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海洋农业

应用双抗体夹心酶联免疫方法检测仿刺参病原菌——黄海希瓦氏菌AP629

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摘要:

“化皮病”是当前仿刺参养殖的最严重的疾病，导致大量死亡，严重影响我国水产养殖的经济效益。以仿刺参病原菌——黄海希瓦氏菌(Shewanella smarflavi)AP629兔源多克隆抗体和鼠源单克隆抗体3D9分别作为包被抗体和检测抗体，建立了黄海希瓦氏菌AP629的双抗体夹心ELISA快速检测方法。多克隆抗体和单抗3D9的最佳稀释倍数分别为1∶400和1∶80，该方法特异性强，与弧菌、气单胞菌、爱德华氏菌、大肠杆菌等均无交叉反应，检测灵敏度高。以PBS和仿刺参体壁匀浆上清液为悬菌介质的最低检出限分别为104 cells/mL和106 cells/mL。对人工感染黄海希瓦氏菌的10头仿刺参进行检测，其检测结果均为阳性，稳定性和重复性良好。该方法的建立有助于快速准确地诊断由黄海希瓦氏菌AP629引起的仿刺参疾病。

关键词: 黄海希瓦氏菌;双抗体夹心ELISA;检测

Detection of Shewanella smarflavi AP629 by Double-antibody Sandwich ELISA Method

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Abstract:

At present, skin ulceration syndrome is the most serious disease in Apostichopus japonicus aquaculture. It caused mass mortality and great economic losses for aquaculture in China. A double antibody sandwich enzymelinked immunosorbent assay (DAS-ELISA) was developed for rapidly detecting S.smarflavi (AP629), a pathogen of Apostichopus japonicus, using polyclonal antibody (pAb) from rabbit and monoclonal antibody 3D9 (mAb 3D9) from mouse against AP629. The optimal dilution of pAb and mAb 3D9 were 1∶400 and 1∶80, respectively. This method has strong specificity, no cross reaction with other bacteria, including Vibrio sp, Aeromonas salmonicida, Edwardsiella ictaluri, and Escherichia coli etc., and high sensitivity in detection. The lowest concentration of strain AP629 that can be detected was 104 cells/mL and 106 cells/mL, respectively using PBS and homogenate of body wall from A. japonicus as medium. 10 artificial infected A.japonicus samples were detected and 100% of them were positive. So it has better stability and repetition. This method is very helpful for rapid and accurate diagnosis of A. japonicus infected by S.smarflavi AP629.

Keywords: Shewanella smarflavi double-antibody sandwich ELISA detection

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