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绿鳍马面鲀与许氏平鲉杀鲑气单胞菌病原的分离和鉴定*

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摘要 2018 年和 2019 年，山东省烟台市蓬莱市一养殖场工厂化养殖的绿鳍马面鲀(*Thamnaconus septentrionalis*)和许氏平鲉(*Sebastes schlegeli*)发病死亡，主要症状为嘴部溃疡、红肿和出血。从发病鱼内脏中均可分离到大量形态一致的优势菌，分别命名为 2018TS-1 和 2019SS-1，分离菌株经 16S rRNA 测序、生理生化鉴定和 *vapA* 基因分析确定为杀鲑气单胞菌杀日本鲑亚种(*Aeromonas salmonicida* subsp. *masoucida*)。人工感染结果显示，2018TS-1 和 2019SS-1 分别能引起绿鳍马面鲀和许氏平鲉的死亡，被感染鱼呈嘴部红肿症状，与自然发病症状一致，其半数致死量分别为 1.78×10^5 和 0.89×10^5 CFU/尾。本研究首次报道了国内工厂化养殖绿鳍马面鲀和许氏平鲉感染杀鲑气单胞菌的病例，是目前人工养殖绿鳍马面鲀的首个疾病报道，也是继大西洋鲑(*Salmo salar*)、大菱鲆(*Scophthalmus maximus*)和裸盖鱼(*Anoplopoma fimbria*)等品种后，在山东省海水养殖鱼类中再次发现杀鲑气单胞菌杀日本鲑亚种的感染。本研究结果丰富了杀鲑气单胞菌杀日本鲑亚种的感染宿主范围，也为绿鳍马面鲀和许氏平鲉养殖的病害防控提供依据。

关键词 绿鳍马面鲀；许氏平鲉；细菌鉴定；杀鲑气单胞菌杀日本鲑亚种

中图分类号 S941.42+9 **文献标识码** A **文章编号** 2095-9869(2023)01-0191-10

绿鳍马面鲀(*Thamnaconus septentrionalis*)曾是一种重要的海洋捕捞鱼类，其营养丰富，出肉率高，肉味鲜美，具有较高的营养和经济价值(徐大凤等, 2018)。随着市场需求量的增加和野生资源的频繁捕捞，野生绿鳍马面鲀资源几近枯竭。资源的枯竭促进了人工养殖的发展，绿鳍马面鲀的人工繁育技术已获成功(关健等, 2011、2012; 薛美岩等, 2012)。工厂化养殖规模逐渐扩大，现已成为一种极具开发潜力且值得推广的优良养殖品种(刘琨等, 2017、2019; 张立宁等, 2020)。但国内外尚未有绿鳍马面鲀病害的报道，这可能与绿鳍马面鲀的生物特性有关。绿鳍马面鲀鱼皮坚韧，对环境中的潜在病原有一定的抵御能力(李平伦

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等, 2003)。此外，虽然有研究报道过甲藻(*Cochlodinium polykrikoides*)对绿鳍马面鲀的毒性作用，以及有研究者发现野生绿鳍马面鲀体内的 2 种粘孢子虫寄生(Zhao *et al*, 2001; Kim *et al*, 2000)，但在养殖条件下绿鳍马面鲀的细菌、病毒和寄生虫等病害尚未见报道。

近年来，随着我国北方网箱养殖的快速发展，许氏平鲉(*Sebastes schlegeli*)的养殖量也在逐年增加，但在养殖过程中各种病害也随之出现(林春媛等, 2011)，在我国许氏平鲉养殖中，已报道哈维氏弧菌(*Vibrio harveyi*)(孟鹏等, 2010)、鱼肠道弧菌(*Vibrio ichthyoenteri*) (王庚申, 2012)、鳗利斯顿氏菌(*Listonella anguillarum*) (王庚申等, 2012)、淋巴囊肿病毒(lymphocystis disease virus) (Zheng *et al*, 2016)、异尖线虫(*Anisakis* spp.) (张雯倩等, 2017)、轮虫弧菌(*Vibrio rotiferianus*) (王凯等, 2019)、美人鱼发光杆菌美人鱼亚种(*Photobacterium damselae* subsp. *damselae*) (Zhang *et al*, 2019)等多种病害，造成巨大的养殖损失。

