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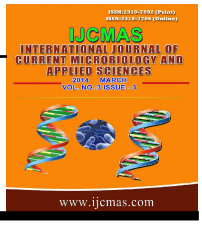

Original Research Article

Bacteria involving in nitrogen fixation and their evolutionary correlation

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A B S T R A C T

Nitrogen fixation is process of changing dinitrogen in to simple soluble intoxic form which is utilized by vegetative plant cell for synthesis of many biomolecules. The evolution of nitrogen fixation required microaerophilic condition because; nitrogenase play key role in this event and it is inhibited by oxygen. As we know that nitrogenase is such enzyme which is blocked by oxygen allosteric effect. So the evolution of nitrogen fixation occurred earlier to the evolution of cyanobacteria. However, evolutions of cyanobacteria require the oxygenic environment or it change anoxygenic environment to oxygenic environment. There was a common ancestor which was first evolved (progenote) which first perform the nitrogen fixation later on change in to cyanobacteria. However some branches of that progenote remain same as *Azotobacter* which fixes nitrogen but it is not a cyanobacteria. In this review we have compile the nitrogen fixer bacteria as well as cyanobacteria and how they are correlated to each other.

Keywords

Nitrogen;
Oxygen;
Nitrogenase;
Bacteria;
Cyanobacteria;
Symbiosis.

Introduction

Nitrogen is a important element for plant growth and production. It is a major component of microbial pigments, secondary metabolites and the key building blocks of proteins. It is also found in other important biomolecules, as Adenosine Tri Phosphate and nucleic acids. Even though it is one of the most abundant elements predominately in the form of nitrogen gas (N_2) in the Earth's atmosphere, plants can only utilize reduced forms of this element. Plants acquire these forms of "combined" nitrogen by: 1) the addition of ammonia and/or nitrate fertilizer (from the Haber-Bosch process) or manure to soil, 2) the release of these compounds during organic matter decomposition, 3) the conversion of atmospheric nitrogen into the compounds by natural processes, such as lightning, and 4) biological nitrogen fixation (Peoples *et al.* 1995). Biological nitrogen fixation is performed by prokaryotes mainly, these prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as *Azotobacter*,

bacteria that form associative relationships with plants, such as *Azospirillum*, *Rhizobium* and *Bradyrhizobium*, that form symbioses with legumes and other plants (Zahran, 2001). Those Microbes which fix dinitrogen in to simple available form are known as diazotrophs, comprises in bacterial and archaeal domains. The nitrogenase enzyme system is responsible for nitrogen fixation in these organisms (Zehr *et al.* 1998).

In recent years, understanding of biological nitrogen fixation has been supported by factors that confound molecular phylogeny such as sequence divergence, paralogy, and horizontal gene transfer (Lilburn *et al.* 2001; Boucher *et al.* 2003; Barcellos *et al.* 2007; Masson-Boivin *et al.* 2009). Obiviously nitrogenise play key role in nitrogen fixation (Goldber *et al*. 1987). The core components of nitrogenase, including *NifH*, *NifD*, *NifK*, *NifE*, and *NifN* genes are universal in nitrogen fixing organisms—typically found within highly conserved operons—and, overall, have remarkably congruent phylogenetic histories (Fani et al. 2000).

The nitrogen fixation evolution

During the evolution of earth there is aggregation of active atoms to form various molecules, nitrogen is one of them. After continuous evolution, organisms were developed and in this process the nitrogen form complexes which become the base of life. However, Nitrogen and oxygen both are evolved nearest to one another and their ratio helps in determination of chemical evolution of galaxies (P´erez-Montero et al. 2012). Steller data suggest a primary origin for N, but there is probably substantial secondary N at the centre of galaxy (Pagel, 1986).

So, we know that chemical evolution leads to the first organism formation (Millar and Urey, 1959). Nitrogen is an important atom which forms many complexes which helps in biomolecule formation. Now the di-nitrogen is available in plenty of in the environment. At that time, evolution occurs and the first microorganism evolved. They require nitrogen for their general metabolism and thus they fix dinitrogen in to simple form.

The nitrogen fixer bacteria

To understand the evolution of photosynthetic bacteria it is necessary to understand how the main groups within Bacteria have evolved from a common ancestor, a critical issue that has not been resolved in the past. These bacteria are anerobic, Facultative anerobic, microaerophilic, aerobic etc.

Strict anaerobes

Clostridium pasteurianum is an obligately anaerobic, free-living nitrogen fixer. It was one of the first nitrogen-fixing organisms isolated, and it has been studied in the laboratory for almost 100 years now. Although nitrogenase from *C. pasteurianum* has been very extensively studied and it possesses a number of unique properties, the nitrogen-fixation (nif) genes of *C. pasteurianum* have been studied only in recent years (Chen et al. 1990). Andother bacteria i.e. *Desulfovibrio* is proterobacteria that fix nitrogen (Ueda et al. 1995).

