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DNA Extraction Methods for Microbial Diversity Assessment from Marine Environments: Current Scenario and Future Perspectives

"Microbial diversity analysis from marine niche is adding huge number of novel microbial genomic sequences reconstructed from metagenomic analysis, wherein the proper representation of the persistent microbes requires the establishment of an efficient nucleic acid extraction method"

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COLUMN ARTICLE

Marine environments are an unprecedented source of indigenous microbial diversity. The nucleic acid information of these microbes, present in the sediments as well as within the living organisms residing in the sediments are the basis for understanding the dynamics-interactions, evolution and their metabolic potentials [1]. However, with the increased interruption by anthropogenic activities the biodiversity is getting highly impacted and these community shifts allow us to identify the extent of impact on the environment [2,3]. Environmental DNA (eDNA) as a whole allows study of all the organisms including those that are not cultivable in the laboratory and were missed from the studies for quite a long time until the concept of molecular markers and direct environmental nucleic acid extraction came into practice.

Sample collection is one of the critical steps in biodiversity studies that has a direct impact on the results and interpretations. Similarly, in the marine sediment involving studies the method used for nucleic acid extraction is shown to have a great impact on the downstream analysis and also in the final result outcomes [4]. The presence of humic acid contents in the sediments highly affects the DNA isolation method and also the stability of the isolated DNA [5]. The SDS based method for DNA extraction [6] has been the most widely used for environment DNA extraction from years with different modifications by different research groups incorporated depending on the sample properties. Several methods have been recently tested in which the cell lysis, DNA extraction and elution modes were varied for developing a standard protocol that would yield a high quality and quantity of DNA for long term storage. Apart from these manual modified protocols, a number of kits are being introduced in market for DNA extraction from these complex niche, and these have proved to be quite competitive in the yield quality if quantity requirement is not too high. The often-used lysis methods include the cryo-mill based pre-processing, SDS liked detergent based chemical lysis, mechanical bead-beating (usually common for the commercial kits), freeze and thaw or a combination of these methods. The next step in the manual method includes removal of the cell debris and protein/ lipid contents, which in the kit includes the spin columns to bind the DNA and remove the other molecules. The final and one of the other critical step reported to affect the quality and quantity of the DNA is the DNA precipitation in the manual approaches. Various groups have tried using different combination of solutions viz., the Na-ethanol, sodium acetate-isopropanol, Na-PEG, only PEG, PEG-NaCl, and similar combinations.

In the process to get improved DNA yield from complex niches, different recent approaches have been introduced, one of them includes the pre-processing of samples for filtering bacterial cells. High quality, humic free DNA from arctic and saltpan regions was obtained by saline wash followed with repeated filtration of the samples before the

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lysis [7]. Another group used the hot alkali treatment to the samples with 1M sodium hydroxide treatment for 20 min at high temperature (> 95°C), which led to better lysis of the dormant and archaeal cells [8]. One more recent study compared the hot alkali method, commercial kit and modified Zhou., *et al*. (1996) method wherein the following modifications were included: bead beating step was added, enzymatic lysis included lysozyme, the proteins were precipitated by a PCI wash instead of the usual CI wash in the protocol and the DNA was precipitated using Na-acetate and isopropanol [9]. They observed higher yield and low smearing in the DNA isolated by modified protocol, and also suggested use of kits for higher number of samples wherein high yield or cost is not a matter of concern.

CONCLUSION

Thus, currently there are a number of options and methods available to choose for DNA extraction from microbes in the marine niche but no gold standard is set. The difficulty is as each and every marine sample varies, even the same region or site has varied diversity at different depths and at areas in a very narrow distance. This wide variation in the indigenous microbes, the presence of archaea with rigid cell wall, endospore forms due to the stressful environment and humic acids are the reason behind the persistent difficulty in obtaining high quality DNA from the marine microflora. It is still gaining attention from researchers worldwide as clearly revealed by the number of papers being published on the methods to extract nucleic acid from sediment samples for accurate assessment of microbial diversity.

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