2018 年 11 月，山东省烟台市蓬莱市一养殖场工厂化养殖的绿鳍马面鲀出现发病死亡情况；2019 年 4 月，该养殖场的许氏平鲉也出现发病死亡情况，典型症状均为嘴部发红。本实验室分别从发病绿鳍马面鲀和许氏平鲉的内脏中分离得到优势菌株 2018TS-1 和 2019SS-1，对其致病性进行了人工感染确认，并通过生理生化鉴定、16S rRNA 和 *vapA* 基因测序分析，对病原菌进行鉴定。研究内容充实了国内工厂化养殖绿鳍马面鲀和许氏平鲉的疾病研究，为这 2 种经济鱼类的疾病防控和健康养殖提供了数据支撑。

1 材料与方法

1.1 病鱼来源

患病绿鳍马面鲀和许氏平鲉均采自山东省烟台市蓬莱市某养殖场，使用水源为浅井地下水，盐度为 28~30，pH 为 7.6~7.8，溶解氧为 8.0 mg/L 左右。绿鳍马面鲀约 5000 尾，体重为 200~300 g，养殖于 8 个 25 m^3 水体的水泥池中，水温为 14℃；许氏平鲉鱼苗 20 000 尾，体重为 30~50 g，养殖于 8 个 25 m^3 水体的水泥池中，水温为 12℃。

1.2 病原菌分离

随机选取具有典型症状的发病鱼各 10 尾，首先在显微镜下观察病鱼的鳃、鳍、体表及内脏等组织，检测是否存在寄生虫感染。随后在无菌条件下，分别取病鱼的肝脏、脾脏和肾脏组织，划线培养于胰蛋白胨大豆琼脂(tryptose soya agar, TSA)和 2216E 培养基，培养温度为 20℃。72 h 后，挑取菌落形态一致的优势菌进行纯化，并用甘油保存于–80℃超低温冰箱备用。

1.3 人工感染

感染实验用绿鳍马面鲀和许氏平鲉购自山东省烟台市某养殖场，体重为 30~50 g，实验前分别养殖于 300 L 循环海水养殖系统中，养殖水温为 16℃。感染实验前，随机取 5 尾鱼解剖，取肝脏、脾脏和肾脏，匀浆，涂布于 TSA 培养基，确定实验鱼未携带病原。将实验鱼随机分为 4 组，每组 10 尾，养殖于 50 L 整理箱，水温为 16~18℃。利用 PBS 缓冲液将培养好的 2018TS-1 和 2019SS-1 菌液浓度调整至 1×10^5、1×10^6、1×10^7 和 1×10^8 CFU/mL。采用背部肌肉注射方法，每尾绿鳍马面鲀注射感染 2018TS-1 菌液 0.1 mL，每尾许氏平鲉注射感染 2019SS-1 菌液 0.1 mL，对照组注射 0.1 mL 的 PBS 缓冲液，每组 2 个平行，饲养于相同条件下。注射感染后观察 21 d，记录各组鱼的发病症状和死亡情况，根据改进的寇氏法(杨茂成, 1990)计算菌株的半数致死量(lethal dose 50%, LD_{50})。取死亡或濒死鱼的肝脏、脾脏和肾脏，进行病原菌的再次分离和 16S r RNA 基因测序鉴定。

1.4 病原菌的鉴定

利用 BIOLOG 微生物鉴定系统(BIOLOG, 美国)，通过 Gen Ⅲ 微孔板试剂条对菌株 2018TS-1 和 2019SS-1 进行生理生化特征鉴定。利用引物 27F (5′-AGAGTTTGATCCTGGTCAGAACGAACGCT-3') 和 1492R (5′-TACGGCTACCTTGTTACGACTTCACCCC-3′) PCR 扩增菌株 2018TS-1 和 2019SS-1 的 16S rRNA 基因(Lane, 1991)，利用引物 A-layer 1F (ACAGTGCACCGAAGGTTGAT) and A-layer 6R (ACGGCAGAGCTTGTCTACCT) PCR 扩增菌株的 *vapA* 基因(Gulla *et al*, 2016)，对获得的扩增产物进行测序。将得到的序列上传至 GeneBank，并进行 BLAST 同源性比对(https://blast.ncbi.nlm.nih.gov)。选取 GeneBank 中的代表性菌株，使用 MEGA 7.0 软件，采用邻位相连法(neighbor-joining)构建系统发育进化树(bootstrap=1000)。

2 结果

2.1 病原分离

绿鳍马面鲀共有 3 个养殖池出现发病情况，发病鱼嘴部充血发红，鱼鳍溃烂，少数头部轻微溃疡，解剖可见肝脏有出血点(图 1)，日死亡率为 0.4%~1.0%，累积死亡率约为 25%；许氏平鲉共有 5 个养殖池出现

