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Molecular Techniques Applied to Investigations of Abundance of the Ammonia Oxidizing Bacteria and Ammonia Oxidizing Archaea Microorganisms in the Environment

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Abstract: *This review shows regards of the recently experienced concerning the environments of ammonia oxidizing bacteria (AOB), ammonia oxidizing archaea (AOA) microorganisms, and denitrifying microbes. The advancements of molecular biology techniques have encouraged staggeringly to the fast recent developments in the sector. Various methods for implementing so are discussed. The function of AOB, AOA, and denitrifying microorganism composition was investigated through a high throughput of the 16S rRNA amplicon sequencing protocol. There is potential to observe the specific species appearance of these microorganisms in each environment and get to the evaluated relative abundance of several kinds. There is information indicated which the structure of denitrifying and nitrifying group was monitored field to significant fluctuations and the complexes, together in space and in time. More effort is required to enhance and isolate those microorganisms that common of the progressions and to function them through the compound of molecular techniques, biochemical and physiological. However, the investigation with deoxyribonucleic acid (DNA), antibodies, and the polymerase chain reaction (PCR) was preferred mainly to report the composition of chemolithoautotrophic bacteria, surveys of their characteristics in environmental that needed quantification at the transcriptional level is presently not available.*

Keywords*: Molecular techniques, ammonia oxidizing bacteria, ammonia oxidizing archaea, chemolithoautotrophic bacteria.*

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I. Introduction

Ammonia oxidation is the rate–limiting, and first steps of nitrification have been measured extensively because of its environmental importance in the large-scale nitrogen cycle and ecological implications [1]. Ammonia oxidizing and nitrite oxidizing microbes are a two-step process catalyzed. It is just the process of oxidative biological related to reduced and oxidized rivers of nitrogen in environmental [2]. This essential process in the nitrogen cycle of global was considered restricted to ammonia oxidizing bacteria (AOB) [3]. In diverse ecosystems, this processes counteract environmentally, it was applied to reduce nitrogen values in wastewater treatment plants. Various researches influences the essence of methods: autotrophic nitrification in microorganisms [4]; environmental of denitrification and nitrate/nitrite ammonification [5]; factors controlling denitrification [6]; aspects of denitrification in sediment and soil [7]; investigations for functional genes and phylogenetic of denitrification and nitrification [8]; molecular basis and cell biology of denitrification [9]; enzymology of ammonia oxidation [10]; enzymology of the nitrogen cycle [11]; aquaculture and nitrifying bacteria [12]; dissimilatory nitrate reductases in microbe [13]; anaerobic ammonia oxidation [14]; inorganic nitrogen metabolism in bacteria [15]; and nitrogen cycling in aquatic ecosystems [16]. The function of microbial communities in freshwater environments was significant in determining the composition [17], presented the diversity of microbial communities and distribution [18].

The functions are an activated related to microorganism groups, e.g., nitrogen fixation; microorganisms are essential processed and in substance, turnover cycling the stream. As gene function target, the ammonia monooxygenase (*amoA)* is more functional than the 16S rRNA scheme, for analysis AOA and AOB population pattern [19]. The high concentrations of ammonia in the streams are toxic to fishes and other aquatic living organisms. The reduction of ammonia in the environment is one of the primary functions of (AOA and AOB) and attained by nitrification, AOA support nitrification in different habitats. However, the separate study of AOB or AOA functions in ammonia oxidation causing significant attention and attracted many researchers [20].

Ammonia oxidizing archaea and AOB involves the key enzyme the *amoA* that is constituted of three *amoA*, *amoB*, and the *amoC* genes encode subunits [21]. *In situ* ratio determinations in streams environments, even so, the indicated that nitrification was appeared almost universally, even in the many oligotrophic environments with very lowest concentrations of nitrates under the growth threshold of AOB [22, 23].

