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半干旱亚湿润干旱沙区樟子松根内真菌群落结构和功能时空动态特征



长江口近岸地区抗生素抗性基因与微生物群落分布 特征

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摘要:由于抗生素的大量使用,环境中微生物对抗生素的抗性不断增加,抗生素抗性基因(ARGs)问题越来越严重,严重威胁生态安全和人类健康.为研究长江口近岸地区水体和底泥沉积物中的 ARGs 和微生物群落的分布特征,通过野外调查采集了8个站点的水样和沉积物样本,对2种磺胺类抗性基因(sull, sul2)、6种四环素类抗性基因(tetM, tetC, tetX, tetA, tetQ)、1种整合子基因 intII、16S rRNA 基因和微生物群落进行检测分析.结果表明,长江口近岸地区 10种抗性基因的检出率为 100%.其中,整合子基因 intII 和水样中多种 ARGs 呈显著正相关关系.变形菌门(Proteobacteria)和拟杆菌门(Bacteroidota)是长江口近岸地区水环境中的优势菌门; Chloroplast 为水体中的主要菌属, Chloroplast 和 Nitrospira 为沉积物中的主要菌属.在水体中,硝化螺旋菌门(Nitrospirota)是4种四环素类抗性基因(tetX, tetA, tetQ)共同的潜在宿主;在沉积物中,Sva0485是 sull 和 intII的共同潜在宿主.微生物群落的分布是长江口近岸地区 ARGs 迁移转化的重要影响因素.

关键词:抗生素抗性基因(ARGs); 荧光定量 PCR(qPCR); 16S 扩增子测序; 长江口近岸地区; 微生物群落中图分类号: X52; X172 文献标识码: A 文章编号: 0250-3301(2023)01-0158-11 **DOI**: 10.13227/j. hjkx. 202203160

Distributions of Antibiotic Resistance Genes and Microbial Communities in the Nearshore Area of the Yangtze River Estuary

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Abstract: Due to the extensive use of antibiotics, the resistance of microorganisms to antibiotics in the environment is increasing, and the problem of antibiotic resistance genes (ARGs) is becoming more and more severe, which seriously threatens ecological security and human health. In order to study the distribution characteristics of ARGs and the microbial community in different media in the coastal area of the Yangtze River Estuary, water and sediment samples from eight sites were collected through a field investigation. Two sulfonamide resistance genes (sul1, sul2) and six tetracycline resistance genes (tetM, tetC, tetX, tetA, tetO, and tetQ), one integrase gene intII, 16S rRNA gene, and the microbial community were detected and analyzed. The results showed that the detection rate of 10 resistance genes in the coastal area of the Yangtze River Estuary was 100%. intII was significantly positively correlated with various ARGs in the water samples. Proteobacteria and Bacteroidota were the dominant bacteria phyla in the water environment of the Yangtze River Estuary. Chloroplast was the main bacteria genus in water, and Chloroplast and Nitrospira were the main bacteria genera in sediment. In water, Nitrospirota was the common potential host of four tetracycline resistance genes (tetX, tetA, tetO, and tetQ). In sediments, Sva0485 was a potential host community shared by sul1 and intII. The distribution of the microbial community was an important factor affecting the migration and transformation of ARGs in the nearshore area of the Yangtze River Estuary.

Key words; antibiotics resistance genes (ARGs); quantitative-polymerase chain reaction (qPCR); 16S amplicon sequencing; nearshore area of the Yangtze River Estuary; microbial communities

2006 年, Pruden 等^[1] 提出将抗生素抗性基因 (antibiotic resistance genes, ARGs) 作为一种新型的 环境污染物,世界卫生组织也于 2015 年明确提出将 微生物抗性作为全球健康风险. 全世界范围内越来越多的研究人员开始关注 ARGs 诱发的相关问题. 近年来,抗生素的滥用导致微生物(包括病原菌和

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非病原菌)对抗生素的抗性逐年增加,环境中的细菌可以通过基因突变和代系遗传等方式获得抗性基因,也可以通过转导、接合和转化等方式获得外源存在的抗性基因^[2].也有研究表明,环境中较低浓度的抗生素可以作为微生物种间或者种内的信号分子,从而调控微生物群落对抗生素进行选择性适应^[3].抗生素的存在会加速上述过程的发生,增加环境中抗生素抗性出现的频率,对环境安全和人类健康造成潜在威胁.近年来,我国各大流域都已存在不同程度的 ARGs 污染^[4~7].

海岸带尤其是河口地区,是连接陆地与海洋的重要过渡区域^[8],接纳了大量来自河流径流和污水排放的污染物,包括多环芳烃^[9]、重金属^[10]、抗生素^[11]和ARGs^[12].含有ARGs的污水最终会通过河口排入海洋环境,并在人类活动和水文气象等多种因素的耦合影响下^[13,14],发生一系列复杂的物理-化学-生物作用过程.抗生素抗性基因导致细菌产生耐药性,并可通过水平转移在细菌间传播扩散^[15],严重威胁生态系统安全和人类健康^[16].因此,厘清河口区域水环境中ARGs和微生物群落的分布特征至关重要.