发病死亡情况，病鱼嘴部发红，鳍部、尾部溃烂，肠道充满淡黄色液体(图 2)，日死亡率约为 1%，累积死亡率约为 40%。

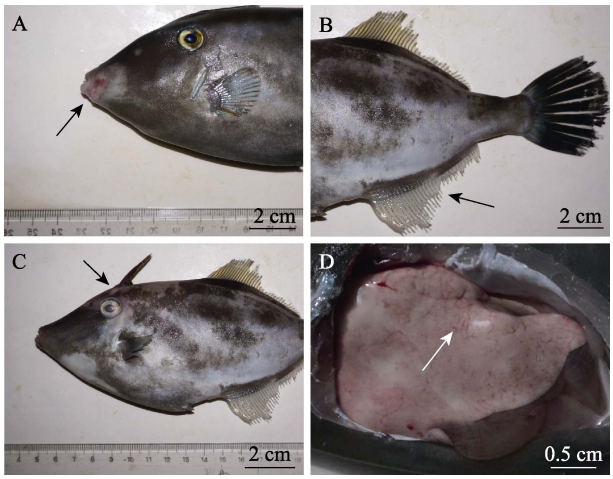

图 1　发病绿鳍马面鲀症状
Fig.1　The clinical signs of diseased *T. septentrionalis*

A：嘴部发红；B：鱼鳍溃烂；C：体表溃疡；D：肝脏出血
A: Red mouth; B: Ulcer in the fin;
C: Ulcer on the body; D: Liver congestion

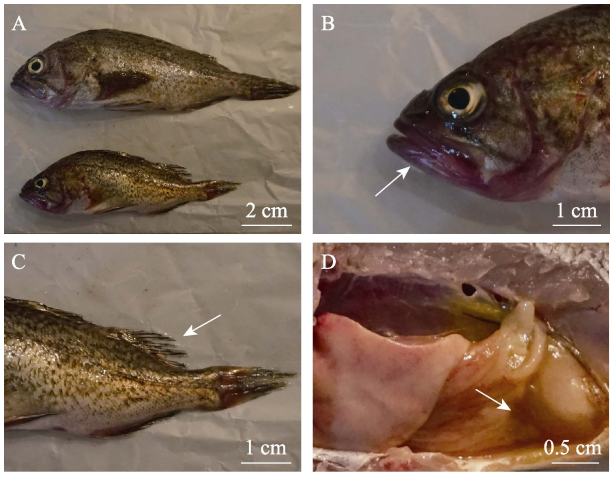

图 2　发病许氏平鲉症状
Fig.2　The clinical signs of diseased *S. schlegeli*

A：发病鱼；B：嘴部发红；C：鱼鳍溃烂；D：肠道积液
A: Diseased fish; B: Red mouth;
C: Ulcer in the fin; D: Intestinal hydrops

肉眼和显微镜观察患病鱼体表和内脏，未发现寄生虫感染。利用 TSA 固体培养基，从所有发病鱼的肝脏、脾脏和肾脏可分离得到大量形态一致的淡黄色圆形不透明菌落。

2.2　人工感染实验

通过背部肌肉注射感染的方式分别检测菌株 2018TS-1 对绿鳍马面鲀和 2019SS-1 对许氏平鲉的致病性。人工感染后死亡的鱼均表现出与自然感染一致的症状，包括典型的嘴部红肿、肝脏出血，另有注射部位红肿溃烂等症状。1×10^7 CFU/尾感染组的绿鳍马面鲀和许氏平鲉在感染后全部死亡，其他感染组部分死亡。2018TS-1 对绿鳍马面鲀的半数致死量为 1.78×10^5 CFU/尾，2019SS-1 对许氏平鲉的半数致死量为 0.89×10^5 CFU/尾，均具有较强的致病性(表 1)。从感染死亡的绿鳍马面鲀和许氏平鲉肝脏、脾脏和肾脏中可重新分离到大量形态一致的优势菌，分离菌落的 16S rRNA 测序结果与 2018TS-1 和 2019SS-1 一致，表明发病鱼死于该分离株的感染。对照组实验鱼均未发生死亡或出现异常症状。

表 1　绿鳍马面鲀与许氏平鲉人工感染实验
Tab.1　Experimental infection of *T. septentrionalis* and *S. schlegeli*

实验鱼 Test fish	感染剂量 Infection dose /(CFU/ind.)	死亡数/总数 Dead fish /total fish	半数致死量 LD_{50} /(CFU/ind.)
绿鳍马面鲀 *T. septentrionalis*	1×10^7	10/10	1.78×10^5
		10/10	
	1×10^6	8/10	
		6/10	
	1×10^5	6/10	
		3/10	
	1×10^4	1/10	
		1/10	
许氏平鲉 *S. schlegeli*	1×10^7	10/10	0.89×10^5
		10/10	
	1×10^6	8/10	
		8/10	
	1×10^5	6/10	
		5/10	
	1×10^4	3/10	
		1/10	

注：2018TS-1 感染绿鳍马面鲀，2019SS-1 感染许氏平鲉。

Note: *T. septentrionalis* was challenged by 2018TS-1; *S. schlegeli* was challenged by 2019SS-1.

