表明 2 个样品组间菌群结构具有显著性差异(P<0.05)。组内样品大多聚集在一起，显示出较好的生物学重复性。

2.2.3　刺参肠道和水体菌群相关性　　根据 OTUs 聚类分析结果，选取样品中有效的 OTUs，绘制得到韦恩图(图 4)，比较刺参肠道和养殖水体样品间的差异。结果显示，刺参肠道和养殖水体样本共有 895 个 OTUs，刺参肠道组特有 2 299 个 OTUs，而养殖水体中特有 641 个 OTUs。刺参肠道和养殖水体样品细菌种类分别占总细菌种类的 83.28%和 40.05%，肠道和养殖水体特异菌群丰度在各样品中分别占比 71.98%和 41.73%。结果显示，刺参肠道所检测到的菌群种类更加丰富。

2.3　刺参肠道与水体核心菌群分析

将核心菌群定义为刺参肠道和养殖水体所有样本都共有的菌群，任何一个样品不含有的菌属都需要排除(李淑贤等, 2022)。经过分析，相对丰度大于 0.1%的共有核心菌群有 13 个属(表 2)。这些菌属主要分布在变形菌门(Proteobacteria)，也有一些分布在拟杆菌门(Bacteroidetes)和放线菌门(Actinobacteria)。

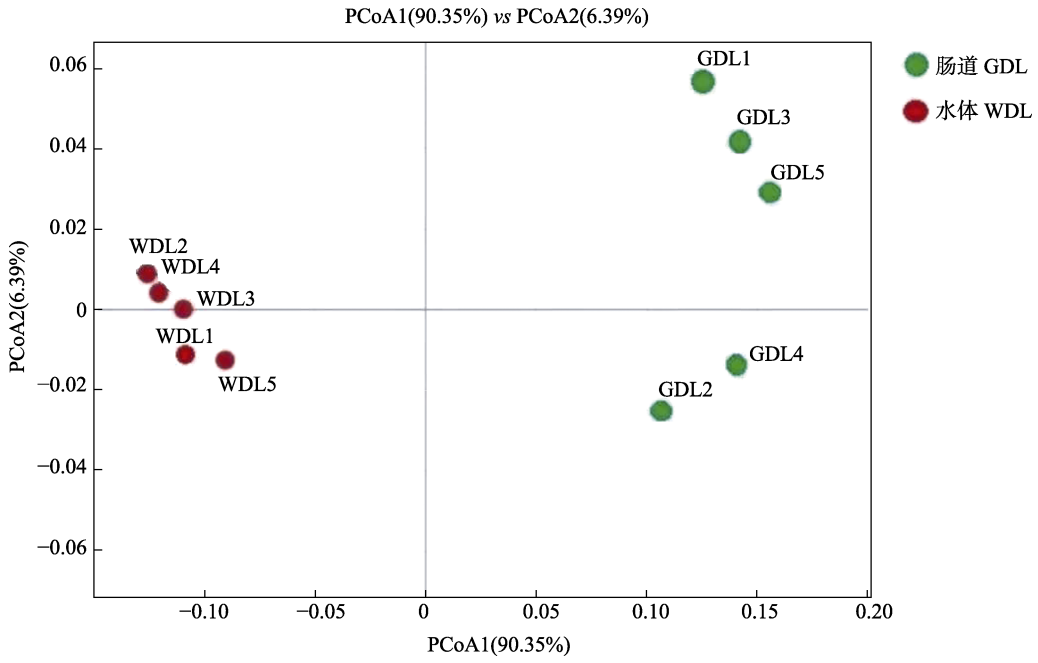

图 3　刺参肠道(GDL)及养殖水体(WDL)菌群 Unifrac PCoA 图

Fig.3　Unifrac PCoA map of the bacterial community about the gut of *A. japonicus* (GDL) and culture water (WDL)

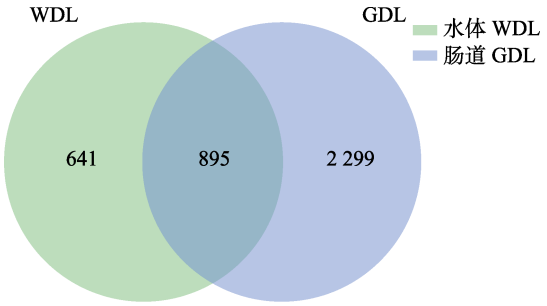

图 4 刺参肠道(GDL)及其养殖水体(WDL)菌群 OTUs 韦恩图
Fig.4 OTUs plot about bacterial community in the gut of *A. japonicus* (GDL) and culture water (WDL)