本文选取长江口近岸地区作为研究区域,采用 荧光 定量 PCR (quantitative-polymerase chain reaction, qPCR)分析水体和沉积物中共 10 种 ARGs 的分布规律,包括 2 种磺胺类抗性基因(sull、sull)、6 种四环素类抗性基因(tetM、tetC、tetX、

tetA、tetO、tetQ)以及1种整合子基因 intII 和16S rRNA 基因,并采用16S 扩增子测序技术分析水环境中微生物群落的分布特征,揭示长江口近岸地区 ARGs 与微生物群落间的影响机制,以期为控制长江口近岸地区水环境中 ARGs 污染提供数据支持和科学依据,并为长江口近岸地区 ARGs 生态风险评价提供参考.

1 材料与方法

1.1 样品采集

本文采集了长江口近岸地区8个采样点的表层水和沉积物样品,具体采样点分布如图1所示.自北向南分别是浏河口(1)、石洞口(2)、吴淞口(3)、竹园排污口(4)、三甲港水闸(5)、朝阳农场(6)、大治河(7)和南汇东滩(8).其中浏河口(1)是浏河与长江的交汇地,吴淞口(3)是黄浦江与长江的交汇地,大治河(7)是大治河与长江交汇地,石洞口(2)和竹园(4)位于城市污水处理厂的排污口,朝阳农场(6)属于潮滩环境,三甲港水闸(5)位于九段沙湿地国家自然保护区的下游断面,南汇东滩(8)属于湿地保护区.

有研究表明,温度对 ARGs 的传播扩散起着重要的作用^[17].因此本次采样时间为 2021 年 7 月 14 日.每个采样点采集表层水(2 L)并装入超纯水洗过的聚乙烯瓶中,同时采集 100 g 沉积物(0~5 cm),并转移至灭菌的塑料袋中.每个采样点采集 3



图 1 采样点分布示意

Fig. 1 Location of sampling sites

1.2 DNA 提取

验室.

1 L 水样经 0.22 μm 孔径的玻璃纤维滤膜过滤 并收集滤膜,沉积物称取 5 mg 湿样,然后根据 DNA 提取试剂盒(Fast DNA Spin Kit for Soil, MP Biomedicals,美国)说明书提取水样和沉积物样的总 DNA. 提取的 DNA 质量和完整性通过 1.5% 的琼脂 糖凝胶电泳进行验证,并通过超微量分光光度计 (NanoDrop2000,美国)进一步定量 DNA 的浓度. 所

1.3 荧光定量 PCR

有 DNA 样品存放于 - 20℃冰箱备用.

本文通过荧光定量 PCR 技术(qPCR)定量分析

了2类8种目标抗性基因和1类整合子基因.本文 使用的 ARGs 片段对应的引物序列、片段大小、退 火温度和文献如表 1 所示. 荧光定量 PCR 反应体系 (20 µL) 为:2×Taq Pro Universal SYBR qPCR Master Mix 10 μL, 上下游引物各 0.4 μL, DNA 模板 2 μL, ddH₂O 7.2 μL. 基因扩增的热循环反应程序为:95℃ 预变性 30 s; 40 个循环(每个循环 95℃ 10 s,退火 30 s, 退火温度见表 1);溶解曲线温度设置为由 60℃增加到95℃.

为了生成用于测定提取 DNA 中每 ng 基因丰度 的 qPCR 标准曲线,将目标基因克隆到质粒(E. coli DH5a)中.使用 NanoDrop2000 测量质粒浓度,并根 据质粒长度和靶序列计算每毫升溶液中靶基因的丰

表 1 目的基因的 qPCR 引物信息和反应条件

	•		="	
	Table 1 Primers, product lengths, and anneal	ling temperatures of q	PCR for target genes	~~~
目的基因	引物序列(5'-3')	片段大小/bp	退火温度/℃	文献
sulI	F-CACCGGAAACATCGCTGCA R-AAGTTCCGCCGCAAGGCT	158	60	[18]
sul2	F-TCCGGTGGAGGCCGGTATCTGG R-CGGGAATGCCATCTGCCTTGAG	191	55	[19]
tetM	F-CCGTTGGGAAGTGGAATGC R-TCCGAAAATCTGCTGGGGTA	196	57	[20]
tetC	F-TGCAACTCGTAGGACAGGTG R-ACCAGTGACGAAGGCTTGAG	140	60	[21]
tetX	F-AGCCTTACCAATGGGTGTAAA R-TTCTTACCTTGGACATCCCG	278	60	[22]
tetA	F-GCTACATCCTGCTTGCCTTC R-CATAGATCGCCGTGAAGAGG	210	60	[20]
tetO	F-GATGGCATACAGGCACAGACC R-GCCCAACCTTTTGCTTCACTA	172	57	[20]
tetQ	F-AGAATCTGCTGTTTGCCAGTG R-CGGAGTGTCAATGATATTGCA	167	53	[23]
intII	F-CCTCCCGCACGATGATC R-TCCACGCATCGTCAGGC	280	60	[22]
16S rRNA	341F-CCTACGGGAGGCAGCAG 534R-TTACCGCGGGCTGCTGGCAC	193	60	[24]