2.3　病原鉴定

BIOLOG GenⅢ试剂条鉴定结果见表 2，由表 2 可知，2 株菌对糊精、D-麦芽糖、蔗糖、D-水杨苷、D-甘露糖、D-果糖、D-甘露醇、明胶、甘油、L-精氨酸和 L-谷氨酸等呈阳性反应。对龙胆二糖、D-松二糖、水苏糖、蜜二糖、D-半乳糖、L-岩藻糖、L-鼠李

糖、肌苷、L-丙氨酸、奎宁酸和 L-焦谷氨酸等呈阴性反应。经 BIOLOG 鉴定系统分析，2 株菌生理生化特征与杀鲑气单胞菌最为接近，可信度为 0.999。

利用 PCR 分别扩增菌株 2018TS-1 和 2019SS-1 的 16S rRNA 基因序列，Gene Bank 登录号分别为 OK258319 和 OK258320。测序结果经过 BLAST 比对分析显示，菌株 2018TS-1 和 2019SS-1 16S rRNA 基因在 Gene Bank 中同源性最高的均为杀鲑气单胞菌。进一步扩增得到了 2 株菌的 *vapA* 基因序列，2018TS-1 和 2019SS-1 的 GenBank 登录号分别为 OK300094 和 OK300095。从 GenBank 中选取代表性菌株，利用 MEGA 7.0 软件分别构建了 16S rRNA 基因和 *vapA* 基因的系统进化树。16S rRNA 系统进化树显示，2018TS-1 和 2019SS-1 均与杀鲑气单胞菌聚为一枝(图 3)，与生理生化鉴定结果吻合。*vapA* 系统进化树结果显示，2019TS-1 和 2018TS-1 均与杀鲑气单胞菌杀日本鲑亚种聚为一枝(图 4)。综合以上结果，将这 2 株病原菌鉴定为杀鲑气单胞菌杀日本鲑亚种(*Aeromonas salmonicida* subsp. *masoucida*)。

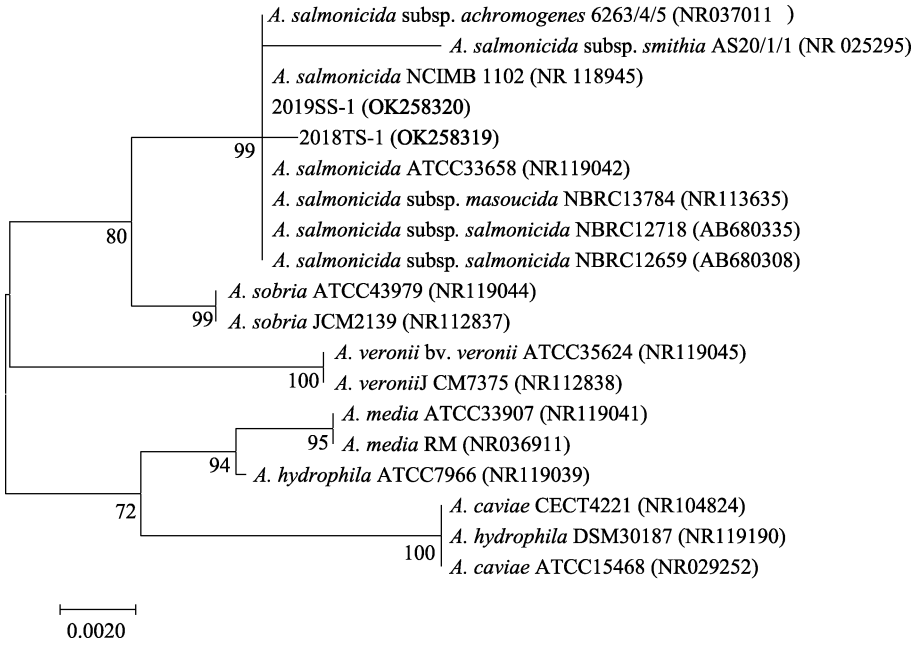

图 3 基于 16S rRNA 基因序列构建的系统发育进化树

Fig.3 Phylogenetic tree constructed by neighbor joining method based on sequences of 16S rRNA

