DOI: 10.19663/j.issn2095-9869.20211227003 http://www.yykxjz.cn/

韩璐璐, 杨成年, 李芳, 阳龙江, 唐征县, 彭小倩, 朱成科, 吕光俊. 翘嘴鲌源维氏气单胞菌的分离鉴定及组织病理观察. 渔业科学进展, 2022, 43(6): 216–227

HAN L L, YANG C N, LI F, YANG L J, TANG Z X, PENG X Q, ZHU C K, LÜ G J. Isolation, identification and histopathological observation of *Aeromonas veronii* from *Culter alburnus*. Progress in Fishery Sciences, 2022, 43(6): 216–227

翘嘴鲌源维氏气单胞菌的分离鉴定及组织病理观察*

韩璐璐 杨成年 李 芳 阳龙江 唐征县
彭小倩 朱成科 吕光俊①

(西南大学水产学院 淡水鱼类资源与生殖发育教育部重点实验室 重庆 402460)

摘要 为确定患病翘嘴鲌(*Culter alburnus*)的病原，本研究从患病鱼肝脏中分离到一株优势菌株CQ200825，对该菌进行形态学观察、生理生化鉴定、16S rRNA 和 *gyrB* 基因序列分析、人工感染实验、毒力基因检测、组织病理观察以及药敏试验。结果显示，菌株 CQ200825 为短杆状的革兰氏阴性菌，通过 16S rRNA 和 *gyrB* 基因序列比对、系统进化树的构建和生理生化特性，确认为维氏气单胞菌(*Aeromonas veronii*)。人工感染实验组与自然发病鱼都表现为鳍条基部出血、肝脾肿大、有腹水等症状，同时从人工感染濒死鱼体内分离到与菌株 CQ200825 理化及分子特性一致的优势菌株，表明 CQ200825 为患病翘嘴鲌的病原菌，并计算出菌株 CQ200825 对翘嘴鲌的半数致死量为 2.7×10^6 CFU/mL。毒力基因检测结果显示，菌株 CQ200825 携带外膜蛋白(*ompA*)、溶血素(*hlyA*)、热稳定性肠毒素(*ast*)和细胞毒性肠毒素(*act*) 4 种毒力基因。组织病理观察发现，患病翘嘴鲌的肝、脾、肾和肠均有不同程度的病变，即肝细胞肿胀、坏死，血管中血细胞凝集，脾脏红白髓界限不清、胞核有轻微肿胀，肾小管上皮细胞发生颗粒变性，肠绒毛大部分坏死脱落。药敏试验结果显示，菌株 CQ200825 对多西环素等 17 种抗菌药物高度敏感，对复方新诺明等 4 种抗菌药物中度敏感，对阿莫西林等 12 种抗菌药物表现为耐药。本研究可为翘嘴鲌维氏气单胞菌病的诊断与防治提供参考依据。

关键词 翘嘴鲌；维氏气单胞菌；分离鉴定；组织病理；药敏试验

中图分类号 S941.42 **文献标识码** A **文章编号** 2095-9869(2022)06-0216-12

翘嘴鲌(*Culter alburnus*)俗称白鱼、翘嘴白等，隶属于鲤形目(Cypriniformes)、鲤科(Cyprinidae)、鲌亚科(Culterinae)、鲌属(*Culter*)，主要分布于我国黑龙江、黄河和长江流域(Tian *et al*, 2020)。其肉质细嫩、营养

* 财政部和农业农村部：国家现代农业产业技术体系、科技部“科技助力经济 2020”重点专项项目、重庆市生态渔产业技术体系建设专项(4321600126)、技术创新与应用发展专项重点项目(cstc2019jscx-gksbX01)和重庆市技术创新与应用发展项目(cstc2020jscx-lyjsAX0011)共同资助 [This work was supported by China Agriculture Research System of MOF and MARA, Ministry of Science and Technology of the People's Republic of China Key Special Project of “Science and Technology for Economy 2020”, Chongqing Ecological Fishery Industry Technology System Construction Special Fund (4321600126), Key Project of Technology Innovation and Application Development (cstc2019jscx-gksbX01), and Chongqing Technology Innovation and Application Development Project (cstc2020jscx-lyjsAX0011)]. 韩璐璐，E-mail: 1599756092@qq.com

① 通信作者：吕光俊，副教授，E-mail: gjlv66@163.com

收稿日期: 2021-12-27, 收修改稿日期: 2022-02-08

丰富，具有高蛋白、低脂肪的特点，是我国重要的名优淡水养殖鱼类之一(张燕萍等, 2015)。随着翘嘴鲌配合饲料及人工繁殖技术的突破，其养殖规模逐年增大，现已在我国安徽、湖北、江苏、浙江及重庆等 10 多个省市大量养殖。然而，随着翘嘴鲌养殖规模的增大和密度的提高，养殖水环境日益恶化，其免疫力逐渐降低，病害也呈暴发趋势，表现为各种细菌性、真菌性疾病和寄生虫病频发，造成经济损失巨大，严重制约了翘嘴鲌养殖业的可持续发展。其中，细菌性疾病对翘嘴鲌的危害最为严重，目前已报道常见病原菌包含嗜水气单胞菌(*Aeromonas hydrophila*)、肠型点状气单胞菌(*A. punotata* f. *intestinalis*)和柱状屈桡杆菌(*Flexibacter columnaris*)等(黄爱华等, 2010; 杨长宏, 2017)。

2020 年 8 月，重庆市荣昌区某养殖场的翘嘴鲌连续多日死亡，累计死亡率达 20%，其临床症状主要表现为鳞片疏松、鳍条基部出血，解剖发现腹腔内有大量腹水，肝脏、脾脏肿大等。本研究针对该疫病采集病鱼，从患病濒死翘嘴鲌肝脏中分离出一株优势菌 CQ200825，对优势菌进行人工感染实验来验证这株菌的致病性，结合细菌的形态学观察、理化特性鉴定、16S rRNA 序列及 *gyrB* 序列分析等数据对菌种类型准确鉴定，并进行了毒力基因检测和组织病理观察，探究其毒力因子及感染后造成的组织病理变化，最后通过药敏试验筛选出敏感药物，以期为该病的诊断和防控提供科学依据。

1　材料与方法

1.1　实验材料

采自重庆市荣昌区某养殖场的患病翘嘴鲌 5 尾，本地繁育和养成，体质量为 200~300 g，发病养殖场采用池塘养殖模式，占地约为 50 m×667 m，养殖水温为 24~28℃。健康翘嘴鲌购自重庆市永川区某养殖场，平均体长为(13.5±1.2) cm，平均体质量为(21.6±1.5) g，共 150 尾，暂养于室内水族缸中，养殖期间水温为 25~27℃，pH 为 7.1~7.6，溶解氧为 8.5~9.2 mg/L，每 2 d 换水 1 次，每次换水量为总体积的 1/3。健康翘嘴鲌暂养 2 周后用于后续的人工感染实验。

1.2　主要试剂与仪器

试剂：PCR 检测试剂盒和 pMD19-T 载体购自 TaKaRa 公司；基因组 DNA 提取试剂盒购自天根生化科技(北京)有限公司；脑心浸出液肉汤(BHI)培养基、细菌微量生化反应管和药敏纸片购自杭州微生物科技有限公司。

仪器：光学显微镜(OLYMPUS，日本)、HM325 型转轮式切片机(Thermo Scientific，美国)、PCR 扩增仪(Bio-Rad，美国)、电泳仪(北京市六一仪器厂)和凝胶成像系统(Bio-Rad，美国)等。