度, 通过对携带目的基因的质粒进行 10 倍的连续梯 度稀释,构建 qPCR 标准曲线. 目的基因的标准曲线 和扩增效率如表 2 所示.

表 2 ARGs 定量所需标准曲线和扩增效率1)

Table 2 Standard curve and amplification efficiency of target genes

基因名称	标准曲线	R^2	扩增效率/%
sul1	y = -3.5738x + 41.395	0. 998 6	91
sul2	y = -3.4855x + 47.301	0. 994 0	94
tetM	y = -3.1425x + 37.189	0.9950	108
tetC	y = -3.4005x + 39.997	0. 998 2	97
tetX	y = -3.5007x + 38.934	0. 997 8	93
tetA	y = -3.3743x + 37.905	0. 998 4	98
tetO	y = -3.4573x + 40.483	0.9996	95
tetQ	y = -3.8476x + 43.346	0. 997 8	82
intII	y = -3.822x + 43.46	0. 999 2	83
16S rRNA	y = -3.2269x + 37.564	0. 999 3	104

¹⁾ y 为 qPCR 检测的 Ct 值, x 为计算后的基因拷贝数经 lg 转化的值

1.4 16S 扩增子测序

为了研究长江口近岸地区水环境的细菌群落多 样性,使用上游引物 341F(ACTCCTACGGGAGGC AGCA) 和下游引物 805R (GGACTACHVGGGTWTC TAAT)对 16S rRNA 基因 V3/V4 区域进行扩增. 扩 增产物送至上海伟寰生物科技有限公司,采用 Illumina NovaSeq PE250 平台进行 16S 扩增子测序.

1.5 数据分析

本文使用 Origin 软件对所有数据进行汇总分 析,绘制柱状图、相关性热图和聚类分析热图,用 Spearman 法进行相关性分析(P<0.05 表示显著相 关),使用R语言包"Hmisc"计算ARGs与微生物群 落的相关系数. ARGs 丰度变化的主成分分析采用 免费的在线数据分析平台 Tutools 进行分析 (https://www.cloudtutu.com), ARGs 与微生物群落

网络分析图的绘制采用生科云在线数据分析平台 (https://www.bioincloud.tech/).

2 长江口近岸地区 ARGs 的赋存

2.1 ARGs 的分布特征

图 2 为长江口近岸地区水环境中 ARGs 相对丰度分布柱状图,W1~W8 表示长江口近岸地区 1~8 号采样点的水样,S1~S8 表示 1~8 号采样点的沉积物样.本文利用 16S rRNA 基因的丰度对其余目的基因的丰度进行标准化处理,用来表征单一细菌ARGs 的相对丰度(基因拷贝数与 16S rRNA 拷贝数的比值)^[18,25],以减少 DNA 提取效率以及水环境中微生物背景值干扰所造成的误差^[26,27].本文选择的10 种 ARGs 在长江口近岸地区 8 个采样点的水样和沉积物中的检出率是 100%.

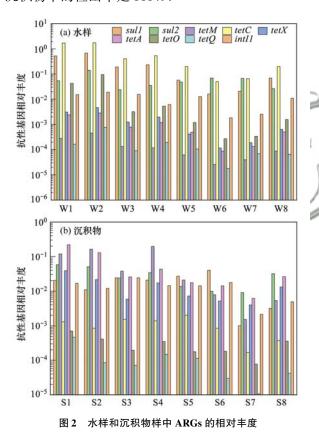


Fig. 2 Relative abundance of ARGs in water and sediment samples

如图 2 所示,在水样中,9 种目标抗性基因中 tetC 的总相对丰度最高,其相对丰度范围在 5.01 × $10^{-2} \sim 1.76$ 之间,为长江口近岸地区表层水中的优势基因.在沉积物中, tetM 和 tetA 的总相对丰度处于较高的水平,相对丰度范围分别在 $1.51 \times 10^{-3} \sim 1.96 \times 10^{-1}$ 及 $6.19 \times 10^{-3} \sim 2.19 \times 10^{-1}$ 之间,为长江口近岸地区沉积物中的优势基因.不同采样点中ARGs 的总相对丰度大小分布规律为: W2 > W1 > W4 > W3 > W5 > W8 > W7 > W6(水样中); S1 > S2

> S4 > S3 > S5 > S6 > S8 > S7 (沉积物中). 在水样中, W2 和 W1 的 ARGs 相对丰度显著高于其他采样点;在沉积物中, S1、S2 和 S4 的 ARGs 相对丰度显著高于其他采样点. 石洞口和竹园采样点位于大型污水处理厂及其邻近水域,而浏河口采样点位于城市内河与长江下游交汇处. 本文分析结果表明ARGs 在长江口近岸地区水环境中的富集与污水处理厂的污水排放以及受人类活动影响的城市内河径流有关. 该结论与前人研究该区域的结论相似[28,29].