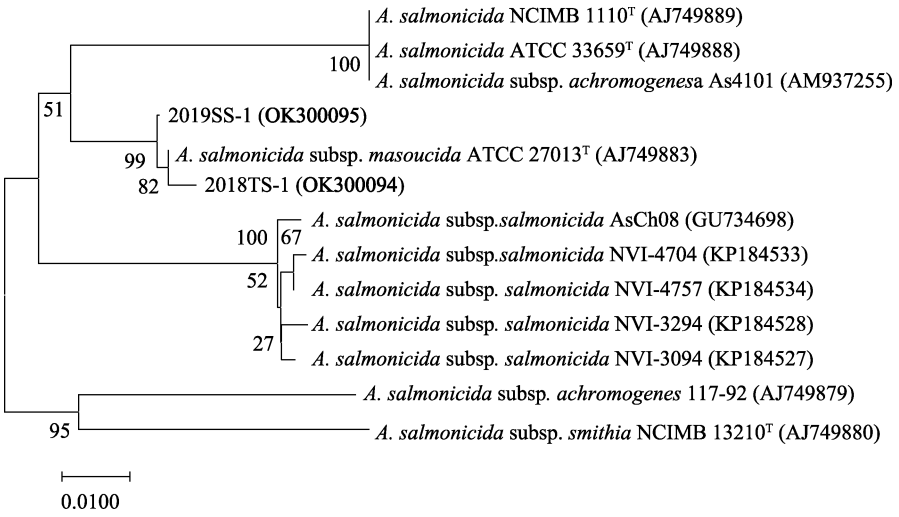

图 4 基于 *vapA* 基因序列构建的系统发育进化树

Fig.4 Phylogenetic tree constructed by neighbor joining method based on sequences of *vapA*

表 2　菌株 2018TS-1 和 2019SS-1 生理生化特征

Tab.2　Physiological and biochemical analysis of 2018TS-1 and 2019SS-1 strain by Biolog

项目 Items	结果 Results		项目 Items	结果 Results	
	2018TS-1	2019SS-1		2018TS-1	2019SS-1
糊精 Dextrin	+	+	D-葡糖酸 D-Gluconic acid	+	+
D-麦芽糖 D-Maltose	+	+	D-葡糖醛酸 D-Glucuronic acid	−	−
D-海藻糖 D-Trehalose	w	+	葡糖醛酰胺 Glucuronamide	−	−
D-纤维二糖 D-Cellobiose	+	+	粘酸 Mucic acid	−	−
龙胆二糖 Gentiobiose	−	−	奎宁酸 Quinic acid	−	−
蔗糖 Sucrose	+	+	糖质酸 D-Saccharic acid	−	−
D-松二糖 D-Turanose	−	−	p-羟基-苯乙酸 p-Hydroxy phenylacetic acid	−	−
水苏糖 Stachyose	−	−	丙酮酸甲酯 Methyl pyruvate	+	+
棉子糖 D-Raffinose	−	−	D-乳酸甲酯 D-Lactic acid methyl ester	−	−
α-D-乳糖 α-D-Lactose	−	−	L-乳酸 L-Lactic acid	−	−
蜜二糖 D-Melibiose	−	−	柠檬酸 Citric acid	−	−
β-甲酰-D-葡糖苷 β-Methyl-D-Glucoside	w	w	α-酮-戊二酸 α-Keto-glutaric acid	−	−
D-水杨苷 D-Salicin	+	+	D-苹果酸 D-Malic acid	−	−
N-乙酰-D-葡糖胺 N-Acetyl-D-Glucosamine	−	−	L-苹果酸 L-Malic acid	+	+
N-乙酰-β-D-甘露糖胺 N-Acetyl-β-D-Mannosamine	−	−	溴-丁二酸 Bromo-Succinic acid	w	+
N-乙酰-D 半乳糖胺 N-Acetyl-D-Galactosamine	−	−	吐温 40 Tween 40	w	w
N-乙酰神经氨酸 N-Acetyl neuraminic acid	−	−	γ-氨基-丁酸 γ-Amino-butryric acid	−	−
α-D-葡糖 α-D-Glucose	w	w	α-羟基-丁酸 α-Hydroxy-butyric acid	−	−
D-甘露糖 D-Mannose	+	+	β-羟基-D, L 丁酸 β-Hydroxy-D, L Butyric acid	−	−
D-果糖 D-Fructose	+	+	α-酮-丁酸 α-Keto-Butyric acid	+	+
D-半乳糖 D-Galactose	−	−	乙酰乙酸 Acetoacetic acid	w	w
3-甲酰葡糖 3-Methyl glucose	−	−	丙酸 Propionic acid	w	w
D-岩藻糖 D-Fucose	−	−	乙酸 Acetic acid	+	+
L-岩藻糖 L-Fucose	−	−	甲酸 Formic Acid	−	w
L-鼠李糖 L-Rhamnose	−	−	pH 6	w	+
肌苷 Inosine	−	−	pH 5	−	−
D-山梨醇 D-Sorbitol	−	w	1% NaCl	+	+
D-甘露醇 D-Mannitol	+	+	4% NaCl	−	−
D-阿拉伯醇 D-Arabitol	−	−	8% NaCl	−	−
肌醇 myo-Inositol	−	−	1%乳酸钠 1% Sodium lactate	w	+
甘油 Glycerol	+	+	梭链胞酸 Fusidic acid	−	−
D-葡糖-6-磷酸 D-Glucose-6-PO4	−	−	D-丝氨酸 D-Serine	−	−
D-果糖-6-磷酸 D-Fructose-6-PO4	−	−	L-天冬氨酸 L-Aspartic acid	+	+
D-天冬氨酸 D-Aspartic acid	−	−	L-谷氨酸 L-Glutamic acid	+	+
D-丝氨酸 D-Serine	−	−	L-组胺 L-Histidine	w	w
明胶 Gelatin	+	+	L-焦谷氨酸 L-Pyroglutamic acid	−	−
甘氨酰基-L-脯氨酸 Glycyl-L-proline	+	+	L-丝氨酸 L-Serine	+	+
L-丙氨酸 L-Alanine	−	−	D-半乳糖醛酸 D-Galacturonic acid	−	−
L-精氨酸 L-Arginine	+	+	L-半乳糖醛酸内酯 L-Galactonic acid lactone	−	−