The environmental experiences were large variations in chemical conditions and several hydrological. Furthermore, The physiochemical characteristic may affect the population of microbes to processes such as: nitrogen cycling [24], NH_4^+ availability [25], dissolved oxygen (DO) concentrations [26], temperature [27], total nitrogen (TN), total phosphorus (TP) [28], light [29], salinity [30], oxidation-reduction potential (ORP) [31], and sulfide concentrations [32]. Some studies were showed that abundance AOA – AOB phylotypes and distinct AOA – AOB groups had been presented in various aquatic environments (freshwater- marine) both in local estuarine gradients and on a wide geographical scale [33, 34]. Moreover, a few is known about whether AOA – AOB microorganisms are essential nitrifies in estuarine and marine environments.

This review is emphasized and highlighted the current achievements in the scope of chemolithoautotrophic bacteria detected through applied approaches of molecular biology, with focus on the natural habitats. There were techniques of engineering such as wastewater treatment plants, oxygen demand, water resources pollutants, and transport of pollutants.

II. Investigations of the Communities' Composition

The caused by their unique requests that are usually unidentified and decreased levels of growth, it is often not simplified to isolate a pure culture of AOB autotrophic microorganisms. The isolate combined with the typically small amount (few than 0.1%), complicated our understandable on the relative abundance and distribution of this microorganism in habitats environmental. However, molecular techniques were appeared to accessible (Fig. 1).