The ability to fix nitrogen (N_2) is found among a wide variety of the prokaryotic eubacteria, but not in eukaryotes. In addition to the prokaryotic eubacteria and eukaryotes, a third 'kingdom'—the archchaebacteria—has been defined based

on the comparison of 16S ribosomal oligonucleotide sequence catalogues. Included in the archaebacterial kingdom are certain obligate halophiles and thermoacidophiles, and the methanogens, strictly anaerobic, methane-producing bacteria. Report of diazotrophy by an archaebacterium, the methanogen *Methanosarcina barkeri*. Because it has been proposed that the archaebacteria, eubacteria and eukaryotes diverged at an early stage in evolution, the discovery of diazotrophy (N_2 fixation) in a member of the archaebacterial group raises interesting evolutionary questions (Murray and Zinder, 1984). In the Methanococcales, diazotrophic growth has been reported for *Methanococcus thermolithotrophicus* and *Methanococcus maripaludis*. *M. Thermolithotrophicus* is the only organism demonstrated to fix nitrogen at 60°C or above. Neither *Methanococcus jannaschii* nor *Methanococcus voltae* fix nitrogen despite the presence of nifH homologues. In *M. jannaschii* it is clear that other nif genes are not present (Leigh, 2000).

Facultative anaerobic

Klebsiella pneumoniae is a gram positive bacteria which contains nif genes although 235 Nif- strains of were characterized (Roberts et al. 1978). Now the clusters of nif gene for *K. Pneumonia* have been constructed (Dixon et al. 1980). Later on *Escherichia coli* transfer in to nitrogen fixer by transferring nitrogen fixing gene from *K. Pneumonia* (Cannon et al. 1976). *Bacillus polymyxa* tested for fixation of molecular nitrogen and it was tested by supplying them with N^{15} (Grau and Wilson 1962). Several *Bacillus* strains, from the rhizosphere of *Ammophila arenaria*, appeared on 'nitrogen-free' agar plates. They were able to grow in nitrogen-poor medium to which 0.1% yeast extract was added. Three of these bacilli were tested for their ability to fix nitrogen using the acetylene reduction assay (Wahab, 1978).

Mixed cultures of *Cellulomonas gelida* plus *Azospirillum lipoferum* or *Azospirillum brasilense* and *C. gelida* plus *Bacillus macerans* were shown to degrade cellulose and straw and to utilize the energy-yielding products to fix atmospheric nitrogen. Cultures inoculated with initially different proportions of *A. brasilense* and *C. gelida* all reached a stable ratio of approximately *Azospirillum*/ *Cellulomonas* cells (Halsall *et al*., 1985).

Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Erwinia herbicola, Citrobacter freundii, Citrobacter intermedius, and *Escherichia coli* bacteria were extensively characterized and tested for acetylene-reducing (nitrogen-fixing) activity under anaerobic conditions. It seems reasonable to suggest that under natural conditions, nitrogen fixation is able to contribute significantly to the nitrogen economy of the cells (Neilson et al. 1976).

Nitrogen fixation genes are shown to undergo a complex positive and negative regulation in *Rhizobium meliloti*. The sequence of *fixK* shows homology with the *Escherichia coli* regulators *fnr* and *crp*, which makes *fixK* the third characterized member of this family of prokaryotic regulators (Batut et al. 1989).

Microaerophilic

Mycobacterium flavum strain, a hydrogen-utilizing bacterium, is capable of fixing molecular nitrogen and resembles other

nitrogen-fixing hydrogen bacteria. It does resemble strains of *Xanthobacter* with respect to cell wall composition, production of carotenoid pigments, carbon source utilization pattern, and deoxyribonucleic acid (DNA) base composition (Malik and Claus 1978). Cultures of *Thiobacillus ferrooxidans* grown in the absence of a source of fixed nitrogen reduced acetylene to ethylene suggesting the presence of nitrogenase (Mackintosh, 1994). *Thiobacillus ferrooxidans* is a gram-negative, highly acidophilic (pH 1.5 to 2.0), autotrophic bacterium that obtains its energy through the oxidation of ferrous iron or reduced inorganic sulfur compounds (Rawlings).

Aerobic bacteria (New species)

Among aerobic bacteria, several diazotrophs have been isolated and characterized as nitrogen-fixer, including *Azatobacter*, *Acetobacter*, *Azoarcus*, *Herbaspirillum Azotobacter Beijerinckia*, *Klebsiella* and Cyanobacteria.

Non-pigmented bacterial strains that specifically induce nitrogen-fixing root nodules on the legume species *Crotalaria glaucoides*, *Crotalaria perrottetii* and *Crotalaria podocarpa* are belong to the genus *Methylobacterium*. They can grow on C_1 compounds such as methanol, formate and formaldehyde but not methylamine as sole carbon source, and carry an *mxaF* gene, encoding methanol dehydrogenase, which supports their methylotrophic metabolism. Presence of a *nodA* nodulation gene, and ability to nodulate plants of *Crotalaria* species and to fix nitrogen are features that separate the strains currently included in this group from other members of the genus *Methylobacterium* (Jourand et al. 2004). A new microaerobic N_2-fixing bacterium was isolated from roots and stems. In view of the distinct characteristics identified within either Frateuria, Gluconobacter, Acetobacter or any known N_2-fixing bacterium a new genus and species are proposed *Saccharobacter nitrocaptans (*Cavalcante and Dobereiner, 1988).