2.4 优势菌群结构特征

在门水平上(图 5a)，刺参肠道和养殖水体的第一优势菌门均为变形菌门，相对丰度占比分别为 47.91%和 79.05%；次优势菌门为拟杆菌门，相对丰度占比分别为 28.57%和 12.09%。刺参肠道中变形菌门和拟杆菌门占优势地位，其相对丰度之和高达 76.48%。此外，还检测出厚壁菌门(Firmicutes)、放线菌门、蓝藻菌门(Cyanobacteria)、芽单孢菌门(Gemmatimonadetes)和 Epsilonbacteraeota 等。

表 2 相对丰度大于 0.1%的共有核心菌属
Tab.2 The core bacterial genera shared by all samples with relative abundance above 0.1%

门 Phylum	科 Family	属 Genus	水体 WDL	肠道 GDL
变形菌门 Proteobacteria	Clade_I	Clade_Ia	46.45%	1.59%
变形菌门 Proteobacteria	Ambiguous_taxa	Ambiguous_taxa	5.87%	7.09%
变形菌门 Proteobacteria	uncultured	uncultured	2.20%	7.52%
变形菌门 Proteobacteria	uncultured_bacterium	uncultured_bacterium	1.20%	3.32%
变形菌门 Proteobacteria	红杆菌科 Rhodobacteraceae	亚硫酸杆菌属 *Sulfitobacter*	0.86%	2.09%
变形菌门 Proteobacteria	红杆菌科 Rhodobacteraceae	陆丹氏菌属 *Loktanella*	0.38%	1.86%
变形菌门 Proteobacteria	Clade_Ⅲ	OM60(NOR5) clade	0.44%	0.42%
拟杆菌门 Bacteroidetes	拟杆菌科 Bacteroidaceae	拟杆菌属 *Bacteroides*	0.13%	8.80%
变形菌门 Proteobacteria	红杆菌科 Rhodobacteraceae	*Planktomarina*	7.57%	0.15%
变形菌门 Proteobacteria	Halieacese	海泥海球菌属 *Halioglobus*	0.19%	4.89%
放线菌门 Actinobacteria	Unknown_Family	*Candidatus_Aquiluna*	1.43%	0.19%
拟杆菌门 Bacteroidetes	黄杆菌科 Flavobacteriaceae	*Ulvibacter*	0.16%	0.22%
拟杆菌门 Bacteroidetes	黄杆菌科 Flavobacteriaceae	*Fluviicola*	0.19%	0.14%

在属水平上(图 5b)，拟杆菌属(*Bacteroides*)和海泥海球菌属(*Halioglobus*)为刺参肠道的主要代表菌属，其相对丰度占比分别为 8.80%和 4.89%。此外，*Lutimonas*、*Woeseia*、亚硫酸杆菌属(*Sulfitobacter*)和陆丹氏菌属(*Loktanella*)，相对丰度占比分别为 3.57%、2.21%、2.09%和 1.86%。养殖水体优势菌属则以 Clade_Ia、*Planktomarina*、NS3a_marine_group、NS5_marine_group、亚硫酸杆菌属和陆丹氏菌属为代表，其相对丰度占比分别为 46.45%、7.57%、4.05%、2.26%、0.86%和 0.38%。

2.5 差异菌群结构特征

刺参肠道及其养殖水体差异菌群分析结果如图 6 所示，在门水平上，刺参肠道检测出的特异性菌门主要为拟杆菌门、厚壁菌门、绿弯菌门(Chloroflexi)、酸杆菌门(Acidobacteria)、硝化螺旋菌门(Nitrospirae)及芽单胞菌门(Gemmatimonadetes)；养殖水体特异性菌门主要隶属于变形菌门。

在属水平上(图 6)，刺参肠道的特异性菌属主要为芽孢杆菌属(*Bacillus*)、乳酸杆菌属(*Lactobacillus*)、海泥海球菌属(*Halioglobus*)、*Lutimonas*、*Woeseia* 和动性球菌属(*Planococcus*)等；养殖水体特异性菌属则主要隶属于 Clade_Ⅱ、NS7_marine_group、NS9_marine_group、NS11_12-marine_group 和 *Planktomarina* 等。

