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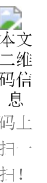
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病原性鳃弧菌(*Vibrio anguillarum*)双重PCR与LAMP检测方法的建立

孙品品¹, 高晓建¹, 张晓君^{1,2}, 马丽娜³, 阎斌伦¹, 白雪松¹, 赵佳铭¹, 毕可然¹, 秦 蕾¹

1. 淮海工学院 连云港 222005; 2. 扬州大学动物科学与技术学院 扬州 225009; 3. 吉林大学再生医学研究所 长春 130021

摘要:

本研究检测了分离自发病大菱鲆、半滑舌鲷及鲤鱼的22株病原鳃弧菌(*Vibrio anguillarum*)毒力相关基因的携带情况,并建立了病原鳃弧菌的分子生物学检测方法。以PCR方法检测8个毒力相关基因的分布,结果显示,22株病原鳃弧菌均可扩增出6个基因(*empA*、*vah1*、*vah4*、*flaA*、*rtxA*和*tonB*)目的条带,未扩增出*virA*和*angM*基因;针对*vah4*和*rtxA*设计引物进行双重PCR扩增,同一PCR反应体系可扩增出两条目的条带,灵敏度为 2.4×10^3 CFU/ml,对照菌无任何扩增条带;以*vah4*设计引物进行LAMP扩增,病原鳃弧菌可扩增出阶梯状条带,呈现阳性反应,6株对照菌无阶梯状扩增条带且呈现阴性反应,LAMP扩增灵敏度为 2.4×10^1 CFU/ml。LAMP检测灵敏度是双重PCR的100倍,LAMP技术与PCR比较,操作简便、快速、灵敏度高且不需昂贵仪器,LAMP检测鳃弧菌的方法更适用于养殖生产实际应用。

关键词: [鳃弧菌](#) [毒力相关基因](#) [双重PCR](#) [环介导恒温扩增技术\(LAMP\)](#)

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Detection of Pathogenic *Vibrio anguillarum* by Using Duplex PCR and LAMP Assays

SUN Jingjing¹, GAO Xiaojian¹, ZHANG Xiaojun^{1,2}, MA Lina³, YAN Binlun¹, BAI Xuesong¹, ZHAO Jiaming¹, BI Keran¹, QIN Lei¹

1. Huaihai Institute of Technology, Lianyungang 222005; 2. College of Animal Science and Technology, Yangzhou University, Yangzhou 225009; 3. Jilin University, School of Pharmaceutical Sciences, Changchun 130021

Abstract:

Vibrio anguillarum widely exists in aquatic environments and has been recognized as one of the prominent waterborne pathogens that undermine the aquaculture industry worldwide. In this study, we investigated the prevalent distribution of eight virulence-associated genes in the *V. anguillarum* strains isolated from *Scophthalmus maximus*, *Cynoglossus semilaevis* and *Cyprinus carpio*, and improved the detection of *V. anguillarum* by using duplex PCR and LAMP assays. Six genes (*empA*, *vah1*, *vah4*, *flaA*, *rtxA*, and *tonB*) were detected in all 22 pathogenic strains of *V. anguillarum*, but *virA* and *angM* were not detected. The duplex PCR assay was established with *vah4* and *rtxA* genes as molecular markers. Two gene fragments from the chromosomal DNA of *V. anguillarum* were detected in one PCR reaction with the detection limit of 2.4×10^3 CFU/ml. The assay in 6 other control strains generated negative results. The LAMP assay was established with *vah4* as the molecular marker. The positive reaction was shown as stair-step amplified bands and the detection limit was 2.4×10^1 CFU/ml. The assay produced negative reactions in 6 types of control pathogenic bacteria (no amplified bands). The LAMP method was 100 times more sensitive than the PCR method. Therefore we concluded that the LAMP assay could be a sensitive, rapid and simple tool for the detection of *V. anguillarum*, and recommend to employ this method in the early diagnosis of *V. anguillarum* infection in aquatic animals.

Key words: [Vibrio anguillarum](#) [Virulence gene](#) [Duplex PCR](#) [LAMP](#)

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地址: 青岛市南京路106号, 黄海水产研究所《渔业科学进展》编辑部 邮编: 266071

电话: 0532-85833580 E-mail: yykxjz@ysfri.ac.cn

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