1.3　病原菌的分离纯化

选择具有典型患病症状的 5 尾濒死翘嘴鲌于超净工作台中，75%酒精擦拭病鱼体表进行消毒，用接种环蘸取病灶明显的脏器组织及腹水，划线接种于 BHI 培养基上，28℃倒置培养 24 h，再挑选优势单菌落进行纯化培养，肉眼观察菌落形态、大小、隆起度等，4℃保存备用。

1.4　理化特性鉴定

挑取纯化培养的分离菌进行革兰氏染色，光学显微镜下观察菌体形态特征。挑取纯化的单菌落接种于 BHI 液体培养基，然后，将培养物接种于细菌微量生化反应管中，28℃培养 24 h，鉴定结果参考《常见细菌系统鉴定手册》(东秀珠等, 2001)和《伯杰细菌鉴定手册》(Holt *et al*, 1994)的标准判断细菌种类。

1.5　16S rRNA 和 *gyrB* 基因序列的扩增及系统发育分析

利用基因组 DNA 提取试剂盒提取菌株 CQ200825 的基因组 DNA 作为 PCR 模板。PCR 反应体系(50 μL)：10×PCR Buffer 5 μL，16S rRNA、*gyrB* 基因上下游引物各 1 μL (引物见表 1) (Li *et al*, 2019; Soler *et al*, 2004)，模板 1 μL，r*Taq* 酶 0.5 μL，dNTPs 4 μL，用 ddH_2O 补足至 50 μL。PCR 反应程序：94℃预变性 5 min；94℃变性 30 s，56℃退火 30 s，72℃延伸 80 s，33 个循环；最后 72℃终延伸 10 min。反应产物用 1%琼脂糖凝胶电泳检测。用凝胶回收试剂盒进行 PCR 产物回收纯化，与 pMD19-T 载体 4℃连接过夜，第 2 天转化 DH5α 感受态细胞，取反应物在氨苄青霉素固体培养物平板上涂布，倒置于 37℃恒温培养箱中 12~16 h，挑选阳性单克隆菌落送华大基因公司测序。将所得序列使用 Blast 软件与已知序列进行比对分析，并使用 MEGA 5.05 软件，选用 NJ 方法构建系统发育树。

1.6　人工感染实验

将分离纯化的 CQ200825 划线接种于 BHI 培养基上，28℃倒置培养 24 h 复苏。挑取单菌落接种于 BHI 液体培养基，28℃、220 r/min 震荡培养 12~14 h，离心收集菌体并用 0.65%生理盐水洗脱，参照麦氏比浊法调整菌悬液浓度为 1.0×10^8、1.0×10^7、1.0×10^6 和

表 1 菌株 CQ200825 管家基因及部分毒力基因引物

Tab.1 Primers of housekeeping genes and some virulence genes of strain CQ200825

基因 Gene	引物序列 Primer sequence (5′~3′)	退火温度 Annealing temperature/℃	产物片段 Fragment size/bp
16S rRNA	F: AGAGTTTGATCCTGGCTCAG R: TACGGTTACCTTGTTACGACTT	56	1513
gyrB	F: TCCGGCGGTCTGCACGGCGT R: TTGTCCGGGTTGTACTCGTC	56	1051
ompA	F: GCTATCCCGGCTCTGTTCGCATCT R: CAGCAGGGTTTCGTCAAGCAGGTC	56	903
hlyA	F: GGCCGGTGGCCCGAAGATACGGG R: GGCGGCGCCGGACGAGACGGGG	62	592
ast	F: ATCGTCAGCGACAGCTTCTT R: CTCATCCCTTGGCTTGTTGT	58	504
act	F: TACCACCACCTCCCTGTCGC R: ATGCTGCTCGCCTTGTGGTT	55	249

1.0×10^5 CFU/mL (马秀玲等, 2014)，实验组腹腔注射菌悬液 0.2 mL/尾，对照组注射相同体积的 0.65%无菌生理盐水，每组实验鱼 30 尾，连续观察 7 d。在人工感染期间，正常充气换水，水温保持在 24~25℃。每天观察实验翘嘴鲌的活动情况并记录其发病与死亡情况，将濒死病鱼病症与原始病症进行对比，并再次进行病原菌的分离与鉴定。使用累积法(Reed-Muench)计算菌株的半数致死量(LD_{50})(杨昆明等, 2018)。

1.7 毒力基因检测

以菌株 CQ200825 的 DNA 为模板，对外膜蛋白(*ompA*)、溶血素(*hlyA*)、热稳定性肠毒素(*ast*)和细胞毒性肠毒素(*act*)进行 PCR 扩增(李芳, 2019; 朱大玲等, 2006; 刘小芳等, 2021; 龙波等, 2016)。引物序列、退火温度和产物片段见表 1。PCR 扩增产物用 1%琼脂糖凝胶电泳检测。凝胶回收试剂盒纯化，克隆经 PCR 验证后送至华大基因公司进行测序。

1.8 组织病理观察

采用组织切片技术(胡骞等, 2020)，取自然患病濒死翘嘴鲌和健康翘嘴鲌的肝、脾、肾和肠组织，用 Bouin´s 液固定 24 h，经乙醇脱水、二甲苯透明、石蜡包埋、切片等步骤处理，切片厚度为 5 μm，经 HE 染色、中性树胶封片，置于光学显微镜下观察拍照。

1.9 药敏试验

采用纸片扩散法检测分离菌株对 33 种抗菌药物的敏感性(李雅军等, 2006)，用无菌生理盐水稀释分离菌株成 1.5×10^8 CFU/mL，取 100 μL 的菌液均匀涂布于平板表面，用无菌镊子夹取药敏纸片贴于培养基表面，28℃恒温倒置培养 24 h 后用游标卡尺测量抑菌圈的直径，最后根据美国临床实验室标准化研究所(CLSI)抗菌药物敏感性实验执行标准(CLSI-M100-S19)判断菌株对各药物的敏感程度(Martinez *et al*, 2016)。

2 结果

2.1 自然发病情况

自然发病的翘嘴鲌游动缓慢，食欲降低或不摄食，鳞片部分脱落，眼球突出(图 1-1)，在眼眶周围、鳃盖及鳍条基部可见明显的充血、出血(图 1-2)。剖检可见腹腔内有大量腹水(图 1-3)，肝脏、脾脏肿大(图 1-4)，有的个体肠道充血，内无食物。

2.2 病原菌的分离与形态学观察

从 5 尾濒死病鱼肝脏中均分离得到均一的优势菌，将其命名为 CQ200825。在 BHI 培养基上 28℃培养 24 h，形成圆形、表面光滑、边缘圆整、直径为 1.0~1.5 mm 的菌落，革兰氏染色呈阴性，细菌形态为短杆状，多数单个排列。

2.3 病原菌的理化特性

菌株 CQ200825 能产生吲哚，不能产生 H_2S。M.R 试验和 V-P 试验阳性；赖氨酸脱羧酶和氧化酶阳性，精氨酸双水解酶、苯丙氨酸脱氨酶、鸟氨酸脱羧酶和脲酶阴性；能利用 D-甘露醇、D-葡萄糖和纤维二糖，不能利用阿拉伯糖、水杨苷、山梨醇和肌醇，具体生理生化特性见表 2。根据生化特性鉴定结果，并参照《常见细菌系统鉴定手册》和《伯杰细菌鉴定手册》可初步确定分离菌株 CQ200825 为维氏气单胞菌(*Aeromonas veronii*)。