注：“+”为阳性，“–”表示阴性反应，“w”表示弱阳性。

Note: +: Positive; –: Negative; w: Weak positive.

3 讨论

杀鲑气单胞菌是一种重要的鱼类病原菌，在世界范围内广泛分布。根据菌株形态学和生理生化特征，如褐色色素产生、溶血性、蔗糖发酵、菌落大小等，分为 5 个亚种，分别为杀鲑亚种(*A. salmonicida* subsp. *salmonicida*)、杀日本鲑亚种(*A. salmonicida* subsp. *masoucida*)、无色亚种(*A. salmonicida* subsp. *achromogenes*)、史氏亚种(*A. salmonicida* subsp. *smithia*)和溶果胶亚种(*A. salmonicida* subsp. *pectinolytica*) (Holt *et al*, 1994; Pavan *et al*, 2000; Nash *et al*, 2006)。其中杀鲑亚种称为典型株，其他菌株均归为非典型株(Wiklund *et al*, 1998)。A 层蛋白(A-layer)是杀鲑气单胞菌的毒力蛋白之一，由毒力阵列蛋白基因 *vapA* 编码 (Lago *et al*, 2012)，*vapA* 基因是杀鲑气单胞菌分型和亚种鉴定的一个重要标准(Gulla *et al*, 2016)，根据 *vapA* 基因的可变区将杀鲑气单胞菌分为 14 个亚型和一些单生菌株，以及无 *vapA* 基因的溶果胶亚种(Gulla *et al*, 2019)。本研究也采用了 *vapA* 基因分型的方法，并将分离到的病原 2018TS-1 和 2019SS-1 确定为杀日本鲑亚种。

传统认为无致病性的杀鲑气单胞菌溶果胶亚种属于嗜温性菌株，而其他致病性亚种均为嗜冷性菌株，通常感染冷水鱼类，如大西洋鲑(*Salmo salar*)、虹鳟(*Oncorhynchus mykiss*)、大西洋鳕鱼(*Gadus morhua*)、北极红点鲑(*Salvelinus alpinus*)、大西洋比目鱼(*Hippoglossus hippoglossus*)、大西洋狼鱼(*Anarhichas lupus*)等(Woo *et al*, 2017)。此次许氏平鲉和绿鳍马面鲀发病时的养殖水温分别为 12℃和 14℃，与先前研究相吻合。但近年来报道发现，杀鲑气单胞菌感染的宿主越来越广泛，在我国热带养殖品种中也有感染的病例报道，如鳜鱼(*Siniperca chuatsi*) (Lin *et al*, 2020)、石斑鱼(*Epinephelus coioides*) (Zhong *et al*, 2021)等，其中，感染石斑鱼的菌株 SRW-OG1 属于嗜温性的杀鲑亚种，可以在 37℃下生长，改变了传统对杀鲑亚种的认识(Zhong *et al*, 2021)。

