Terminal restriction fragment length polymorphism (T-RFLP) analysis: Characterizing the unseen

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Received: 21 June 2006 / Final revision: 17 January 2007 / Accepted 18 January 2007

Abstract The golden era of microbiology during early 1900s was based on the isolation and characterization of purified single cultures. However, since the understanding of limitations of culturing methods to decipher the majority of the microbial diversity, microbiologists have been keen to assess the abundance and distribution of microbial diversity by alternative methods¹. The quest has been further fueled by the idea of relating such information about the community structure to the ecosystem function. Therefore, the goal of new cohort of modern day microbiologists is to understand microbial community structure and dynamics within their natural habitats. Consequently, a wide variety of culture independent approaches and methods for microbial community structure determination have been developed and applied to varied ecosystems. By far the majority of such methods use direct isolation of genetic content from the environmental samples and PCR amplification of genes of interest for bypassing the culturing biases. Existing methods include Amplified Ribosomal DNA Restriction Analysis (ARDRA), Single Stranded Conformation Polymorphism (SSCP), Thermal and Denaturing Gradient Gel Electrophoresis (TGGE and DGGE), Amplified Length Heterogeneity (ALH) analysis and Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis².

T-RFLP has been recently introduced and offers rapid characterization of microbial diversity and its dynamics. Further, it is most useful for analysis of large number of samples.

Terminal Restriction Fragment (TFR) patterns are generated and analyzed by a series of PCR amplification, restriction digestion, capillary gel electrophoresis and libraries match. An important requirement of T-RFLP analysis procedure is the sequence information of target gene from a wide variety of genetic backgrounds to design the degenerate or universal PCR primers. The primers used for this study are labeled on the 5' end with a fluorescent molecule (Fluorophore). Fluorophore labeled amplicons are subjected to downstream steps and terminal fragment length (in bases) is calculated with an automated system by comparison with TRF peak retention time of an internal DNA size standard. The TRF patterns are then analyzed by matching with the existing database of the target gene restriction pattern³.

For a relatively new technique, T-RFLP analysis has gained widespread applications in various aspects of microbiology ranging from disease monitoring to determination of plant-microorganism interactions. Rhizosphere(s) are naturally occurring ecosystems and allow realistic evaluation of plant- microorganism cross talk at the natural habitat. Rasche *et al.* have recently shown how genetically modified crops mis-effect the microbial diversity within the rhizosphere as compared to their isogenic, non-manipulated wild-type⁴. T-RFLP analysis also encompasses a wide variety of ecologically significant phenomenon are tricky to understand. Microbial evolution with respect to various kinds of environmental stresses have been studied with T-RFLP analysis, wherein soil samples with their constitutive microbial communities were subjected to a pre-determined stress (qualitative and quantitative) for a certain length of

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time and resulting fluctuation of microbial diversity was analyzed. Microbial flux in response to toxic chemical pollutants and their degradation is yet another major area covered by T-RFLP analysis. Biological remediation of hazardous pollutants by environmental release of degradative microorganisms has a major constrain of stringent monitoring for the mis-effects on the existing microbial flora. With T-RFLP it has become conveniently feasible to perform such monitoring studies. One of the studies for on-site *p*-nitrophenol biodegradation with bioaugmentation of a degradative strain showed no adverse effect on the pre-existing microbial community due to the above intervention⁵.

The T-RFLP technique has undergone several up gradation to further improve the accurate determination of microbial community structure. One of the major advancements has been the application of Real Time PCR and Reverse Transcriptase PCR with T-RFLP analysis for quantitative purposes⁶. Also the domain of T-RFLP based analysis, which has largely used certain phylogenetic marker gene such as 16S rRNA gene, is expanding to accommodate studies with genes of metabolic significance. Considering the significant accuracy of microbial community structure and dynamics determination, T-RFLP analysis would prove to be an important tool in studies pertaining to such issues. However, it is important to note that T-RFLP analysis requires very careful optimization of all the different steps involved in the process including T-RFLP profile characterization, database search etc. Like the other PCR base fingerprinting methods using multi-template DNA, T-RFLP has been reported to be prone to selective amplification of some of the templates and thus mis-effecting the prediction of

community structure. In the light of all the limitations and advantages that T-RFLP offers, the possibility of rapidly studying several samples makes it most widely applicable technique for the future and offers the characterization of the unseen microbial diversity and its dynamics.

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