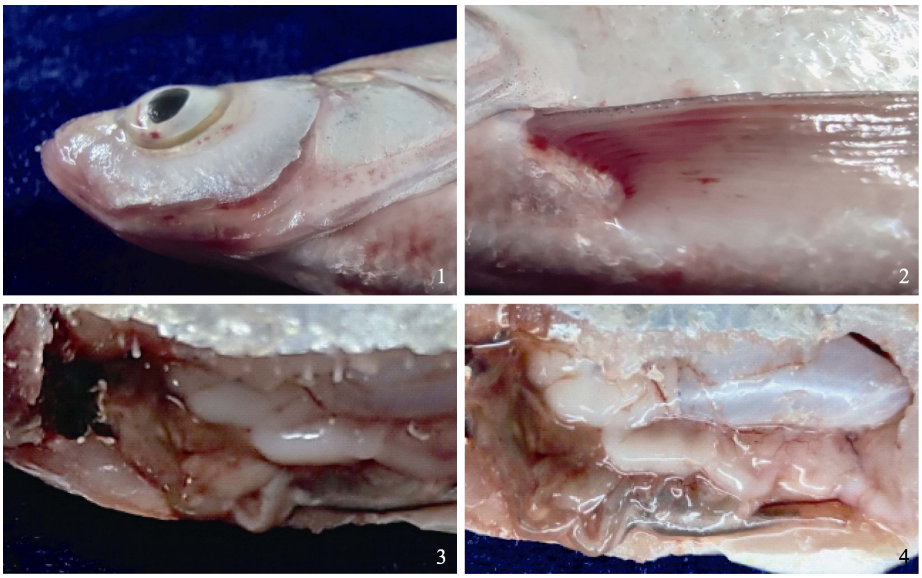

图 1　自然发病翘嘴鲌的临床症状

Fig.1　The clinical signs of naturally diseased *C. alburnus*

1：眼球突出；2：鳍条基部充血、出血；3：有腹水；4：肝脾肿大

1: Exophthalmos; 2: Hyperemia and bleeding at the base of the fin; 3: Ascites; 4: Hepatosplenomegaly

表 2　CQ200825 的生理生化鉴定结果

Tab.2　Biochemical and physiological identification of strain CQ200825

鉴定项目 Identification items	菌株 CQ200825 CQ200825 isolate	维氏气单胞菌 *A. veronii**	鉴定项目 Identification items	菌株 CQ200825 CQ200825 isolate	维氏气单胞菌 *A. veronii**
阿拉伯糖 Arabinose	_	_	脲酶 Urease	_	_
蔗糖 Sucrose	+	+	氧化酶 Oxidase	+	+
硫化氢 H_2S	_	_	V-P 试验 V-P test	+	+
吲哚 Indol	+	+	M.R 试验 M.R test	+	+
精氨酸双水解酶 Arg dihydrolase	_	_	苯丙氨酸脱氨酶 Phe deaminase	_	_
赖氨酸脱羧酶 Lys decarboxylase	+	+	鸟氨酸脱羧酶 Orn decarboxylase	_	_
D-甘露醇 D-mannitol	+	+	纤维二糖 Cellobiose	+	+
D-葡萄糖 D-glucose	+	+	丙二酸盐 Malonate	_	_
水杨苷 Salicin	_	_	山梨醇 Sorbitol	_	_
肌醇 Inositol	_	_	棉籽糖 Raffinose	_	_
乳糖 Lactose	_	_	木糖 Xylose	_	_

注：+：阳性；_：阴性；*：参考 Holt 等(1994)。

Notes: +: Positive reaction; _: Negative reaction; *: Reference: Holt *et al*, 1994.

2.4　16S rRNA 和 *gyrB* 基因序列分析及系统进化树的构建

以分离菌株 CQ200825 基因组 DNA 为模板，分别用 16S rRNA 和 *gyrB* 基因的引物进行 PCR 扩增，其长度约为 1500 bp 和 1100 bp (图 2)。对目的条带进行割胶回收、纯化、与 pMD19-T 载体连接、转化 DH5α 感受态细胞、挑选阳性克隆测序，发现菌株 CQ200825 所获得的 16S rRNA 和 *gyrB* 基因序列片段大小分别为 1513 和 1051 bp。

分别将菌株 CQ200825 的 16S rRNA 和 *gyrB* 基因序列与 NCBI 基因库中的序列进行比对，结果显示，菌株 CQ200825 16S rRNA 基因与 GenBank 登录的维氏气单胞菌(登录号：NR044845)的相似性达到 99.60%；菌株 CQ200825 *gyrB* 基因与 GenBank 登录的维氏气单胞菌(登录号：KR537456)的相似性达到 99.63%。同时，使用 MEGA5.05 软件构建系统发育树，结果显

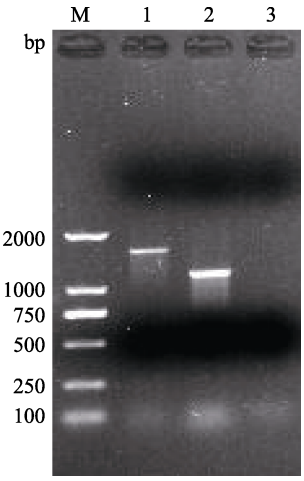

图 2 CQ200825 的 16S rRNA 和 *gyrB* 基因 PCR 扩增结果
Fig.2 PCR amplification results of 16S rRNA and *gyrB* genes of CQ200825

M：DL-2000 DNA marker；1：16S rRNA；2：*gyrB*；3：阴性对照 Negative control

示，菌株 CQ200825 均与维氏气单胞菌聚为一支(图 3、图 4)。根据以上结果可进一步确定菌株 CQ200825 为维氏气单胞菌。

2.5 人工感染实验

对健康翘嘴鲌腹腔注射分离菌株 CQ200825，感染结果见图 5。结果显示，1.0×10^8 CFU/mL 实验组翘嘴鲌 2 d 内全部死亡；1.0×10^7、1.0×10^6 和 1.0×10^5 CFU/mL 实验组翘嘴鲌 7 d 内死亡率分别为 76.7%、23.3%和 6.7%；对照组未出现死亡现象，表明菌株 CQ200825 有较强的致病性。人工感染造成翘嘴鲌鳍条基部充血、出血，解剖发现腹腔内有大量腹水，肝脾肿大，与自然发病翘嘴鲌症状基本一致。同时，从濒死鱼体内分离到一株优势菌株，经理化特性和分子特征鉴定与菌株 CQ200825 一致，这表明菌株 CQ200825 为自然发病翘嘴鲌的病原菌。根据累积法计算得到菌株 CQ200825 对翘嘴鲌的 7 d LD_{50} 为 2.7×10^6 CFU/mL。

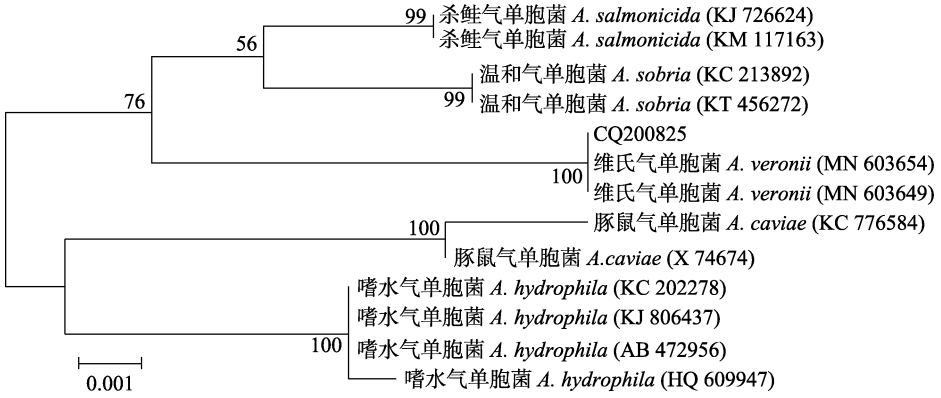

图 3 基于 16S rRNA 序列构建的系统发育树
Fig.3 Phylogenetic tree analysis based on 16S rRNA sequence