2.6 菌群功能注释分析

基于 KEGG 代谢通路数据库，共注释到 300 条三级代谢通路，2 组样本在其中 146 条差异代谢通路存在极显著差异($P<0.001$)。对丰度排名前 30 的三级差异代谢通路进行分析(图 7)，其中，刺参肠道中特有的三级代谢通路主要表现为碳水化合物消化吸收(carbohydrate digestion and absorption)、蛋白酶体(proteasome)、RNA 转运(RNA transport)、蛋白质消化吸收(protein digestion and absorption)、鞘脂类代谢(sphingolipid metabolism)和初级胆汁酸生物合成(primary bile acid biosynthesis)等。养殖水体中的特异性三级代谢通路具体表现为四环素生物合成

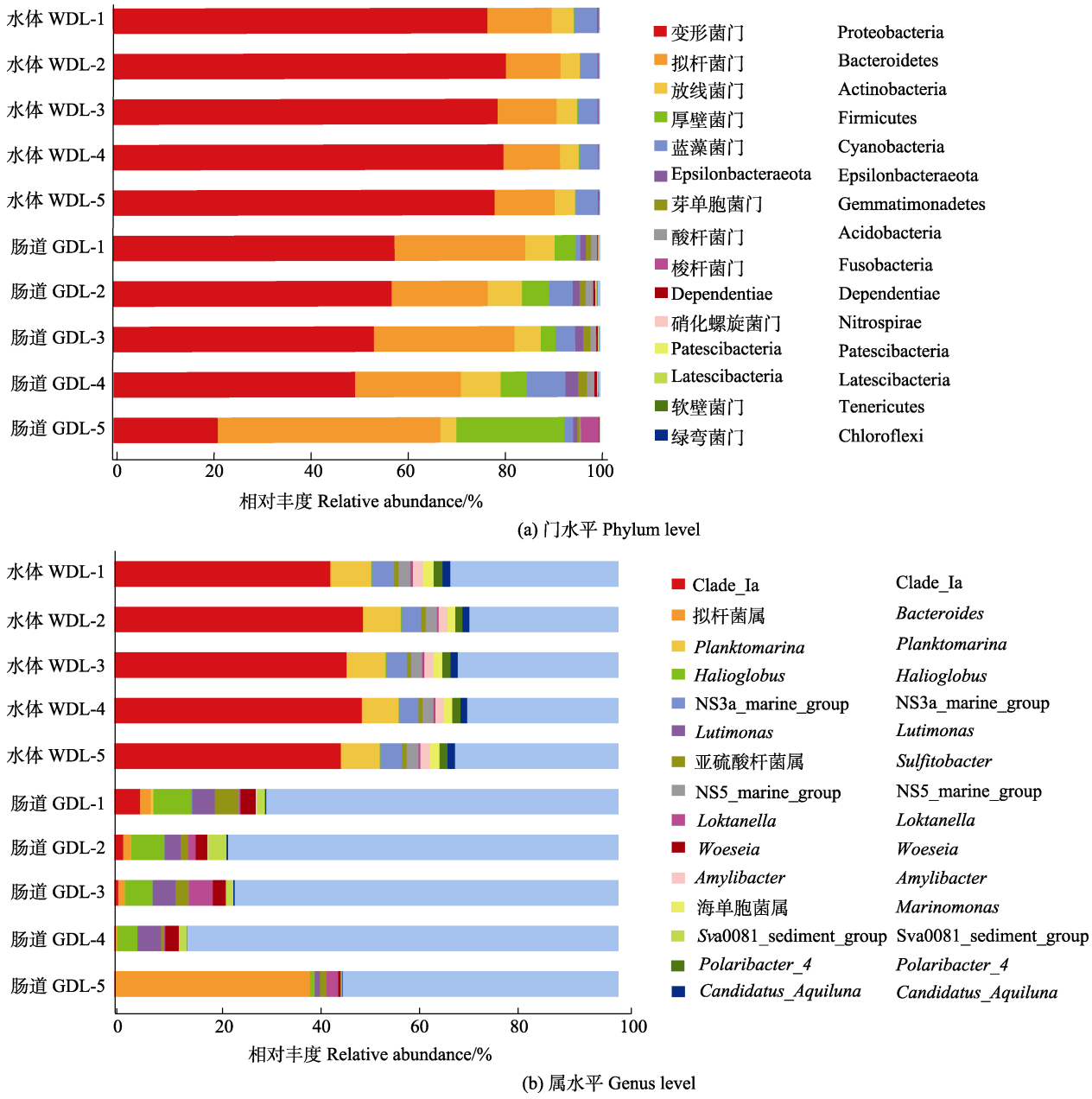

图 5　刺参肠道(GDL)和养殖水体(WDL)优势菌群在门(a)和属(b)水平上的相对丰度

Fig.5　Relative abundance of the dominant bacterial in the gut of *A. japonicus* (GDL) and culture water (WDL) at the phylum (a) and genus (b) levels