在国内，杀鲑气单胞菌杀日本鲑亚种已被报道可感染多种水产养殖动物，包括刺参(*Apostichopus japonicus*) (杨嘉龙等, 2007)、大西洋鲑(Du *et al*. 2015)、虹鳟(刁菁等, 2018)、中国大鲵(*Andrias davidianus*) (赵光伟等, 2019)等，本实验室也在山东省的养殖裸盖鱼(*Anoplopoma fimbria*) (王晓冉等, 2017)、半滑舌鳎(*Cynoglossus semilaevis*)和大菱鲆(Wang *et al*, 2020)中发现了杀鲑气单胞菌杀日本鲑亚种感染的病例。通过本研究进一步发现，杀鲑气单胞菌杀日本鲑亚种还可感染绿鳍马面鲀和许氏平鲉，这表明病原的宿主范围在不断扩大。杀鲑气单胞菌的基因组中含有大量的可移动元件，包括插入元件(insertion sequences, ISs)、基因组岛(genomic islands, GEIs)、转座子(transposons)和前噬菌体(prophages)等(Reith *et al*, 2008; Emond-Rheault *et al*, 2015)，这些可移动原件增强了杀鲑气单胞菌的变异能力，也进一步增强了细菌对宿主、环境的适应能力(Juhas *et al*, 2009; Bellanger *et al*, 2014; Darmon *et al*, 2014)，这可能是导致近年来杀鲑气单胞菌感染宿主种类和流行区域不断扩大的重要原因。

本次从患病绿鳍马面鲀和许氏平鲉中分离出来 2 株致病菌 2018TS-1 和 2019SS-1，人工感染实验结果表明，菌株 2018TS-1 和 2019SS-1 能引发绿鳍马面鲀和许氏平鲉死亡，其症状与自然条件下发病症状一致，从人工感染发病鱼中可再次分离到优势菌 2018TS-1 和 2019SS-1，表明这 2 株菌是此次发病的病原。通过生理生化鉴定、16S rRNA 和 *vapA* 基因分型，确定病原为杀鲑气单胞菌杀日本鲑亚种。本研究是人工养殖绿鳍马面鲀病害的首例报道，为绿鳍马面鲀的病害防控提供了基础信息。尽管日本和韩国均发现过杀鲑气单胞菌感染许氏平鲉的病例(Izumikawa *et al*, 1997; Han *et al*, 2010)，但本研究是国内首次报道许氏平鲉感染杀鲑气单胞菌。同时，本次在同一养殖场的不同养殖品种均发现杀鲑气单胞菌感染，表明场内可能存在水平传播，在养殖管理中，防疫措施仍需进一步加强。

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Isolation and Identification of *Aeromonas salmonicida* from *Thamnaconus septentrionalis* and *Sebastes schlegeli*

