The Quarterly Scientific Journal of Applied Biology
Vol.35, No.1, Ser.70, Spring 2022, P: 14-16
Journal homepage: https://jab.alzahra.ac.ir
10.22051/JAB.2021.34043.1392

Research Paper

The study and comparison of the impact of primer in the evaluation of bacterial community structure in semi-arid area by Illumina MiSeq

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Received: 2021.06.10 **Accepted: 2021.9.4**

Introduction

Soil microorganisms are very crucial for appropriate ecosystem functioning, and one of their main parts is the biogeochemical cycling of nutrients. Microorganisms mediate around 80–90% of all routes in soil (Wurst et al., 2012). Microorganisms have serious roles in soil structure preservation, organic matter construction and decay, nitrogen fixation, toxic complexes break, and inorganic compound alterations (Wurst et al., 2012).

There is no accurate information on the amount and dimension of the diversity of soil microorganisms. Furthermore, the exact identification of total soil microbial diversity through culture dependent methods is almost impossible. In part, this is due to the fact that only 1% of the soil microbial population can be recovered *via* cultivation (Torsvik & Øvreås, 2002 & Nannipieri, 2003). New approaches of extracting nucleic acids from soils, along with developing next generation sequencing (NGS) approaches now permit more deepness of information and understanding of the microbial community in soil. NGS allows high throughput analysis of complex microbial communities in soil *via* small amplicons of typically hypervariable domains of prokaryotic 16S rRNA genes. Additionally, NGS generates extra

information that can be used to obtain more information about microbial communities in complex environments in comparison with other techniques such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction length polymorphism (TRLP). In NGS approaches, each DNA molecule is sequenced as an individual read, permitting the identification of specific species and describing the top 99.99% of the microbiota (Fredriksson et al. 2013). The aim of this study is to compare the impact of primers in assessing soil bacterial community construction.

Methods and materials

The study site is located in the southeast of Iran in the semi-arid steppe area of Khabr National Park and Ruchun Wildlife Refuge, Kerman Province, Iran. This park spreads from 28°28′ to 28°58′ N and from 56°02′ to 56°38′ E. The mean annual temperature and rainfall differs among 17.5–21.0°C and 200–350 mm, respectively. Two cold and warm zones were carefully chosen for sampling in this study. Soil samples were taken from cold and warm areas which grazed (disturbed) or not grazed (undisturbed) in spring and fall. The PowerSoil®DNA Isolation Kit was used to extract DNA from soil based on the manufacturer's instructions and stored at –20°C before usage. To assess the diversity and composition of the bacterial communities in soil samples, a DNA library was generated *via* the 27f/519r and 515f/806r primer set which amplifies V1–V3 and V4-region of 16S rRNA gene, respectively. Amplicons were sequenced using Illumina MiSeq platforms following Illumina. Samples were sequenced at 2×300 paired-end reads. Data gained from sequencing were analyzed via Quantitative Insights Into Microbial Ecology (QIIME, v 1.9.0).

Results and Discussion

The results showed the effect of area, grazing and season on bacterial community construction. NGS data analysis presented that primers which amplified V4-V5 area identified more bacteria (97.9%) in comparison to V1-V3 primers (90.4%). V1-V3 primers had better efficacy to identify Proteobacteria (37.85%) compared to V4-V5 which identified more Actinobacteria (33.6%). Actinobacteria and Proteobacteria are the dominant phyla in semiarid environments and alkaline dry soils. Furthermore, V4-V5 primers identified more bacteria in class, family and genus taxonomic groups compared to V1-V3 primer. Results indicated the importance of primers in assessing microbial community structure. It can be concluded that choosing one special primer set making underestimating or overestimating of some specific groups of bacteria. Then, although V4-V5 primer had better efficiency in bacteria

identification, it is recommended to use both primers to estimate bacterial community construction accurately.

Keywords**:** *Biodiversity, Microbial Community, Next Generation Sequencing, Soil Microbiology, Taxonomy*

Declaration of conflict of interest**:** *The authors declare that they have no conflicts of interest.*