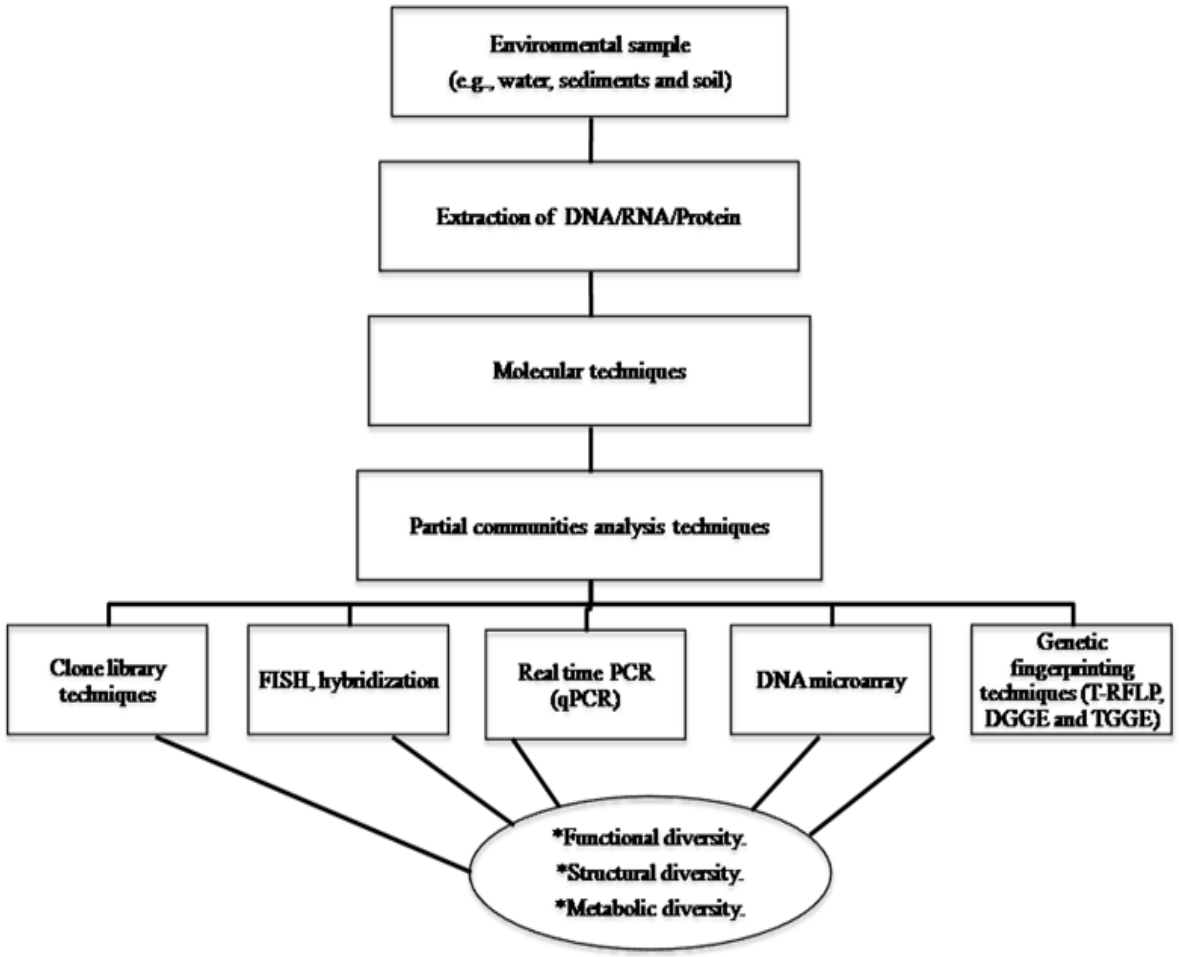

Figure1. Molecular techniques model to functional diversity of microbes and characterize the structural in the environments.

2.1 Immunofluorescence Technology

The function of fluorescent polyclonal antibodies (FPA) that identify ammonia monooxygenase (AMO) were functional immunofluorescences to identify ammonia oxidizing microbes [35]. The FPA production depends on the pure cultures accessibility. The diversity of essential serological was shown within the isolates from the various sediment samples [36]. FPA was changed for selective calculating of many *Nitrosomonas spp* supported the affiliated and suspended microorganism for species-specific counting [37]. For years ago, *Nitrosomonas spp* were considered to be natural compounds of the microbes [37], and at the minimum the most obvious [38], the AOB in freshwater habitats, whereas *Nitrosospira spp*. were found to be dominated in sedimentary [39, 40]. Through microscopy of immunofluorescence with partial sequence 16S ribosomal RNA sequences analysis and DNA hybridization [41, 42]. Furthermore, the novel studies of other places have changed this show.

2.2. Phylogenetic Technology

The understanding that microorganisms of nitrifying (nitrite and ammonia oxidizers) appeared to the sole of possessing operated for 16S rRNA molecular methods were significantly facilitated analyses of communities [43, 44]. The understanding of these sequences enables several techniques for analyzing the group structures of nitrifying microorganisms. Many of the experiments have been made for the clade of the AOB within the genus *β-Proteobacteria*.

2.2.1. PCR–based Technology

Up to 1997, 30 oligonucleotides of the 16S rRNA gene sequences are known [45], with understanding specificity for AOB of the genus *β–Proteobacteria* were proposed for PCR or hybridizations with whole cells or DNA and RNA extraction. Almost all the understanding about the AOB diversity groups in natural habitats has been collected to apply oligonucleotides. The primer of the oligonucleotide for *Nitrosococcus oceani* was described [46, 47], however, have not been extensively used [48].

The PCR with primer sequences of known characteristics was applied to amplification 16S rRNA, AOB, and AOA groups from natural habitat. The use of direct nested PCR facilitates the detection of the family *Eubacteriaceae* [49] was achieved. In a few situations, modified PCR showed positive significance from samples where direct PCR unsuccessful, that was primarily ascribed to decreased AOB abundance [50]. When surveys of various environmental samples (Soil and water, nutrient-enriched or poor) it showed apparently that *Nitrosospira*-like AOB is nearly omnipresent [51].

Nitrosospira-like AOB showed to be the dominant AOB and the universally present worldwide. However, *Nitrosomonas*-like AOB might be identified from several environmental samples. *N. eutropha/europaea* has long been recognized that at lowest [52, 53], owing to their possibility to construct microbial extracellular polymers substances [41]. As a strategy of survivability, the exopolymers might be supported recovery after extreme dryness stress in sediments [54], survivability in famine situations [55].