Bacteria with animals

Nitrogen-fixing symbiosis in animal systems is only advantageous in specialised ecological niches in which wood is the sole dietary intake. In the case of shipworms, the symbiosis has many of the advanced features associated with nitrogen fixing root nodules (Sprent and Raven, 1985). Some *Tetraponera* ants (Formicidae, Pseudomyrmecinae) subsist almost entirely on amino acid deficient honeydew secretions of pseudococcids and harbour a dense aggregation of bacterial symbionts in a unique pouch–shaped organ at the junction of the midgut and the intestine. The organ is surrounded by a network of intruding tracheae and Malpighian tubules, suggesting that these bacteria are involved in the oxidative recycling of nitrogen–rich metabolic waste. We have examined the ultrastructure of these bacteria and have amplified, cloned and sequenced ribosomal RNA–encoding genes, showing that the ant pouch contains a series of close relatives of Flavobacteria and *Rhizobium*, *Methylobacterium*, *Burkholderia* and *Pseudomonas* nitrogen–fixing root–nodule bacteria. We argue that pouch bacteria have been repeatedly 'domesticated' by the ants as nitrogen–recycling endosymbionts. This ant–associated community of mutualists is, to our knowledge, the first finding of symbionts related to root–nodule bacteria in animals (Borm et al 2002).

Life on earth evolves in soup like material most probably the liquid media in which all the nutrient dissolved. First living organism "progenote" evolved in such medium, it had to digest the readymade nutrient. However, most of nitrogen was present in combined form so its availability was less. Gradually earth become cool and combined nitrogen trapped in rock. Along with that rock microbe "progenote" changes its form from non photosynthetic to photosynthetic and utilize nitrogen for their need. Although some microbe retains the same quality only change its behaviour as anerobic, aerobic, facultative aerobic etc. They still fix nitrogen but their method is different from Cyanobacteria in case of temporal variation. The most important enzyme for nitrogen fixation retain same in all the nitrogen fixer.

Nitrogenase, which catalyzes the ATP-dependent reduction of dinitrogen (N_2) to ammonia (NH_3), accounts for roughly half of the bioavailable nitrogen supporting extant life. The fundamental requirement for fixed forms of nitrogen for life on Earth, both at present and in the past, has led to broad and significant interest in the origin and evolution of biological N_2fixation. One key question is whether the limited availability of fixed nitrogen was a factor in life's origin or whether there were ample sources of fixed nitrogen produced by abiotic processes or delivered through the weathering of bolide impact materials to support this early life. If the latter, the key questions become what were the characteristics of the environment that precipitated the evolution of this oxygen sensitive process, when did this occur, and how was its subsequent evolutionary history impacted by the advent of oxygenic photosynthesis and the rise of oxygen in the Earth's biosphere. Since the availability of fixed sources of nitrogen capable of supporting early life is difficult to glean from the geologic record, there are limited means to get direct insights into these questions. Indirect insights, however, can be gained through phylogenetic studies of nitrogenase structural gene products and additional gene products involved in the biosynthesis of the complex metal-containing prosthetic groups associated with this enzyme complex. Insights gained from such studies, as reviewed herein, challenge traditional models for the evolution of biological nitrogen fixation and provide the basis for the development of new conceptual models that explain the stepwise evolution of this highly complex life sustaining process. Nitrogen fixation (*nif*) genes were spread by lateral gene transfer relatively late in evolution. A report was established for this hypothesis. The distance between fast growing rhizobia and slow growing rhizobia was which state that nif genes may have evolved to a large degree in a similar fashion as the bacteria which carry them.

Cyanobacteria have played a significant role in Earth history as primary producers and the ultimate source of atmospheric oxygen. To date, however, how and when the group diversified has remained unclear. For evolution of Cyanobacteria there is oxygenic environment evolution and for nitrogen fixation there is compartmentization is necessary. We can say that nitrogen fixation process evolve first than oxygenic environment. Geochemical evidence suggests that oxygen first reached levels that would compromise nitrogen fixation (and hence select for heterocyst differentiation). Integrating phylogenetic analyses and geological data, we suggest that the clade of cyanobacteria marked by cell

differentiation diverged once between 3.6 and 3.4 billion year ago. These microbes conduct photosynthesis: using sun light, water and carbon dioxide to produce carbohydrates andoxygen. In fact, all the plants on Earth incorporate symbiotic cyanobacteria (known as chloroplasts) to do their photosynthesis for them down to this day. For some untold eons prior to the evolution of these cyanobacteria, during the Archean eon, more primitive microbes lived the real old-fashioned way: anaerobically. These ancient organisms and their "extremophile" descendants today thrived in the absence of oxygen, relying on sulfate for their energy needs.

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