(tetracycline biosynthesis)、抗坏血酸和盐酸的代谢(ascorbate and aldarate metabolism)、磷脂酰肌醇(phosphatidylinositol signaling system)、磷酸肌醇代谢(inositol phosphate metabolism)和安沙霉素类生物合成(biosynthesis of ansamycins)等。

3　讨论

3.1　刺参肠道及其养殖水体优势菌群

微生物作为海洋生态系统中的主要分解者，推动着生态系统物质循环和能量流动，对于维持生态系统平衡及养殖生物健康等方面起着十分重要的作用(李革雷等, 2012)。开展刺参肠道及其养殖水体菌群特征研究，不仅可以为养殖过程中病害防治及污染治理提供一定的理论支撑，还可以为养殖环境微生态调控构建提供一定的实践基础。

本研究中，刺参肠道及其养殖水体样品中相对丰度占比前 10 位的优势细菌种类基本一致，且优势菌群均主要隶属于变形菌门和拟杆菌门。该结果与已报道的福建吊笼养殖刺参(陆振等, 2016)和池塘养殖刺参(丁斯予等, 2019)肠道优势菌门相一致，表明不同地域和不同养殖模式下，变形菌门和拟杆菌门均作为

图 6 刺参肠道(GDL)及养殖水体(WDL)差异菌群 LEfSe 分析

Fig.6 LEfSe analysis about the specific bacterial community of the *A. japonicus* gut (GDL) and culture water (WDL)

核心菌群存在于刺参肠道及养殖水体中。变形菌门作为细菌中最大和最具多样性的一个族群，具有较强的适应性和丰富的代谢多样性，在生物化学物质循环过程中起重要作用(白洁等, 2009; 刘思亮等, 2002; Gupta, 2000)，同时还可以产生多种消化酶分解各种纤维素和半纤维素，以帮助宿主从食物中消化吸收养分(Rimoldi *et al*, 2018; Morriso *et al*, 2009; 杨坤杰等, 2016)。拟杆菌门作为微生物群落中最大的革兰氏阴性菌，具有水解淀粉和几丁质的能力，可帮助宿主降解碳水化合物、蛋白质和大量宿主本身难以消化的植物多糖等其他物质，为宿主提供能量，促进生长，对生物体内肠道健康平衡具有积极作用(Gibiino *et al*, 2018; Cottrell *et al*, 2000; O'Sullivan *et al*, 2002; Sonnenburg *et al*, 2005)。变形菌门和拟杆菌门作为核心菌群大量存在于刺参肠道和养殖水体中，对维持养殖环境微生态平衡和刺参机体健康具有重要作用。

3.2 刺参肠道特异性菌群

不同环境介质中所具有的不同生活条件，以及某些菌群特定的生物学功能限制会导致特异性菌群的产生，这些特异性菌群也会对宿主产生一定的影响(Zhou *et al*, 2022)。本研究中，刺参肠道中特异性菌属有 158 种，且多数为厌氧和需盐种类，以芽孢杆菌属、乳酸杆菌属、海泥海球菌属、*Lutimonas* 和 *Woeseia* 等为代表。