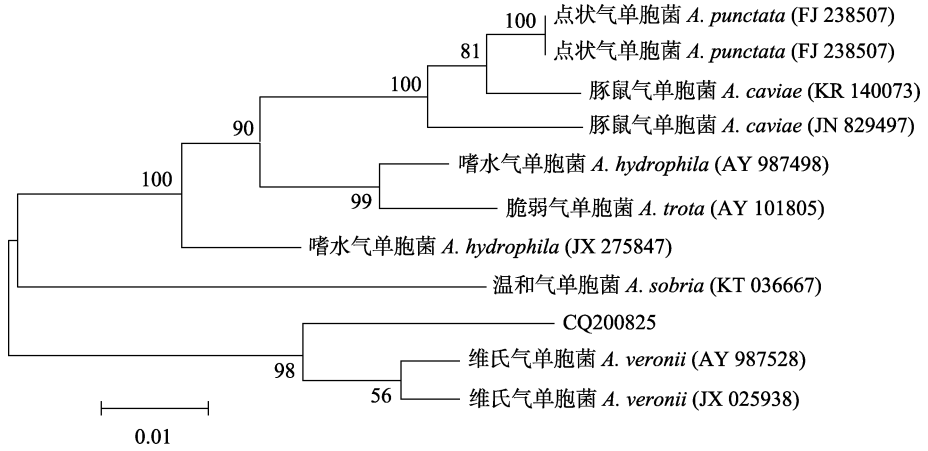

图 4 基于 *gyrB* 基因序列构建的系统发育树
Fig.4 Phylogenetic tree analysis based on *gyrB* sequences

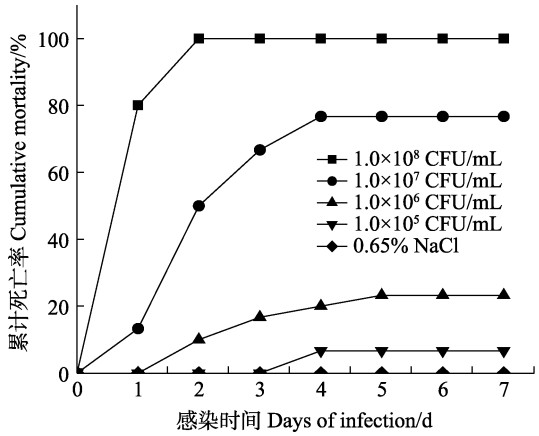

图 5　菌株 CQ200825 人工感染实验结果
Fig.5　The artificial infection experiment results of the strain CQ200825

2.6　毒力基因检测结果

根据已报道的维氏气单胞菌 *ompA*、*hlyA*、*ast*、*act* 基因设计引物进行 PCR 扩增，经测序分别获得大小为 903、592、504 和 249 bp 的 DNA 片段(图 6)。

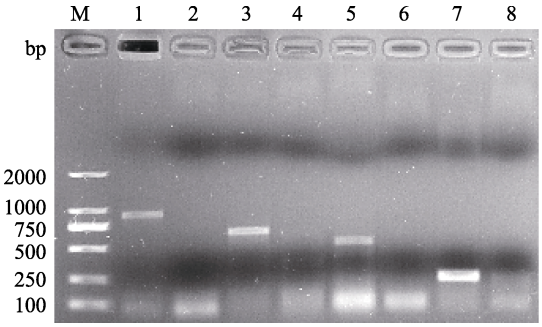

图 6　CQ200825 的毒力基因 PCR 扩增结果
Fig.6　PCR amplification results of virulence genes of CQ200825

M：DL-2000 DNA marker；1、3、5、7 分别为 *ompA*、*hlyA*、*ast*、*act*；2、4、6、8 分别为 *ompA*、*hlyA*、*ast*、*act* 的阴性对照
M: DL-2000 DNA marker; 1, 3, 5 and 7 were *ompA*, *hlyA*, *ast*, and *act*, respectively; 2, 4, 6 and 8 were negative controls of *ompA*, *hlyA*, *ast*, and *act*, respectively

2.7　组织病理学

组织病理学观察表明，患病翘嘴鲌的肝、脾、肾和肠均发生了不同程度的病变。健康鱼肝细胞呈圆形或多角形，排列整齐(图 7-1)；患病鱼肝细胞肿胀、坏死，血管中血细胞凝集(图 7-2)。健康鱼脾脏红白髓分明，结构清晰(图 7-3)；患病鱼脾组织坏死，细胞排列紊乱，红白髓界限不清，胞核有轻微肿胀(图 7-4)。健康鱼肾小管结构清晰，肾小囊腔体积正常(图 7-5)；患病鱼肾小管上皮细胞发生颗粒变性，肾小球萎缩(图 7-6)。健康鱼肠道各部分结构清晰、完整(图 7-7)；患病鱼肠道结构遭到严重破坏，浆膜脱落，肠绒毛大部分坏死脱落，杯状细胞和上皮细胞坏死(图 7-8)。

2.8　药敏试验结果

本研究检测了菌株 CQ200825 对氟苯尼考等 33 种抗菌药物的敏感性，包括红霉素等 3 种大环内酯类、头孢他啶等 12 种 β-内酰胺类、多四环素等 3 种四环素、复方新诺明等 2 种磺胺类、庆大霉素等 6 种氨基糖苷类、恩诺沙星等 3 种喹诺酮类、氟苯尼考以及林可霉素、多黏霉素等 2 种其他类抗菌药物。结果显示，分离菌株对多西环素、恩诺沙星、氟苯尼考等 17 种抗菌药高度敏感，对红霉素、复方新诺明、磺胺异恶唑和新霉素中度敏感，对青霉素、阿莫西林、庆大霉素等 12 种抗菌药表现为耐药，具体结果见表 3。

3　讨论

本研究从患病翘嘴鲌肝脏中分离得到一株致病菌 CQ200825，人工感染症状与自然发病鱼基本一致，7 d LD_{50} 为 2.7×10^6 CFU/mL，表现出较高的致病力。经形态学观察和生理生化特性分析发现，菌株 CQ200825 与维氏气单胞菌非常相似，因为维氏气单胞菌区别于气单胞菌属其他细菌的理化特征是精氨酸双水解酶阴性、赖氨酸脱羧酶阳性(Holt *et al*, 1994)。为进一步确定其分类地位，本研究进行了 16S rRNA 基因序列分析，结果显示，该序列与已知维氏气单胞菌序列相似性达到 99.60%。但由于 16S rRNA 基因保守性较高，无法区分亲缘关系较近的细菌类群(Martino *et al*, 2011)，而 *gyrB* 基因较 16S rRNA 具有更高的替换率，适合亲缘关系较近的种属鉴定(Tancsics *et al*, 2014)。因此，本研究针对 *gyrB* 基因序列进行分析，结果显示，其与已知维氏气单胞菌序列相似性达到 99.63%。结合形态学、生理生化特性以及 16S rRNA 和 *gyrB* 序列分析综合判定分离菌株 CQ200825 为维氏气单胞菌。

维氏气单胞菌亦被称为维罗纳气单胞菌，隶属于气单胞菌科(Aeromonadaceae)、气单胞菌属(*Aeromonas*)，是致病性气单胞菌的代表种，普遍存在于海水、淡水及土壤等环境中，该细菌能够感染各种水产动物，尤其在水温高、水质条件恶劣、水产动物体表受伤的情况下发病率较高(凡飞等, 2015)。王兴丽等(2016)研究发现，该菌能引起虹鳟(*Oncorhynchus mykiss*)体表溃