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Abstract The greenfin horse-faced filefish (*Thamnaconus septentrionalis*) and rockfish (*Sebastes schlegeli*) occupy important positions in the offshore net fishery in Shandong Province. Interest in their mariculture has been developing rapidly in recent years as candidates for submerged cage open-sea aquaculture. With the development of breeding techniques and the expansion of large-scale farming, fish disease may become a serious constraint that limits sustainable aquaculture and leads to great economic losses. Epidemiological investigation is the basis of disease control and should be carried out throughout the culture process. In this study, we describe the diseases of *T. septentrionalis* and *S. schlegeli* caused by *Aeromonas salmonicida* subsp. *masoucida*. In November 2018, an outbreak of *T. septentrionalis* disease was observed in a farm located in Penglai, Shandong Province, and an outbreak of *S. schlegeli* disease occurred in the same farm in April 2019, with daily mortalities of 0.4%~1% and about 1%, respectively. The main symptoms in the diseased fish were ulcers, redness, swelling, and bleeding in the mouth. Most diseased fish in the ponds showed "red mouth". No parasites were observed by the naked eye or light microscope. From the liver, spleen, and kidney of all the diseased fish, many homogeneous colonies were observed after three days incubation on TSA and 2216E agar plates. All strains had the same shape, color, and size, and the 16S rRNA genes of all strains were the same, with high identity with *A. salmonicida*. The virulence of the isolates was tested experimentally via injection with *T. septentrionalis* (infected by 2018TS-1) and *S. schlegeli* (infected by 2019SS-1) in the laboratory to calculate the median lethal dose (LD_{50}). The results showed that the LD_{50} of 2018TS-1 to *T. septentrionalis* was 1.78×10^5 CFU/fish, and that of 2019SS-1 to *S. schlegeli* was 0.89×10^5 CFU/fish. The dead fish of the experimentally infected group showed ulcers and red mouth, the same symptoms as in naturally infected fish. Dominant colonies isolated from experimentally infected fish were all identified as *A. salmonicida* by 16S rRNA gene sequencing, which indicated that 2018TS-1 and 2019SS-1 were the pathogens of *T. septentrionalis* and *S. schlegeli*, respectively. Bacterial identification was carried out by 16S rRNA gene analysis and Biolog Gen Ⅲ characterization. The 16S rRNA gene sequences of 2018TS-1 and 2019SS-1 (Gene Bank: OK258319 and OK258320) isolated from *T. septentrionalis* and *S. schlegeli* were analyzed using MEGA5, and the phylogenetic tree derived from 16S rRNA gene sequences clustered the isolates with *A. salmonicida*. Among the Biolog Gen Ⅲ tests, 31 produced positive reactions or weak positive reactions for both strains (Dextrin, D-Maltose, D-Trehalose, D-Cellobiose, Sucrose, β-Methyl-D-Glucoside, D-Salicin, α-D-Glucose, D-Mannose, D-Fructose, D-Mannitol, Glycerol, Gelatin, Glycyl-L-Proline, L-Arginine,

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D-Gluconic Acid, Methyl Pyruvate, L-Malic Acid, Bromo-Succinic Acid, Tween 40, α-Keto-Butyric Acid, Acetoacetic Acid, Propionic Acid, Acetic Acid, pH 6, 1% NaCl, 1% Sodium Lactate, L-Aspartic Acid, L-Glutamic Acid, L-Histidine, and L-Serine), and two weak positive reactions for 2019SS-1, while the others were negative. According to the Biolog database, both strains were identified as *A. salmonicida*. Based on the molecular analysis of 16S rRNA genes and Biolog Gen Ⅲ phenotype results, the isolates were identified as *A. salmonicida*. The *vapA* gene, which encodes the outer membrane protein (A-layer protein) and causes the auto-aggregation of bacteria, is a conserved gene with some variation region in *A. salmonicida*. *vapA* gene typing is an effective and important method for classifying the molecular types and subspecies of this fish. *vapA* gene typing was also used in this study to identify subspecies of strains isolated from *T. septentrionalis* and *S. schlegeli*. The *vapA* gene sequences of 2018TS-1 and 2019SS-1 (Gene Bank: OK300094 and OK300095) were analyzed using MEGA5 with type strains obtained from Gene Bank. The phylogenetic tree derived from the *vapA* gene sequences clustered 2018TS-1 and 2019SS-1 with type strain ATCC 27013, indicating that the strains isolated from *T. septentrionalis* and *S. schlegeli* belonged to *A. salmonicida* subsp. *masoucida*, similar to the A-layer type Ⅶ strains, which are all from the northeast Asian and Canadian coasts in the Pacific Ocean. Based on the experimental infection, 16S rRNA sequence analysis, Biolog Gen Ⅲ characterization, and *vapA* gene typing, we confirmed that *A. salmonicida* subsp. *masoucida* is the pathogen of *T. septentrionalis* and *S. schlegeli*, and the cause of these two diseases on the farm. This is the first report of *T. septentrionalis* and *S. schlegeli* infected by *A. salmonicida* in industrial aquaculture, as well as the first report of a disease of *T. septentrionalis* in culture. It has been reported that *A. salmonicida* subsp. *masoucida* can infect Atlantic salmon (*Salmo salar*), turbot (*Scophthalmus maximus*), sablefish (*Anoplopoma fimbria*), and tongue sole (*Cynoglossus semilaevis*) cultured in Shandong Province. In this study, we expanded the host list of *A. salmonicida* subsp. *masoucida* to include two new species in aquaculture, *T. septentrionalis* and *S. schlegeli*, on the same farm, indicating that *A. salmonicida* subsp. *masoucida* may translate and adapt to a new host in a short period. Considering the increasing host and economic losses caused by *A. salmonicida* in fish culture, the prevention of *A. salmonicida* subsp. *masoucida* should be an important objective for mariculture in the future.

Key words *Thamnaconus septentrionalis*; *Sebastes schlegeli*; Bacteria identification; *Aeromonas salmonicida* subsp. *masoucida*