本文长江口近岸地区水样和沉积物中 ARGs 的 绝对丰度平均值分别为 2.32 × 10⁴ ~ 7.66 × 10⁴ copies· mL^{-1} 和 1.41 × 10⁷ ~ 6.59 × 10⁷ copies· g^{-1} . Lu 等[30]研究了 ARGs 在渤海和黄海的空间分布,结 果表明在渤海,目的基因的总绝对丰度范围在2.05 ×10² ~ 7.25 × 10³ copies · mL⁻¹之间,而在黄海中,目 的基因的总绝对丰度范围在 21.1~8×10³ copies·mL⁻¹之间. 在渤海和黄海的河口区域,目的 基因的总绝对丰度范围分别在 1.23 × 104 ~ 3.94 × 10^{5} copies • mL⁻¹ 和 8.91 × 10^{4} ~ 3.67 × copies·mL-1之间. Chen 等[3] 对东海海湾沉积物中的 27个目的基因进行了研究,在杭州湾和象山湾河口 区域,目的基因的总绝对丰度范围分别在8.39×106 ~ 2. 33 \times 10⁷ copies·g⁻¹ 和 1. 25 \times 10⁷ ~ 2. 08 \times 10⁷ copies·g⁻¹之间. Lu 等^[25]对辽河口和大辽河口磺胺类 抗性基因(sul1、sul2 和 sul3)进行了表征,辽河和大 辽河口水样 ARGs 的绝对丰度平均值 < 1 × 103 copies·mL⁻¹. 杨颖^[32]调查了北江流域四环素抗性基 因的污染情况,发现在所检出的四环素抗性基因中, 含量最高的是 tetC,与 16S rRNA 拷贝数比值范围在 8.3×10⁻²~13.2之间. 范长征等^[5]分析了湘江长沙 河段水体及底泥中4种常见四环素类抗生素的含量 特征及其季节变化,其优势基因 tetC 和 tetA 的相对丰 度范围在 8.2×10⁻⁵~7.3×10⁻³之间. 长江口近岸地 区 ARGs 的污染情况在全国处于较高的水平.

为了更深入地研究长江口近岸地区水环境中ARGs的分布特征以及水体和沉积物ARGs分布的相关性,本文采用主成分分析法(PCA)^[33]对长江口近岸地区8个采样点不同介质中ARGs的变化特征进行聚类分析.如图3所示,X轴表示第一主成分(PC1),Y轴表示第二主成分(PC2).点与点之间的距离表示差异程度.PC1和PC2总共解释了基因丰度变异的85.2%,其中,PC1的值为54.6%.可以得出,表层水样品在PC1上聚集,沉积物样品在PC2上聚集.说明不同采样点不同介质中的ARGs分别聚类在一起,而且水样与沉积物样之间没有关联性.

只有个别采样点的 ARGs 分布超出了 95% 置信椭圆 的范围,分别是水样中的 W2 和沉积物样中的 S1. 水样中 ARGs 的分布变化可能是由于石洞口污水处 理厂的污水排放提供了大量的营养物质,促进了当 地的细菌生长. 沉积物样中 ARGs 的分布变化可能 是由于浏河口接纳了大量来自城市内河径流和人类 活动引起的陆源排放的污染物,包括重金属、抗生 素、抗性细菌等. 浏河口和石洞口样点中 ARGs 的相 对丰度平均值分别为: 2.62×10^{-1} 和 4.50×10^{-1} (水 样中),5.27×10⁻²和4.32×10⁻²(沉积物中).这两个 样点的 ARGs 污染水平处于同一数量级,其差异很 小.而且这两个样点以及吴淞口和竹园排污口的污染 水平显著高于其他4个样点,所以污水处理厂的污水 排放和城市内河径流带来的陆源污染物是 ARGs 污 染的主要来源. Proia 等[34] 通过研究比利时 Zenne 河 中 ARGs 的分布规律,发现污水处理厂处理水的排放 会增加 ARGs 的丰度. Guo 等[35] 研究了 ARGs 在长江 口的赋存特征,结果表明,污水影响区域和内河交汇

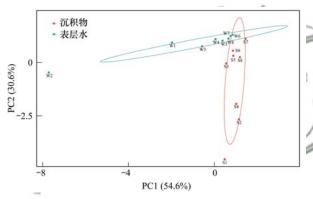


图 3 不同介质中 ARGs 丰度的聚类分析

Fig. 3 Cluster analysis of ARGs abundance in different media

区域会对 ARGs 的丰度造成较大的影响.