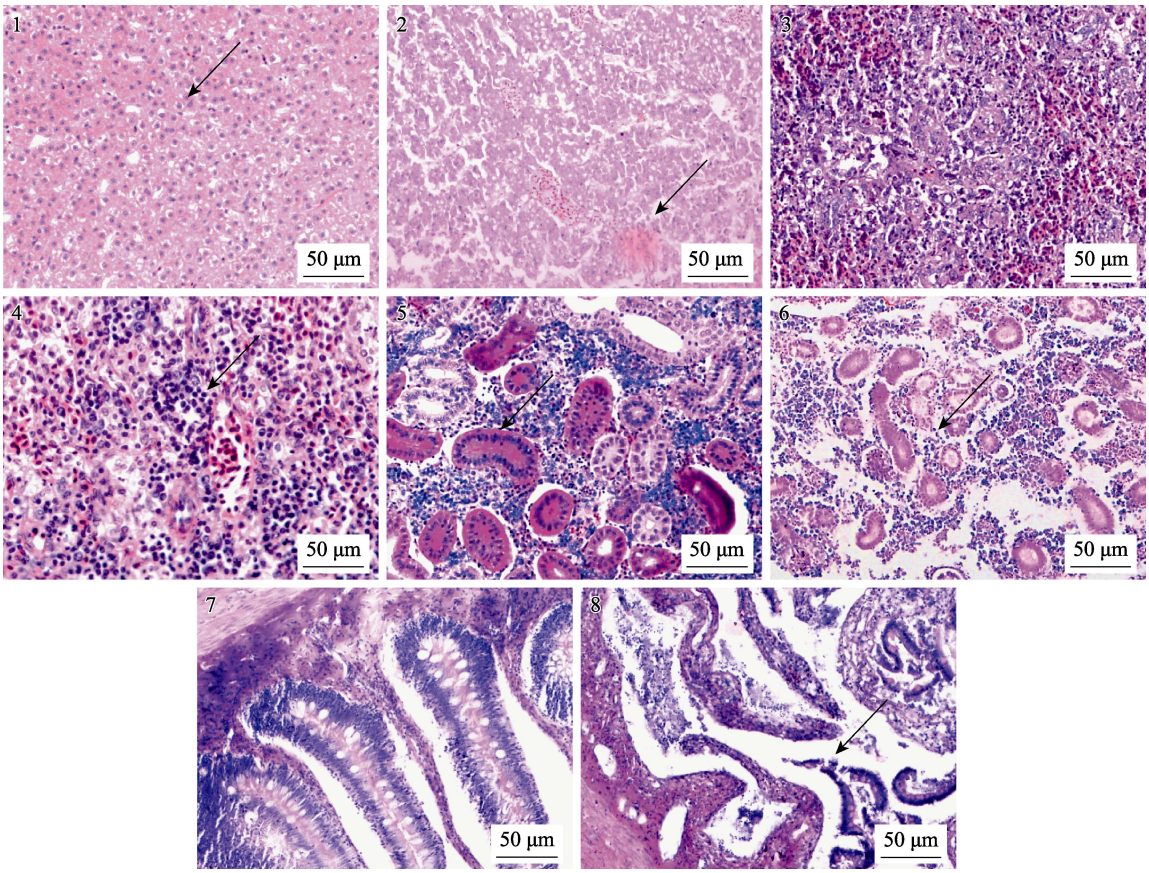

图 7 健康翘嘴鲌和患病翘嘴鲌组织病理学观察

Fig.7 Histopathological observation of healthy *C. alburnus* and diseased *C. alburnus*

1：健康鱼肝细胞呈圆形或多角形，排列整齐(箭头)；2：患病鱼肝细胞肿胀、坏死，血管中血细胞凝集(箭头)；3：健康鱼脾脏红髓和白髓结构清晰，淋巴细胞排列整齐；4：患病鱼脾脏组织坏死，胞核有轻微肿胀(箭头)；5：健康鱼肾小管结构清晰(箭头)；6：患病鱼肾小管上皮细胞发生颗粒变性(箭头)；7：健康鱼肠道各部分结构完整；8：患病鱼肠绒毛大部分坏死脱落，杯状细胞坏死(箭头)

1: Liver cells of healthy fish were round or polygonal and arranged neatly (arrow); 2: Liver cells of diseased fish were swollen and necrotic, and hemocytes agglutinated in blood vessels (arrow); 3: The structure of red pulp and white pulp in spleen was clear and the lymphocytes were arranged neatly in healthy fish; 4: Spleen tissue of diseased fish necrosis and the nucleus had slight swelling (arrow); 5: Renal tubules of healthy fish were clear (arrow); 6: The epithelial cells of renal tubules showed granulosa degeneration in diseased fish (arrow); 7: Parts of intestines were structurally intact in healthy fish; 8: Most of the intestinal villus were necrotic and exfoliated, and goblet cells were necrotic in diseased fish (arrow)

疡、鳃丝和内脏出血等症状。郭莹等(2020)研究发现，该菌能引起罗氏沼虾(*Macrobrachium rosenbergii*)肌肉发白、游泳足和附肢发红等症状，严重时出现大量死亡。据报道，感染维氏气单胞菌患病或死亡的水产动物还有锦鲤(*Cyprinus carpio* var. *koi*)、花斑副沙鳅(*Parabotia fasciata*)、斑点叉尾鮰(*Ictalurus punctatus*)、克氏原螯虾(*Procambarus clarkii*)、鳜(*Siniperca chuatsi*)等(Han *et al*, 2021; 高通等, 2020; 田浪等, 2018; 蒋国民等, 2021; 高金伟等, 2016)。

维氏气单胞菌致病机理较为复杂，可能是由于其携带了多种毒力因子，包括外膜蛋白、溶血素、气溶素、细胞毒性肠毒素、丝氨酸蛋白酶等(Nawaz *et al*, 2010)。因此，检测菌株是否携带毒力基因对了解维氏气单胞菌致病性及防治至关重要。经 PCR 检测并测序，菌株 CQ200825 携带 *ompA*、*hlyA*、*ast* 和 *act* 4 种毒力基因。这 4 种毒力基因作为质粒基因，是气单胞菌致病性发挥关键作用的毒力因子，一般与其感染过程中的入侵、粘附、定植、释放及损伤宿主组织等活动有关。有研究表明，*hlyA* 是导致宿主组织广泛出血和溶血的关键毒力基因，同时也可以导致宿主肠炎(常藕琴等, 2014)，*ast* 和 *act* 是气单胞菌导致肠炎的关键毒力因子，且肠炎的严重程度与毒力因子的数量和种类有着密切联系(Nawaz *et al*, 2010)。Chopra 等(2000)研究表明，气单胞菌属细菌对宿主的感染力较

表 3　CQ200825 株对 38 种抗菌药物的耐药性分析

Tab.3　Antimicrobial susceptibility of CQ200825 isolate to 38 kinds of antibiotics