The possibility of identification of members of the *N. eutropha* / *europaea* parentage increased with raising nutrient concentrations [56, 57]. Occasionally, although sequences of *Nitrosomonas*-like might not be determined in ecological DNA investigations, enrichment cultures obtained from same habitats detected *Nitrosomonas spp* [58, 59]. The considered of obvious that even within various types of sediment (profundal and littoral sediments) distinct communities correlated to either *N. eutropha* or *N. europaea* occurred [60].
Interestingly, during a continuously high number of ecological investigations sequences related to the same AOB clusters were obtained from many various sites (samples of aquatic habitats) with the diversity structure of their 'total' bacterial groups. The statistics were showed the representation of higher abundance within the microbial communities, which just some strains are in culture as described.

In the investigation applying 16S rDNA amplicons to the descriptions AOB group in the root zone and the sediment of lakes in the Netherlands, not confirmations were observed which any specific classification/phylogenetic clusters were particular for these periodically low amounts of oxygen conditions [61]. The fertilized of soil huge elevated in nitrification concentration, however not in population size, was assessed by using PCR of competitive depended on 16S rRNA and *amoA* genes. This was proposed phenotypic modification within the AOB group [62]. It could be exciting to find if such changes might be monitored when the aim of the primary proteins mRNAs of ammonia oxidation (AMO, HAO).

Over the past decades after the implementation of terminal restriction fragment length polymorphism (T-RFLP) [63, 64], temperature gradient gel electrophoresis (TGGE), and denaturing gradient gel electrophoresis (DGGE) [65] in environmental microbiology. These approaches are recent generally used in most microbiological laboratories globally as molecular tools to correlate between the diversity of microorganism communities and to observer population dynamics. Recent progress in these approaches has demonstrated their significance in environmental microbiology.

2.2.2. Fluorescent in situ Hybridization (FISH)

Various oligonucleotides investigations to determined ammonia oxidizing group of the subclass *β-Proteobacteria* have been reported [66, 67], but there is barely a little FISH applications for evaluating of the abundance of nitrifying microorganism in ecosystems. According to the appearance search of the basic local alignment search tool (BLAST), together probes aligned sequences of 16S rRNA in ammonia oxidizing group and some unclassified clones. They might be used together because their characteristics are slightly various. The application of determined probes that identify diverse communities of ammonia oxidizing group could be possible only in environments with high ammonia oxidizing group abundances, for example, wastewater treatment plants. Until a little investigation for ammonia oxidizing group of the *Proteobacteria* has been reported, that could be particularly functional for the counting AOA and AOB groups in aquatic samples.

Microarray technology has considered a high-throughput platform and valuable tool to estimate possible candidate proteins and genes [68, 69]. DNA microarrays are extensively applied for gene expression [70, 71].

III. Conclusions

Molecular biology approaches have achieved the investigation of unidentified microorganisms and their function in various natural habitats more obtainable. Moreover, we are distanced to knowing the difficulty of microbial in every environmental. Nearly more than one hundred of the diverse genomes per gram occur in soils, and the numbers might be higher in habitats of aquatic likewise. Target DNA (functional genes or 16S rRNA sequences encoding) might be amplified by cloned, sequenced and PCR. The technology such as T-RFLP, TGGE, and DGGE might be facilitated to shown sample sets for the evaluation of regional and seasonal variations. Wherefore most obtained of sequences for functional genes will be novel. The status did not become dramatic in the 16S rRNA sequences situation. However, the functions of each environment will increase unique sequence data of the 16S rRNA databanks abundances. Recently molecular technology, like microarrays with various copies of all denitrification and nitrification genes, will potentially help to show of microorganisms for their specific metabolic facilitate in the nitrogen cycle. The knowledge is needed not concerning the bacterial structure, however, the concerning their activities. The excitement about the molecular biology approaches which allow us to investigate microorganisms directly in their environmental, we require extra attempts to develop and characterize molecular methods, those organisms that are still unidentified.

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