

863课题进展

3种DNA提取方法对养殖池塘不同生境菌群PCR-DGGE分析的影响

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摘要:

比较3种DNA提取方法(溶菌酶法、CTAB法及珠磨法)对养殖池塘几种生境(底泥、饲料、草鱼肠道内容物及肠道壁)菌群PCR-DGGE分析的影响。结果表明,以3种提取方法获得的DNA为模板均能进行16S rDNA V3区特异性片段扩增;但不同DNA提取方法对池塘不同生境菌群DGGE指纹图谱存在显著影响。DGGE指纹图谱显示草鱼肠道内容物菌群(溶菌酶法)与肠道壁菌群(溶菌酶法)存在较高一致性,底泥菌群(CTAB法)及饲料菌群(溶菌酶法)与鱼体肠道菌群(包括内容物菌群和肠道壁菌群)则存在明显差异,但肠道菌群与底泥菌群的相似度相对更高。研究提示采用微生物分子生态学工具对养殖池塘不同生境进行菌群结构分析时,需提前优化DNA提取方法;在以投饲为主的养殖鱼塘中,草鱼肠道菌群更多源自于池塘底泥。

关键词: 养殖池塘; 菌群; PCR-DGGE

Effects of Three Different DNA Extraction Methods on the Analysis of Bacteria Community from Different Micro-ecological Environments in a Farming Pond by PCR-DGGE

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Abstract:

This study compared the effects of three different DNA extraction methods (lysozyme digestion, CTAB method and bead mill) on analyzing bacteria from different micro-ecological environments in an farming pond (pond sludge, feed, intestinal content and intestinal wall of grass carp) by PCR-DGGE. The results showed that 16S rDNA V3 fragments were successfully amplified from samples extracted by three DNA extraction methods in all micro-ecological environments; but different DNA extraction methods had remarkable influence on DGGE fingerprints of bacteria under different micro-ecological environments. The cluster analysis of DGGE fingerprints showed that the bacterial community of intestinal content (lysozyme digestion) was almost identical to that of intestinal wall (lysozyme digestion) in grass carp, while the bacterial community of pond sludge (CTAB method) or feed (lysozyme digestion) showed obvious difference to the intestinal bacterial community of grass carp. However, the bacterial community of pond sludge was more similar to intestinal bacterial community compared to that of feed. This study indicated that DNA extraction method should be optimized before analyzing the structure of bacterial community under different micro-ecological environments in an farming pond, and most of the intestinal microbiota in grass carp were derived from the pond sludge in the feed-fed farm pond.

Keywords: aquaculture pond bacterial community PCR-DGGE

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