2.2 intII 整合子基因与 ARGs 的相关性

水平转移是 ARGs 在环境介质以及微生物中扩散传播的主要途径,而整合子(integrons)是促进水环境中 ARGs 水平转移的重要移动基因元件^[36-38].

长江口近岸地区8个采样点的 intII 的相对丰 度从高到低为: W2 > W3 > W1 > W5 > W8 > W4 > W7 > W6(水样中); S3 > S6 > S1 > S4 > S5 > S2 > S8 > S7 (沉积物中). 采用 Spearman 等级相关性检 验[39]分析了 intII 与其他 ARGs 的相关关系(如表 3),说明 intII 与多种 ARGs 都存在显著的相关关 系,尤其是在水样中. sull 基因在水样和沉积物中 都与 intI1 呈显著相关性(P<0.05),这可能是由于 sul1 的基因序列通常与 intI1 的基因序列重叠所导 致,前人的研究也证实了这一点[40~48].然而,在沉积 物中,四环素类抗性基因和 intII 无显著相关性(P >0.05),与前人的研究结论不符^[44]. Yang 等^[44]以 长江中下游的15个淡水湖泊为研究区域,对18种 不同的 ARGs 进行检测分析. 本研究结果证明四环 素类抗性基因 tetG 与 intII 之间存在显著的相关关 系(P<0.05). 这说明不同种抗性基因的抗性机制 不同,导致其传播扩散的影响因子不同. 虽然 intl1 是促进水环境中 ARGs 水平转移的重要移动基因元 件,但自然界中还广泛存在其他可移动遗传元件,包 括整合子、转座子、噬菌体、质粒和基因岛 等[45,46]. 而且,其他的环境因素包括抗生素、重金属 和多环芳烃等也可能影响表层沉积物中 ARGs 的传 播扩散[47,48].

表 3 表层水和沉积物中 intII 与其余 ARGs 的相关性分析 $^{1)}$

Table 3 Correlation analysis between intII and other ARGs in water and sediment samples

类型		sulI	sul2	tetM	tetC	tetX	tetA	tetO	tetQ
表层水	r	0.881 **	0.048	0. 881 **	0. 881 **	0.786*	0.786*	0.786*	0.690
10/2/10	P	< 0.01	0.911	< 0.01	< 0.01	0.021	0.021	0.021	0.058
沉积物	r	0.786*	0.143	0.405	0.667	0.048	0.143	0.143	0.310
00/1/193	P	0.021	0.736	0.320	0.071	0.911	0.736	0.736	0.456

1)r 表示相关系数;* 表示显著性差异,P < 0.05;** 表示极显著性差异,P < 0.01

3 长江口近岸地区微生物群落分布

3.1 微生物群落多样性分析

本文采用 16S 扩增子测序技术对长江口近岸地区 8 个采样点的微生物群落多样性进行分析. 细菌群落丰富度指数(Chao1)以及多样性指数(Shannon和 Simpson)[49]计算公式如下:

Chao1 =
$$S_{obs} + n_1(n_1 - 1)/2(n_2 + 1)$$
 (1)

式中, S_{obs} 为观测到的 OTU(操作分类单元)数, n_1 为只有一条序列的 OTU 数, n_2 为只有两条序列的 OTU 数.

$$H_{\text{Shannon}} = -\sum_{i=1}^{S_{\text{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N}$$
 (2)

$$D_{\text{Simpson}} = \sum_{i=1}^{S_{\text{obs}}} n_i (n_i - 1) / N(N - 1)$$
 (3)

式中, S_{obs} 为观测到的 OTU 数, n_i 为第 i 个 OTU 中所含的序列数, N 为所有序列数.

如表 4 所示, 沉积物的 Chao1、Shannon 和Simpson 指数都高于表层水,这说明长江口近岸地区沉积物的细菌丰富度和生物多样性要远高于表层水. 竹园采样点表层水的 Shannon 指数在 8 个采样点中最高(6.80), 沉积物中的 Shannon (7.36) 指数仅次于吴淞口采样点(7.38), 这可能是因为污水处理厂的污水排放促进了细菌的繁殖, 增加了竹园周围水环境的微生物群落多样性. 吴淞口采样点表层水中 Simpson 指数远远低于其他采样点, 这说明吴