抗菌药物种类 Classes of antibiotics	抗菌药物 Antibiotics	判断标准 Assessment criteria			抑菌圈直径 Diameter of inhibition/mm	敏感性 Sensitivity
		R	I	S		
大环内酯类 Macrolides group	红霉素 Erythromycin	≤13	14~16	≥23	15.44±0.47	M
	麦迪霉素 Midecamycin	≤13	14~17	≥18	9.00±0.42	R
	吉他霉素 Kitasamycin	≤21	22~30	≥31	18.20±0.25	R
β-内酰胺类 β-lactam group	头孢他啶 Ceftazidime	≤14	15~17	≥18	30.98±1.49	S
	头孢哌酮 Cefoperazone	≤15	16~20	≥21	30.51±0.31	S
	氨曲南 Aztreonam	≤15	16~21	≥22	39.93±1.08	S
	青霉素 Penicillin	≤19	20~27	≥28	0	R
	头孢唑啉 Cefazolin	≤15	16~17	≥18	14.72±0.42	R
	头孢氨苄 Cefalexin	≤14	15~17	≥18	0	R
	头孢曲松 Ceftriaxone	≤25	25~26	≥27	33.83±1.02	S
	头孢拉定 Cefradine	≤14	15~17	≥18	0	R
	羧苄西林 Carbenicillin	≤19	20~22	≥23	16.87±0.33	R
	哌拉西林 Piperacillin	≤17	18~20	≥21	27.01±0.43	S
	氨苄西林 Ampicillin	≤13	14~16	≥17	0	R
	阿莫西林 Amoxicillin	≤18	19~25	≥26	0	R
四环素类 Tetracyclines group	四环素 Tetracycline	≤14	15~20	≥21	21.65±0.16	S
	多西环素 Doxycycline	≤12	13~15	≥16	22.03±0.46	S
	米诺环素 Minocycline	≤14	15~17	≥18	21.02±0.51	S
磺胺类 Sulfonamides group	复方新诺明 Cotrimoxazole	≤23	24~32	≥33	25.84±1.29	M
	磺胺异恶唑 Sulfisoxazole	≤14	15~23	≥24	22.72±1.18	M
氨基糖苷类 Aminoglycosides group	庆大霉素 Gentamicin	≤12	13~14	≥15	11.19±0.66	R
	妥布霉素 Tobramycin	≤12	13~14	≥15	18.27±0.33	S
	新霉素 Neomycin	≤12	13~16	≥17	15.45±0.64	M
	丁胺卡那 Amikacin	≤14	15~16	≥17	22.43±0.40	S
	卡那霉素 Kanamycin	≤13	14~17	≥18	18.99±0.81	S
	链霉素 Streptomycin	≤11	12~14	≥15	20.62±0.54	S
喹诺酮类 Quinolones group	恩诺沙星 Enrofloxacin	≤12	13~15	≥16	38.19±0.54	S
	环丙沙星 Ciprofloxacin	≤15	16~20	≥21	40.12±1.80	S
	吡哌酸 Pipemidic acid	≤21	22~28	≥29	31.32±1.00	S
氯霉素类 Chloromycetins group	氟苯尼考 Florfenicol	≤12	13~17	≥18	36.67±0.97	S
林可霉素类 Lincomycin group	林可霉素 Lincolmensin	≤23	24~30	≥31	9.06±0.82	R
其他类抗生素 Other antibiotic group	杆菌肽 Bacitracin	≤8	9~12	≥13	0	R
	多粘菌素 B polymyxin B	≤8	9~11	≥12	13.83±0.09	S

注：S 表示高度敏感；M 表示中度敏感；R 表示耐药。

Notes: S: Highly susceptible; M: Moderately susceptible; R: Resisitant.

强，可能与 *act* 可刺激宿主的促炎反应有关。同时结合组织病理学研究发现，患病鱼的肝、脾、肾及肠均有不同程度的病变，其中以肝、肠损伤最为严重，表现为肝细胞肿胀、坏死，血管中血细胞凝集；肠道浆膜脱落，肠绒毛大部分坏死脱落，可以推测肝脏和肠道是该菌的主要靶器官，同时，肝、肠的感染也可能是脾、肾等器官病变的原因之一，这与大口黑鲈(*Micropterus salmoides*)和达氏鲟(*Acipenser dabryanus*)

感染维氏气单胞菌引起的组织病理变化特征相一致(Pei *et al*, 2021; 刘亚等, 2018)。

尽管免疫接种是控制水产养殖动物暴发性流行病有效的方法，但存在免疫效果不理想、免疫力持久性差等问题，使得免疫接种的开发和应用受到很大限制。目前，国内外对维氏气单胞菌病的防治仍为主要使用抗菌药物。本研究药敏试验分析了 33 种药物，其中，高度敏感抗菌药物 17 种，包括多西环素、恩诺沙星、氟苯尼考等，占比为 51.52%；中度敏感药物 4 种，包括红霉素、复方新诺明、磺胺异恶唑和新霉素，占比为 12.12%；耐药抗菌药物 12 种，包括青霉素、阿莫西林、庆大霉素等，占比为 36.36%。维氏气单胞菌四川分离株对丁胺卡那、庆大霉素中度敏感，对四环素、环丙沙星、磺胺异恶唑等耐药(龙波等, 2016)；维氏气单胞菌印度分离株对四环素、环丙沙星等高度敏感，对丁胺卡那耐药(Mallik *et al*, 2020)。而本研究中的 CQ200825 株对丁胺卡那、四环素、环丙沙星等高度敏感，对磺胺异恶唑中度敏感，对庆大霉素耐药。这可能是由于不同来源、不同地理环境中的菌株对不同抗菌药物的敏感程度存在差异。因此，在选择抗菌药物治疗时，首先应参考菌株药敏试验结果，结合无公害食品渔用药物使用准则(NY 5071-2002)及农业农村部渔业渔政管理局印发《水产养殖用药明白纸 2020 年 1、2 号》等相关规定，进行科学、规范用药。

参 考 文 献

CHANG O Q, LIU X Y, PAN H J, *et al*. Histopathological and pathogeny preliminary research on mulberry heart of Siberian sturgeon (*Acipenser baeri*). Journal of Fisheries of China, 2014, 38(6): 904–911 [常藕琴, 刘晓勇, 潘厚军, 等. 西伯利亚鲟桑葚心病理组织学和病因的初步研究. 水产学报, 2014, 38(6): 904–911]

CHOPRA A K, XU X, RIBARDO D, *et al*. The cytotoxic enterotoxin of *Aeromonas hydrophila* induces proinflammatory cytokine production and activates arachidonic acid metabolism in macrophages. Infection Immunity, 2000, 68(5): 2808–2818

DONG X Z, CAI M Y. Manual for identification of bacteriology. Beijing: Science Press, 2001, 114–115 [东秀珠, 蔡妙英. 常见细菌系统鉴定手册. 北京: 科学出版社, 2001, 114–115]

FAN F, ZHANG S W, LI L T, *et al*. Screening and characterization of a bacterium named 4-1-3 antagonizing *Aeromonas veronii*. Progress in Fishery Sciences, 2015, 36(2): 146–152 [凡飞, 张生武, 李联泰, 等. 凡隆气单胞菌(*Aeromonas veronii*)拮抗菌 4-1-3 的筛选鉴定及其活性物质初探. 渔业科学进展, 2015, 36(2): 146–152]

GAO J W, LIANG L G, WANG Y B, *et al*. Identification and susceptibility test of pathogenic *Aeromonas veronii* isolated from *Siniperca chuatsi*. Microbiology China, 2016, 43(12): 2686–2692 [高金伟, 梁利国, 王亚冰, 等. 鳜源致病性维氏气单胞菌的鉴定及药敏试验. 微生物学通报, 2016, 43(12): 2686–2692]