据报道，芽孢杆菌(*Bacillus*)属革兰氏阳性菌，含有较高的蛋白酶、脂肪酶和淀粉酶活性，对有机物质具有很强的降解能力，可以产生抗逆性的芽孢，促进蛋白质和脂肪在胃肠道的消化吸收，调节肠道菌群平衡，在维持动物肠道和促进机体健康生长中起重要作用(董春光等, 2015)。在动物肠道以及水体中增加芽孢杆菌的比例，可显著提高水产动物对饲料的利用率，改善生长性能和体成分，提高机体的抗氧化能力和免疫能力，抑制有害菌的生长，提高水产动物的产量(Gullian *et al*, 2004; Ziaeinejad *et al*, 2006; Kennedy *et al*, 1998; 张干等, 2019)。因此，芽孢杆菌现已作为益生菌在水产动物中得到普遍应用(Gomez-Gil *et al*, 2000; Hong *et al*, 2005; 姜燕等, 2022; 杜佗等, 2017)。乳酸杆菌属对病原菌具有较强的抑制作用，不仅可以分泌乳酸、过氧化氢和细菌素等抗菌化合物，改变细胞膜渗透性，抑制病原菌的生长(Xin *et al*, 2020)，还可以与肠道内的病原菌竞争营养物质或粘附位点，从而达到抑制病原菌的作用(Nikoskelainen *et al*, 2001)。Yan 等(2007)研究发现，乳酸杆菌能保护肠道上皮细胞，鼠李糖乳酸杆菌(*Lactobacillus rhamnosus*)分泌的 2 种蛋白 p75 和 p40 均能抑制细胞因子诱导的上皮细胞凋亡，并显著降低 TNF-α 引起的肠上皮细胞损伤。此外，乳酸杆菌还可以增强肠道上皮的屏障功能，调节黏膜免疫系统，从而发挥调节肠道微生态的作用。Martin 等(2008)研究发现，副干酪乳杆菌(*Lactobacillus paracasei*)和鼠李糖乳杆菌(*L. rhamnosus*)可以产生结肠黏膜细胞的重要能量来源，从而维持结肠上皮细胞的健康，增强肠道上皮的屏障功能，为宿主营造一个健康的肠道微生态环境，有效防止有害微生物对宿主的侵染。刺参肠道中存在的大部分特异性菌群可通过自身生理活动影响宿主的营养、代谢、免疫等生理过程，对机体的生长健康尤为重要。

