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罗非鱼组织内无乳链球菌实时荧光定量PCR检测方法建立

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Real-time quantitative PCR for detection of *Streptococcus agalactiae* from tilapia tissue

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摘要

为建立一种罗非鱼组织内无乳链球菌（*Streptococcus agalactiae*）的定量检测方法，以无乳链球菌cfb基因为靶标建立了*Taq*Man荧光探针实时定量PCR方法，并对该方法的特异性、敏感性及实用性进行了验证。应用建立的方法检测无乳链球菌标准菌株和阳性菌株均为阳性，阴性对照菌株均为阴性；无乳链球菌基因组DNA最低检测质量浓度为3.42×10^{-7} ng•μL^{-1}，每个反应体系检测下限低于10个细胞；人工感染罗非鱼肝、脾、中肠和肾组织样品无乳链球菌检测结果均为阳性，组织样品每微克DNA可检测到无乳链球菌细胞数量分别为2.95×10^{3}个、2.45×107个、2.34×10^{3}个和4.54×10^{4}个，对照组为阴性。结果表明建立的罗非鱼组织内无乳链球菌实时荧光定量PCR检测方法具有准确性高、特异性和敏感性强等特点，可对罗非鱼组织内无乳链球菌进行快速定量检测，可用于罗非鱼无乳链球菌病的监测与预防。

关键词 ： 罗非鱼，无乳链球菌，实时荧光定量PCR，*Taq*Man荧光探针

Abstract :

By designing primers and probe based on the conserved region of *cfb* gene of *Streptococcus agalactiae* isolated from tilapia, we developed a real-time fluorescent PCR assay for detection of *S.agalactiae* from tilapia tissue and verified the specificity, sensitivity and practicability of the assay. The standard and positive strains of *S.agalactiae* were positive, and all negative controls were negative. The minimum detectable concentration of *S.agalactiae* genome DNA was 3.42×10^{-7} ng•μL^{-1}, and the detection limit of the assay was less than 10 cells per reaction system. The genome DNA samples of midgut, liver, spleen and kidney which were collected from tilapia artificially infected with *S.agalactiae* were tested by real-time PCR and were positive. The quantities of *S.agalactiae* detected in per μg DNA of tissue samples were 2.95×10^{3} cells, 2.45×10^{7} cells, 2.34×10^{3} cells and 4.54×10^{4} cells, respectively, and the controls were negative. The results show that the real-time quantitative PCR assay which we developed was accurate, specific and sensitive, and could detect *S.agalactiae* from various tilapia tissues rapidly. The method can be used for surveillance and prevention of *S.agalactiae* disease of tilapia.

Key words : tilapia *Strepltococcus agalactiae* real-time quantitative PCR *Taq*Man probe

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