GAO T, WANG J, WANG K Y, *et al*. Isolation, identification and drug sensitivity analysis of pathogenic *Aeromonas veronii* from diseased *Parabotia fasciata Dabry*. Chinese Journal of Preventive Veterinary Medicine, 2020, 42(3): 239–244 [高通, 王均, 汪开毓, 等. 花斑副沙鳅源致病性维氏气单胞菌的分离、鉴定及药物敏感性分析. 中国预防兽医学报, 2020, 42(3): 239–244]

GUO Y, GUO Q S, ZHOU M, *et al*. *Aeromonas veronii* from *Macrobrachium rosenbergii*: Isolation and identification. Chinese Agricultural Science Bulletin, 2020, 36(36): 137–143 [郭莹, 郭青松, 周淼, 等. 罗氏沼虾维氏气单胞菌的分离鉴定. 中国农学通报, 2020, 36(36): 137–143]

HAN Z R, SUN J F, JIANG B Y, *et al*. Concurrent infections of *Aeromonas veronii* and *Vibrio cholerae* in koi carp (*Cyprinus carpio* var. *koi*). Aquaculture, 2021, 535: 736395

HOLT J G, KRIEG N R, SNEATH P H A, *et al*. Bergey's manual of determinative bacteriology (9th ed). Baltimore: Willians and Wilkins, 1994

HU Q, HU R X, JIN Y L, *et al*. Isolation, identification and pathohistological observation of *Aeromonas veronii* from *Procambarus clarkii*. Acta Hydrobiologica Sinica, 2020, 44(4): 811–818 [胡骞, 胡瑞雪, 金玉立, 等. 克氏原螯虾源维氏气单胞菌的分离鉴定及组织病理学观察. 水生生物学报, 2020, 44(4): 811–818]

HUANG A H, ZHANG D Z. The diagnosis and prevention of common diseases of *Erythroculter ilishaeformis*. Fishery Guide to be Rich, 2010(21): 45–47 [黄爱华, 张大中. 翘嘴红鲌常见疾病的诊断与防治. 渔业致富指南, 2010(21): 45–47]

JIANG G M, LI J L, LIU L, *et al*. Isolation and identification of bacterial pathogens from *Procambarus clarkii* and study on variations in serum immune factors. Journal of Fisheries of China, 2021. http://kns.cnki.net/kcms/detail/31.1283.S.20210611.1637.002.html [蒋国民, 李金龙, 刘丽, 等. 克氏原螯虾(*Procambarus clarkia*)细菌性病原分离鉴定及血清免疫因子变化研究. 水产学报, 2021. http://kns.cnki.net/kcms/detail/31.1283.S.20210611.1637.002.html]

LI F, WU D, GU H R, *et al*. *Aeromonas hydrophila* and *Aeromonas veronii* cause motile *Aeromonas* septicaemia in the cultured Chinese sucker, *Myxocyprinus asiaticus*. Aquaculture Research, 2019, 50(5): 1515–1526

LI F. Motile *Aeromonas* septicemia and *Clinostomum* disease in farmed Chinese sucker, *Myxocyprinus asiaticus*. Doctoral Dissertation of Southwest University, 2019 [李芳. 胭脂鱼

运动型气单胞菌败血症及弯口吸虫病初步研究. 西南大学博士研究生学位论文, 2019]

LI Y J, ZHANG B, WANG H. Update of CLSI/NCCLS drug susceptibility standards in 2006 (CLSI/NCCLS M100-S16 replace M100-S15). Jiangxi Journal of Medical Laboratory Sciences, 2006, 24(4): 355–356 [李雅军, 张斌, 王华. 2006 年 CLSI/NCCLS 药敏标准的更新(CLSI/NCCLS M100-S16 代替 M100-S15). 江西医学检验, 2006, 24(4): 355–356]

LIU X F, REN Y, ZHANG D F, *et al*. Detection, genotyping and pathogenicity of virulence genes in *Aeromonas* species isolated from diseased freshwater fish. Journal of Fisheries of China, 2021, 45(3): 462–471 [刘小芳, 任燕, 张德锋, 等. 鱼源气单胞菌的毒力基因检测、分型及致病力. 水产学报, 2021, 45(3): 462–471]

LIU Y, YANG R, CHEN Y Y, *et al*. Isolation, identification of *Aeromonas veronii* in *Acipenser dabryanus* and its histological observations. Journal of Southern Agriculture, 2018, 49(6): 1235–1241 [刘亚, 杨锐, 陈叶雨, 等. 达氏鲟维氏气单胞菌的分离鉴定及病理组织学观察. 南方农业学报, 2018, 49(6): 1235–1241]

LONG B, WANG J, HE Y, *et al*. Isolation, identification and pathogenicity of *Aeromonas veronii* isolated from *Micropterus salmoides*. Chinese Journal of Veterinary Science, 2016, 36(1): 48–55 [龙波, 王均, 贺扬, 等. 加州鲈源维氏气单胞菌的分离、鉴定及致病性. 中国兽医学报, 2016, 36(1): 48–55]

MA X L, CHEN R J, WANG F, *et al*. Determination of bacterial suspension concentration and the scope of application using absorbance method. Journal of Microbiology, 2014, 34(4): 90–92 [马秀玲, 陈蕊君, 王飞, 等. 吸光度法快速确定菌悬液浓度及其适用范围. 微生物学杂志, 2014, 34(4): 90–92]

MALLIK S K, JOSHI N, SHAHI N, *et al*. Characterization and pathogenicity of *Aeromonas veronii* associated with mortality in cage farmed grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) from the Central Himalayan region of India. Antonie van Leeuwenhoek, 2020, 113(12): 2063–2076

MARTINEZ R, ROCHA J F, BEJARANO D, *et al*. Identification of SNPs in growth-related genes in Colombian creole cattle. Genetics and Molecular Research, 2016, 15(3): 120–125

MARTINO M E, FASOLATO L, MONTEMURRO F, *et al*. Determination of microbial diversity of *Aeromonas* strains on the basis of multilocus sequence typing, phenotype, and presence of putative virulence genes. Applied and Environmental Microbiology, 2011, 77(14): 4986–5000

NAWAZ M, KHAN S A, KHAN A A, *et al*. Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. Food Microbiology, 2010, 27(3): 327–331

PEI C, SONG H L, ZHU L, *et al*. Identification of *Aeromonas veronii* isolated from largemouth bass *Micropterus salmoides* and histopathological analysis. Aquaculture, 2021, 540: 736707

SOLER L, YÁÑEZ M A, CHACON M R, *et al*. Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes. International Journal of Systematic and Evolutionary Microbiology, 2004, 54(5): 1511–1519

TANCSICS A, BENEDEK T, FARKAS M, *et al*. Sequence analysis of 16S rRNA, *gyrB* and *catA* genes and DNA-DNA hybridization reveal that *Rhodococcus jialingiae* is a later synonym of *Rhodococcus qingshengii*. International Journal of Systematic and Evolutionary Microbiology, 2014, 64(1): 298–301

TIAN J, PENG D, WEN H, *et al*. A comparative study on protein-sparing effects among juvenile *Erythroculter ilishaeformis* line，*Ancherythroculter nigrocauda* line and their hybrid F_1 fed diets with different protein to carbohydrate ratios. Aquaculture Nutrition, 2020, 26(4): 993–1006

TIAN L, WEN G L, ZHANG S B, *et al*. Isolation and biological characterization of the pathogenic *Aeromonas veronii* from *Ictalurus punctatus*. China Animal Husbandry and Veterinary Medicine, 2018, 45(11): 3203–3210 [田浪, 温贵兰, 张升波, 等. 斑点叉尾鮰源致病性维氏气单胞菌的分离与生物学特性鉴定. 中国畜牧兽医, 2018, 45(11): 3203–3210]