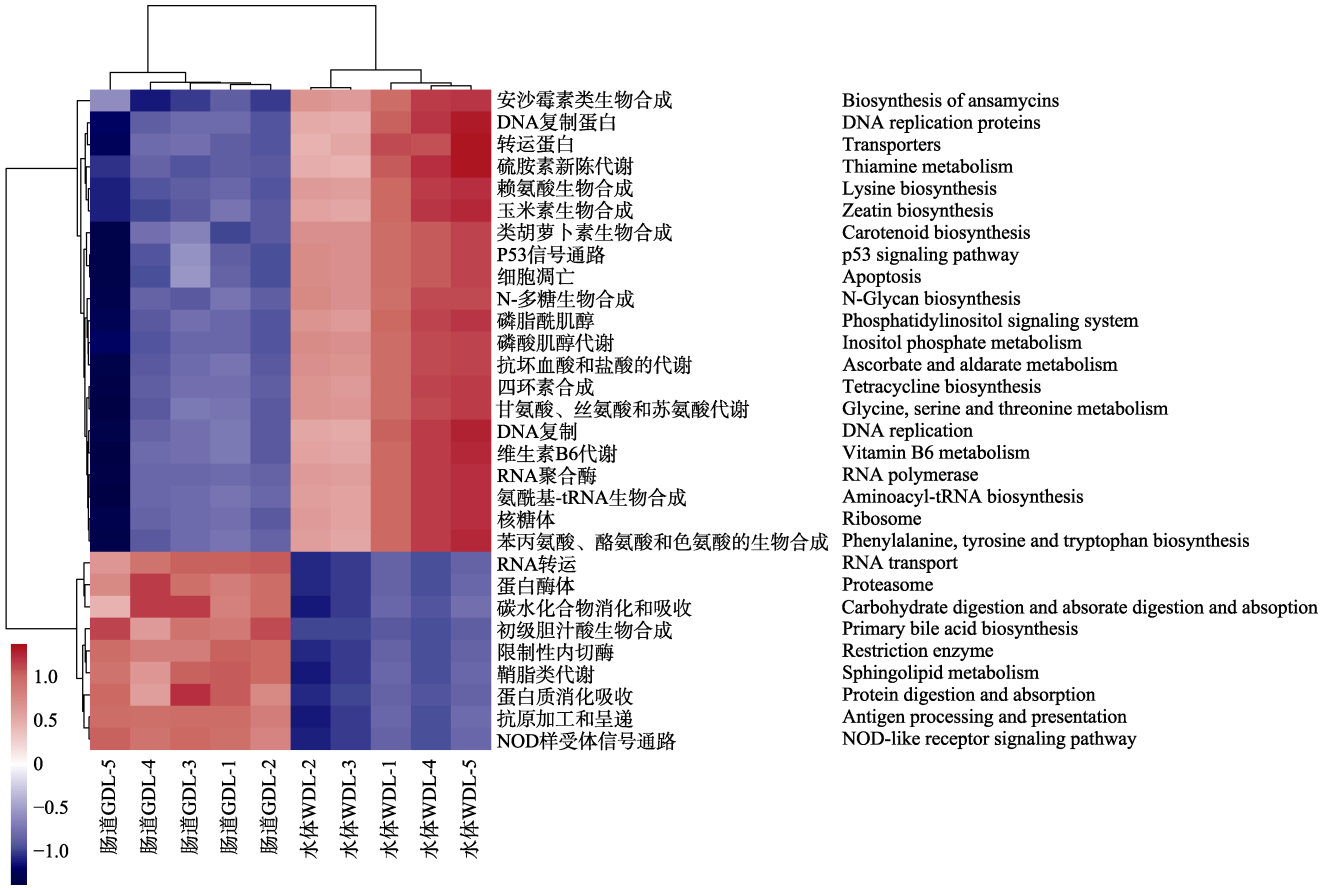

图 7　KEGG 三级代谢通路刺参肠道(GDL)和养殖水体(WDL)特异性代谢通路的分级聚类热图

Fig.7　Hierarchically clustered heatmap of the specific metabolic pathways of the *A. japonicus* gut (GDL) and culture water (WDL) at the KEGG C level

上面的聚类分支代表样本来自不同的分组，左侧聚类树代表功能的分类，
红色表示菌群功能相对丰度较高，蓝色表示菌群功能相对丰度较低。

The cluster branches above represent samples from different groups, the cluster tree on the left represents the classification of functions, red indicates that the relative abundance of bacterial community function is relatively high, and blue indicates that the relative abundance of bacterial community function is relatively low.

3.3　刺参肠道菌群功能

本研究基于 KEGG 差异代谢通路分析得出，刺参肠道菌群的功能主要表现在碳水化合物消化吸收、蛋白质消化吸收、鞘脂类代谢和初级胆汁酸生物合成等与新陈代谢相关通路的差异化表达。其中，值得注意的是三级代谢通路中碳水化合物消化吸收在刺参肠道内显著上调。曾晨燨等(2020)研究表明，菌群功能可以反映出细菌群落整体代谢水平。碳水化合物消化吸收通路的显著上调可能与刺参肠道内检测到较高相对丰度的拟杆菌门(相对丰度 28.57%)和厚壁菌门(相对丰度 8.09%)细菌相关。研究表明，拟杆菌门细菌可以刺激肠道内壁产生岩藻糖基化聚糖，通过碳水化合物分子水解获得碳和能量，在发酵碳水化合物的同时有效分解纤维壁的多糖，从而增强宿主对营养物质的消化吸收，提高营养利用率(刘荣瑜等, 2022; 黄媛媛等, 2022)。厚壁菌门细菌在碳水化合物的营养代谢过程中发挥着重要的作用，具有多种与淀粉降解酶有关的基因，通过分解纤维素产生挥发性脂肪酸，帮助刺参消化吸收营养物质(McDermott *et al*, 2014; Hooper, 2004; Sears, 2005; 杨求华等, 2016)。刺参生长过程对碳水化合物、蛋白质和脂类物质需求较高(Xia *et al*, 2015)，其肠道内含有大量微生物群落，能够产生和分泌多种酶类，具有消化和转化营养物质的功能。然而，引起刺参机体碳水化合物、蛋白质、鞘脂类等有机物的消化代谢不仅仅是由单一细菌产生的，而是多种细菌群落共同作用的结果，其相关机制还有待进一步研究。

4　结论

综上所述，刺参肠道与环境优势菌群组成存在一定的相关关系，同时也表现出独特的结构和功能特

征。本研究仅局限于考虑刺参养殖水体单一环境介质，后续将进一步结合投喂饵料情况开展吊笼养殖刺参肠道微生物群落动态变化研究，以期为北方刺参吊笼健康养殖提供一定的理论参考。

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Characteristics and Correlation Analysis of Bacterial Community Structure in the Gut of *Apostichopus japonicus* and Culture Water in Suspension Cages from North China

