Metagenomic analysis of Antimicrobial Resistant Genes in Wastewater Microbiome

Maanasa. M, Divakar. K^{*}

Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur, Tamil Nadu, India

*Corresponding author

ABSTRACT

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread. This is caused mainly due to over usage of antimicrobials. The other reasons for the AMR are natural occurrence, clinical misuse, self-medication and environmental pollution. To overcome this hazardous problem at the global level, functional genomics and metagenomics is used. Metagenomics is a molecular tool used to analyze the DNA acquired from environmental samples, in order to study the community of microorganisms present in a niche. As part of the research work, effluent from anaerobic sludge treatment plant rich in anaerobic bacteria (culturable/unculturable) was selected as source for extracting metagenomic DNA (approximately \sim 40kb). The extracted \sim 40kb DNA fragments were not species specific (independent of type of microorganism present in the sludge), selection were made only based on the size of DNA. Sequence unbiased fosmid library was constructed using pCC2FOS fosmid vector and expressed using Maxplax phage expression on E. coli EPI 3000 cells against β-lactum antibiotics. Total of 43 colonies which showed positive for resistance against tested β -lactum antibiotics were selected further investigation. The fosmid DNA was purified from all the 43 clones, the sequence of fosmid DNA was determined using next generation sequencing on Illumina Nextseq500 platform. Sequence analysis using MG-RAST revealed the presence of 29 gene sequences associated to ARGs. Among 29 genes 6 were found to have closely associated to already reported, well known ARGs. Remaining 23 genes were found to have very less sequence similarity with the reported ARGs and showed high sequence similarity with oxidoreductases. Metagenomics can be applied to formulate a strong correlation between antimicrobial resistance and microbiome. This study could lead to discovery of complex microbial communities and their functional components involved in antimicrobial resistance in bacteria.

Keywords: Antibiotic resistance; Metagenomics; Microbiome; Wastewater;



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