WANG X L, WANG K Y, CHEN D F, *et al*. Isolation and identification of *Aeromonas veronii* from rainbow trout and histopathology study of the diseased fish. Chinese Journal of Preventive Veterinary Medicine, 2016, 38(11): 879–883 [王兴丽, 汪开毓, 陈德芳, 等. 一株虹鳟源维氏气单胞菌的分离鉴定及组织病理学观察. 中国预防兽医学报, 2016, 38(11): 879–883]

YANG C H. Artificial propagation test of *Erythroculter ilishaeformis*. Anhui Agricultural Science Bulletin, 2017, 23(7): 128–129, 175 [杨长宏. 翘嘴红鲌的人工繁殖技术试验. 安徽农学通报, 2017, 23(7): 128–129, 175]

YANG K M, GUO A M, MA J X, *et al*. Isolation, identification and antibiotic sensitivity of bacillary enteritis in *Perca fluviatilis* Linnaeus. Progress in Fishery Sciences, 2018, 39(6): 42–51 [杨昆明, 郭爱民, 马江霞, 等. 河鲈细菌性肠炎致病菌的分离、鉴定及药敏实验. 渔业科学进展, 2018, 39(6): 42–51]

ZHANG Y P, WU B, FANG C L, *et al*. The estimation of biological parameters for *Culter alburnus* in the waterway connecting the Poyang Lake and the Yangtze River. Progress in Fishery Sciences, 2015, 36(5): 26–30 [张燕萍, 吴斌, 方春林, 等. 鄱阳湖通江水道翘嘴鲌(*Culter alburnus*)的生物学参数估算. 渔业科学进展, 2015, 36(5): 26–30]

ZHU D L, LI A H, WANG J G, *et al*. The correlation between the distribution pattern of virulence genes and the virulence of

Aeromonas hydrophila strains. Acta Scientiarum Naturalium Universitatis Sunyatseni, 2006, 45(1): 82–85 [朱大玲, 李爱华, 汪建国, 等. 嗜水气单胞菌毒力与毒力基因分布的相关性. 中山大学学报(自然科学版), 2006, 45(1): 82–85]

(编辑 马璀艳)

Isolation, Identification and Histopathological Observation of *Aeromonas veronii* from *Culter alburnus*

HAN Lulu, YANG Chengnian, LI Fang, YANG Longjiang, TANG Zhengxian, PENG Xiaoqian, ZHU Chengke, LÜ Guangjun①

(*Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, College of Fisheries, Southwest University, Chongqing* 402460, *China*)

Abstract *Culter alburnus* is a common freshwater aquaculture fish in China. It is popular because of its tender meat, rich nutrition, and high-protein and low-fat contents. With the breakthrough in compound feed and artificial breeding technology, the scale of *C. alburnus* cultivation is increasing, and it is cultivated in more than 10 provinces and many cities in China, including Anhui, Hubei, Jiangsu, Zhejiang, and Chongqing. However, with the increase in culturing scale and density of *C. alburnus*, the water quality is continuously deteriorating, leading to decreased immunity and spread of diseases, especially various bacterial, fungal and parasitic diseases, among which bacterial diseases are the most serious, causing huge economic losses. In August 2020, *C. alburnus* died in a farm, and the cumulative death rate was 20% in Rongchang District, Chongqing. The main clinical symptoms was loose scales, bleeding at the base of the fin, a large amount of ascites in the abdominal cavity, and hepatosplenomegaly. In order to identify the pathogen responsible for the death of *C. alburnus*, the pathogenic mechanism was investigated and appropriate drugs to treat the disease were screened. A dominant strain named CQ200825 was isolated from the liver of the diseased fishes. An artificial infection experiment was performed to verify pathogenicity of the strain CQ200825. This was combined with the morphological, physiological, and biochemical analysis of the bacteria for identification; 16S rRNA sequence and *gyrB* sequence analysis was also done. Several analyses were performed for accurate identification of the species, virulence gene detection, histopathological observation, and to probe its virulence factor and tissue pathological changes caused after infection. Finally, effective drugs were screened by drug susceptibility test. The results showed that naturally occurring fishes swam slowly, had decreased appetite, or did not feed at all. Their scales were partially detached and the eyeballs were protruding. An obvious hyperemia and hemorrhage around the orbit, gill cover, and base of the fin was also observed. Autopsy revealed a large amount of ascites in the abdominal cavity and the liver and spleen were swollen. The bacterium grew at 28℃ for 24 h on BHI medium, and its colony diameter was 1.0~1.5 mm, which had smooth surface and rounded edges. Strain CQ200825 was gram negative, and its size was about 0.5~1.0×1.2~2.4 μm; the strain was short rod-shaped, and most single permutation. The physiological and biochemical characterization of the strain CQ200825 showed that it could produce indole but not H_2S. The M.R test and V-P test results were positive. The test results for the production of lysine decarboxylase and oxidase

① Corresponding author: LÜ Guangjun, E-mail: gjlv66@163.com

were positive, whereas those for arginine hydrolase, phenylamine deaminase, ornithine decarboxylase, and urease were negative. The strain could utilize D-mannitol, D-glucose, and cellobiose, but could not utilize arabinose, salicin, sorbitol, and inositol. The evolutionary tree constructed by 16S rRNA and *gyrB* gene sequences of the strain showed that it clustered with *Aeromonas veronii*, and the similarity was 99.60% and 99.63%, respectively. Based on morphology and physiological and biochemical properties, the strain CQ200825 was identified as *A. veronii*. Both the artificially infected and the naturally pathogenetic fishes showed symptoms of hemorrhage at the base of the fin, hepatosplenomegaly, and ascites. Meanwhile, a dominant strain was isolated from the artificially infected dying fishes, which was the same as the strain CQ200825 based on its physicochemical and molecular properties, indicating that the strain CQ200825 was the pathogen of the diseased *C. alburnus*. The LD_{50} value of strain CQ200825 against *C. alburnus* was 2.7×10^6 CFU/mL. Four virulence genes were found in the strain CQ200825, including the outer membrane protein (*ompA*), haemolysin (*hlyA*), cytotonic heat-stable enterotoxin (*ast*), and cytotoxic enterotoxin (*act*), and sequencing analysis revealed that the lengths of these genes were 903 bp, 592 bp, 504 bp, and 249 bp, respectively. Pathological analysis revealed that the liver, spleen, kidney, and intestine of the diseased *C. alburnus* had different degrees of pathological changes. Liver cells of the diseased fish were swollen and necrotic, and hemocytes agglutinated in blood vessels; splenic tissue was necrotic and the cells were abnormal, the boundary of red and white pulp was unclear, and the nucleus had slight swelling; the epithelial cells of renal tubules showed granulosa degeneration and glomerular atrophy; and the intestinal structure was severely damaged, serous membrane was removed, most of the intestinal villus were necrotic and exfoliated, and goblet cells epithelial cells were necrotic. The results of the drug susceptibility test revealed that the strain CQ200825 was highly sensitive to 17 antibacterial drugs, namely doxycycline, enrofloxacin, florfenicol, ceftazidime, kanamycin, cefoperazone, streptomycin, ciprofloxacin, and pipemidic acid; moderately sensitive to four antibacterial drugs, including erythromycin, cotrimoxazole, sulfisoxazole, and neomycin; and resistant to 12 antibacterial drugs namely penicillin, amoxicillin, gentamicin, midecamycin, kitasamycin, carbenicillin, cefazolin, and lincolmensin. This study can provide reference for the diagnosis and prevention of *A. veronii* infection in *C. alburnus*.

Key words　*Culter alburnus*; *Aeromonas veronii*; Isolation and identification; Histopathology; Drug susceptibility test