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Abstract Owing to its high economic and nutritional value, *Apostichopus japonicus* is an important mariculture species in North China. Because of the rapid development of its aquaculture industry in recent years, the limitations of traditional aquaculture modes such as pond aquaculture, cofferdam aquaculture, and beach aquaculture have become increasingly prominent. Therefore, the high-efficiency and healthy northern suspension cage *A. japonicus* breeding model, with the best comprehensive benefits and the least management problems, came into being. Microorganisms, as an essential part of the aquaculture pond ecosystem, not only play an important role in the material circulation and energy flow of the ecosystem, but also have great significance in maintaining ecosystem balance. As a representative invertebrate, echinoderms such as *A. japonicus* have a simple digestive structure. Bacteria account for a large proportion of the gut microbiome of *A. japonicus*, providing more than 70% of their energy demand. The bacterial community is closely related to the healthy growth of the host and plays an important role in digestion and metabolism, defense against pathogens, and immune function. However, the complex bacterial community in the gut of aquaculture species depends on the culture environment, and there is a close symbiotic relationship between the environmental and gut bacterial community which affects the survival and growth of organisms, disease occurrence, and material circulation. Previous studies have shown that the complex bacterial community in the gut of *A. japonicus* primarily comes from their habitat and maintains a relatively stable dynamic balance with the external environmental community. In order to improve the growth capacity of *A. japonicus* and quality of the culture water, it is important to understand the structural characteristics of the bacterial communities of *A. japonicus* and their culture water to support the development of the *A. japonicus* aquaculture industry. This information will provide a theoretical reference for the healthy aquaculture of *A. japonicus* and assist with disease prevention and control. Clarifying the complex relationship between the structure and functional characteristics of bacterial communities and the aquaculture environment, as well as the important role of the bacterial community in growth, will support future research on the bacterial community mechanisms, explore ways to improve the ecological regulation of breeding yield, and promote the healthy development of the *A. japonicus* culture industry. To date, there have been limited studies on the correlation between the gut bacterial community structure of *A. japonicus* and its culture environment.

At present, most of the existing studies are based on the traditional pure culture or separation and

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enrichment culture methods, which cannot accurately reflect natural bacterial communities. In recent years, high-throughput sequencing technology has introduced a new way to comprehensively analyze the structural and functional characteristics of bacterial communities by combining several bioinformatics methods. With the continuous development of molecular sequencing technology, 16S rRNA high-throughput sequencing technology has become a valuable tool to study the structure of bacterial communities. It has been widely used to study a variety of ecosystems and bacterial community diversity, providing a novel means to study the species diversity and quantity of bacterial communities, and the structural and functional characteristics of bacterial communities. Most of the relevant existing studies are based on the structure and diversity of the bacterial community of *A. japonicus* cultured in the south, whereas only a few studies have been conducted on the structure and functional characteristics of bacterial communities of *A. japonicus* cultured in suspension cages in the north. Therefore, in order to investigate the relationship between the bacterial community structure of *A. japonicus* and the culture water, this study analyzed their structural and functional characteristics in cage-cultured *A. japonicus* in North China using high-throughput sequencing technology, and preliminarily discussed the correlation between them. The results showed that the diversity and richness of the *A. japonicus* gut bacterial community were significantly higher than those of the culture water (P<0.05). The dominant bacteria in the gut of *A. japonicus* and the culture water were Proteobacteria and Bacteroidetes. There were 13 common core bacteria with a relative abundance greater than 0.1%. In addition, the bacterial communities showed some independence; the specific phyla in the gut belonged to Firmicutes and Chloroflexi, represented by *Bacillus*, *Lactobacillus*, *Halioglobus*, *Lutimona*s, and *Woeseia*. Based on an analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway database, a total of 300 tertiary metabolic pathways was annotated, among which 146 tertiary metabolic pathways had highly significant differences (P<0.001). The specific metabolic pathways in the gut of *A. japonicus* were mainly carbohydrate digestion and absorption, protein digestion and absorption, and sphingolipid metabolism. This study showed that the bacterial community in the gut of *A. japonicus* is similar to that of the culture water, but there were significant differences in the relative community abundance. The results of this study provide a theoretical basis for the healthy cultivation of northern *A. japonicus* in suspension cages.

Key words *Apostichopus japonicus*; Gut bacterial community; Suspension cage culture; High-throughput sequencing; Community structure