Título: 16S rRNA gene PCR primer choice and its influences on archaeal phylogenetic analyses

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Resumo:

Molecular phylogeny studies have an important role in describing uncultured microorganisms and their distribution among different environments. The 16S rRNA gene has proven to be a valuable phylogenetic marker in studies describing microbial communities and in the last years many “universal” domain specific PCR primers have been proposed. In the present study, an environmental sample was submitted to PCR reactions using two different *Archaea*-specific primer pairs and the sequences obtained were analyzed to verify how the choice of PCR primers influences the analysis. DNA from a lake sediment sample was extracted and submitted to PCR reactions using primer pairs 21f-958r and 340f-1000r. PCR fragments were cloned in *E. coli*, extracted and the DNA was submitted to Sanger sequencing procedures. The resulting sequences were aligned with ClustalX and submitted to further analysis using the Mothur software. Phylogenetic trees were constructed with MEGA5 software. Rarefaction curves showed that sequences obtained with the 21f-958r primer pair have a tendency to reach the plateau sooner, indicating a smaller richness when compared to the results obtained with the 340f-1000r primer pair. This can be also observed in the richness and diversity indexes, which indicated a more diverse community of the sequences obtained with the 340f-1000r primer pair. Beta diversity analysis ∫-Libshuff indicated statistical differences between the groups of sequences obtained with different primer sets. Venn diagrams confirmed these observations, since very few OTUs were shared between groups of sequences obtained with different primer pairs. When sequences were classified and phylogenetic trees were constructed, we were able to see two very different community profiles. Using the 340f-1000r primer pair, 78,4% of the sequences were from the Euryarchaeota phylum, with the other 21,6% classified as Thaumarchaeota, Creanarchaota or the Miscellaneous Crenarchaeotal Group (MCG). When using the 21-958r pair, only 15,2% of the sequences were classified as Euryarchaota, with the remaining 84,8% distributed among the other groups. Since all sequences were obtained from the same environmental DNA, we conclude that 16S rRNA gene PCR primer choice has a critical role on the phylogenetic and diversity analysis.

Palavras-chaves: archaea; primer bias; molecular phylogeny

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