表 4 细菌群落丰富度指数和多样性指数

Table 4	Racterial	community	richness	and	diversity	index

Table 4	растепат с	community rici	nness and divers	sity index
样品	类型	Chao1 指数	Shannon 指数	Simpson 指数
浏河口(1)	表层水	1 554	6. 18	0. 993
W14-1 11 (1)	沉积物	2 431	7.06	0. 998
石洞口(2)	表层水	928	5. 42	0. 985
H 1171 H (2)	沉积物	2 521	7. 31	0. 999
吴淞口(3)	表层水	427	2. 40	0.709
Λημ H (3)	沉积物	3 166	7. 38	0. 998
竹园排汚口(4)	表层水	1 929	6. 80	0. 997
11 km 11L1 2 tm (±)	沉积物	2 838	7. 36	0. 999
三甲港水闸(5)	表层水	1 066	5. 81	0. 992
	沉积物	2 534	7. 10	0. 998
朝阳农场(6)	表层水	818	4. 96	0. 969
#11H/14-91(0)	沉积物	2 272	6. 70	0. 995
大治河(7)	表层水	885	5. 20	0. 982
XIII (沉积物	2 304	7. 28	0. 999
南汇东滩(8)	表层水	727	4. 84	0. 979
ITI ILZIVIAE (O)	沉积物	1 735	6. 90	0. 997

淞口水样中的群落物种多样性和均匀度偏低,其他 采样点的 Simpson 指数都比较接近,表明物种多样 性程度较均匀.

3.2 微生物群落结构分析

本文对长江口近岸地区 8 个采样点的微生物群落结构特征进行分析. 总体而言,长江口水环境中的微生物群落由 81 个门、231 个纲、569 个目、961 个科、1 960个属和4 788个种组成. 在门分类水平上,不同介质中微生物群落的相对丰度如图 4 所示.

在水样中,变形菌门(Proteobacteria)、放线菌 门(Actinobacteriota)、拟杆菌门(Bacteroidota)和蓝 菌门(Cyanobacteria)为主要菌群,其总相对丰度平 均值为85.87%,其相对丰度平均值占比分别为 46%、13.03%、13.00%和13.84%.经过石洞口污 水处理厂处理水排放后的下游吴淞口采样点中变形 菌门(Proteobacteria)占比显著提高,占总比例的 83.27%,为绝对优势菌门,污水处理厂对变形菌门 (Proteobacteria)有着显著的影响^[50]. 在沉积物中, 变形菌门 (Proteobacteria)、拟杆菌门 (Bacteroidota)、疣微菌门(Verrucomicrobiota)和酸 杆菌门(Acidobacteriota)为主要菌群,其总相对丰度 平均值为64.06%,其相对丰度平均值占比分别为 36.03%、10.72%、9.23%和8.08%.并且,变形菌 门(Proteobacteria)和拟杆菌门(Bacteroidota)作为长 江口水环境中的优势菌群,可能参与众多物质的生 物化学循环过程[51].

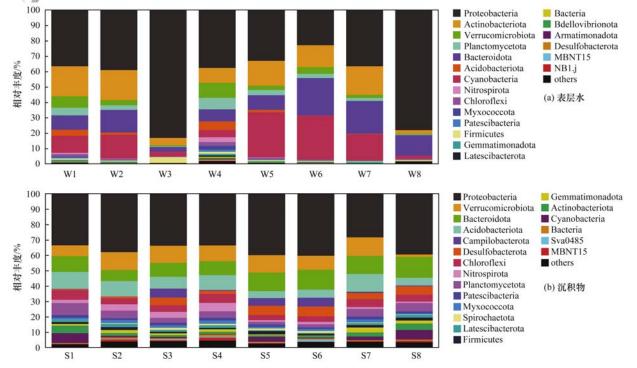


图 4 长江口近岸地区水环境中微生物群落在门水平上的相对丰度

Fig. 4 Relative abundance of microbial communities in the nearshore area of the Yangtze River Estuary at the phylum level

本文在属分类水平上对样品及其菌属进行了聚类分析,并根据各样品中微生物菌属的相对丰度绘制热图,如图 5 所示. 在水体中,W1 和 W5 采样点之间的菌属差异较小,其中相对丰度较高的菌属是Chloroplast、Flavobacterium 和 Rheinheimera. 而 W2和 W3以及 W4和 W5采样点之间的菌属差异较大.

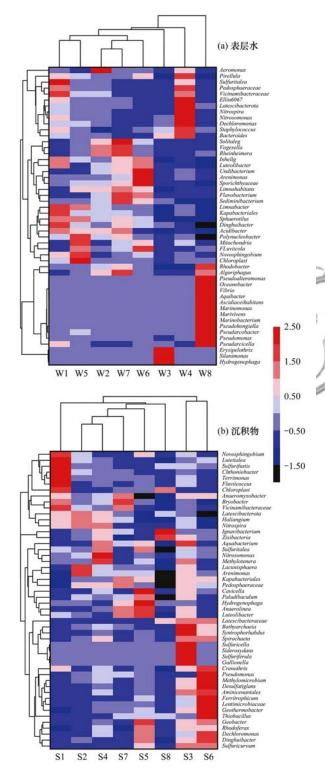
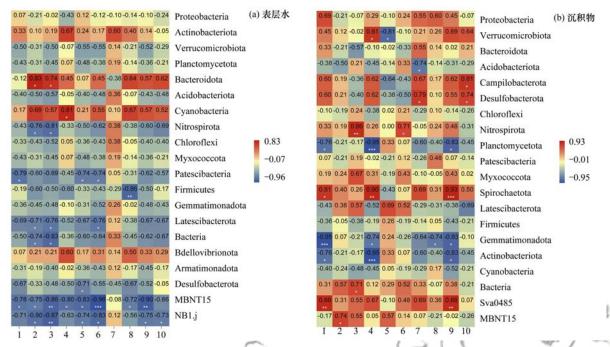


图 5 长江口近岸地区属水平下的聚类分析热图

Fig. 5 Cluster analysis heat map of the nearshore area of the Yangtze River Estuary at the genus level

W2 采样点相对丰度较高的是 Rheinheimera 和 Flavobacterium, 分别占总量的 31.62% 和 19.87%; W3 采样点的主导菌属为 Hydrogenophaga, 其相对丰 度占总量的87.17%,且显著高于其他采样点.在 W4 采样点相对丰度较高的是 Chloroplast 和 Nitrospira, 分别占总量的 14.84% 和 12.83%; 而 W5 采样点主导菌属是 Chloroplast, 其相对丰度占总量 的 45.50%. W4 和 W5 采样点之间的微生物菌属存 在显著差异,说明污水处理厂的污水排放对水环境 中微生物群落结构变化有较大的影响. 有研究发现, 污水处理厂可以直接将微生物释放到环境中[52,53]. 在沉积物中,S2 和 S4 采样点微生物分布的相似性 较高,差异较小,其主导菌属均是硝化螺菌属 (Nitrospira),相对丰度分别占总量的 12.46% 和 11.75%,这可能是由于污水处理厂的厌氧生物处理 工艺增加了水环境中污泥富集硝化螺菌属的风险. 硝化螺菌属(Nitrospira)通常是污水处理厂活性污泥 体系中的优势菌属[54].

3.3 长江口近岸地区 ARGs 与微生物相关性分析 选取相对丰度前 20 的菌门与 ARGs 进行 Speraman 相关性分析,相关性热图如图 6 所示. 20 种菌门与 10 种 ARGs 之间有 200 种对应关系. 在水 样中,菌门与部分 ARGs 间(29/200)呈显著相关(P <0.05),放线菌门(Actinobacteriota)、拟杆菌门 (Bacteroidota)和蓝菌门(Cyanobacteria)与大部分 ARGs 呈正相关. 硝化螺旋菌门(Nitrospirota)、髌骨 门 (Patescibacteria)、厚壁菌门 (Firmicutes)、 Latescibacterota, Bacteria, Desulfobacterota, MBNT15 和 NB1. i 与 ARGs 呈显著负相关. 在沉积物中, 菌门 与部分 ARGs 间(25/200) 呈显著相关(P<0.05), 变形菌门(Proteobacteria)、Campilobacterota、 Desulfobacterota、硝化螺旋菌门(Nitrospirota)、 Spirochaetota、Sva0485 和 MBNT15 与多种 ARGs 呈 显著正相关关系. 拟杆菌门(Bacteroidota)、浮霉菌 (Planctomycetota) 芽 单 胞 菌 门 (Gemmatimonadota)和放线菌门(Actinobacteriota)与 多种 ARGs 呈显著负相关关系. 当微生物菌门与目 的 ARGs 呈显著正相关关系时,这些菌门可能是 ARGs 的潜在宿主. Wang 等[55]研究了洪湖中微生物 群落和 6 种目的 ARGs 之间的相关关系, 发现厚壁 菌门(Firmicutes)、变形菌门(Proteobacteria)和芽单 胞菌门(Gemmatimonadota) 丰度与 ARGs 丰度呈显 著正相关,表明所属这些门的细菌可能是 ARGs 的 宿主. 微生物菌门与目的 ARGs 呈负相关关系可能 意味着该菌门的丰度变化会影响 ARGs 的丰度和水 平转移. Chen 等[56] 的研究表明, 硫杆菌属



1. sull , 2. sul2 , 3. tetM , 4. tetC , 5. tetX , 6. tetA , 7. tetO , 8. tetQ , 9. intII , 10. 16S rRNA; *表示 P < 0.05 , **表示 P < 0.01 , ***表示 P < 0.001

图 6 ARGs 与微生物菌门相关性分析

Fig. 6 Correlation analysis between ARGs and microbial phylum

(Thiobacillus) 对土霉素具有一定的生物降解作用, 从而可能间接抑制 ARGs 的水平基因转移,降低 ARGs 的相对丰度.

为了分析长江口近岸地区 ARGs 潜在的宿主信息,本文采用网络共现图挖掘了 ARGs 与微生物群落的共现模式(图7). 基于 Spearman 相关性系数计算结果(r>0.7, P<0.05),选取 ARGs 与相对丰度排名前20的微生物菌门做网络共现分析. 结果如图7所示,其中的连接线表示节点之间的相关系数大于0.7,显著性小于0.05的相关关系,红线表示正

相关,蓝线表示负相关.水样中共检测到5个潜在宿主,沉积物中共检测到3个潜在宿主.在水样中,磺胺类抗性基因 sul1 未检测到宿主信息, sul2 只有1个宿主信息(放线菌门),四环素类抗性基因(tetX、tetA、tetO 和 tetQ)的潜在宿主较多.在水样中,硝化螺旋菌门(Nitrospirota)是 tetX、tetA、tetO 和 tetQ 共同的潜在宿主.微生物氮循环的频繁发生会产生硝化螺旋菌门(Nitrospirota)^[8],因此,微生物氮的循环可能会间接影响水体中四环素类抗性基因的分布.在沉积物样品中,只有 sul1、tetC 和 intl1 存在潜在

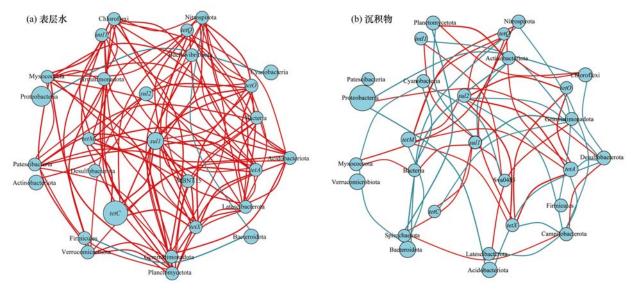


图 7 不同介质中 ARGs 与微生物群落网络分析

Fig. 7 Network analysis of the relationship among ARGs and microbial communities in different media

宿主菌门. 磺胺类抗性基因 sull 的潜在宿主较多(2个),说明在沉积物中 sull 受微生物群落变化的影响较大. sull 的潜在宿主分别为 Spirochaetota 和 Sva0485. 硝化螺旋菌门(Nitrospirota)是 tetC 的潜在宿主. Sva0485 是 sull 和 intll 的共同潜在宿主. 由于不同介质中微生物群落结构不同, ARGs 的分布和诱导的潜在机制可能有所不同, 从而导致 ARGs 与微生物群落的相关性以及 ARGs 的潜在宿主菌存在差异. 共同潜在宿主的存在可能会对 ARGs 的分布特征和水平转移产生影响, 会导致细菌获得多重耐药性, 在一定程度上加速细菌的产生, 生态安全和人类健康可能遭到威胁[57]. 长江口近岸地区多重耐药菌对 ARGs 的影响机制值得进一步研究和探索.

4 结论

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- (1)2 种磺胺类抗性基因(sul1、sul2)、6 种四环素类抗性基因(tetM、tetC、tetX、tetA、tetO、tetQ)以及1种整合子基因 intII 和 16S rRNA 基因在长江口近岸地区水环境中均有检出. 城市内河径流和污水处理厂的污水排放对长江口近岸地区 ARGs 的分布有较大的影响.
- (2)在水体中,整合子基因 *intII* 与 7 种 ARGs (*sul1*、*sul2*、*tetM*、*tetC*、*tetX*、*tetA*、*tetO*)存在显著正相关关系(*P*<0.05);在沉积物中整合子基因 *intII* 只与一个磺胺类抗性基因 *sul1* 存在显著正相关关系.整合子基因 *intII* 可能已经不是沉积物中调控 ARGs 迁移转化的重要影响因子.
- (3)微生物群落多样性分析上,长江口近岸地区沉积物中细菌的丰富度和多样性要远高于表层水体.微生物群落结构上,变形菌门(Proteobacteria)和拟杆菌门(Bacteroidota)是长江口近岸地区水环境中的优势菌门; Chloroplast 为水体中的主要菌属,Chloroplast 和 Nitrospira 为沉积物中的主要菌属.微生物群落多样性以及群落结构变化与污水处理厂的污水排放有关.
- (4)在水体中,硝化螺旋菌门(Nitrospirota)是 4 种四环素类抗性基因(tetX、tetA、tetO 和 tetQ)共同 的潜在宿主;在沉积物中,Sva0485 是 sull 和 intll 的共同潜在宿主. 微生物群落的丰度变化对长江口 近岸地区大部分 ARGs 的迁移转化有较大影响.

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