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A REVIEW

Morphological study of nitrogen fixers

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Abstract : Biological diversity, the common term of biodiversity, in the simplest word means the variation and variety of life forms. The biodiversity being the amalgamation of dual word *i.e.* 'Bios' meaning "Life" and 'Diversitas' meaning "Variety" or "Difference". Nitrogen fixation on one hand and Photosynthesis on the other hand is the foundation of all the living system in this planet. Nitrogen fixation can be defined as a chemical process involving the conversion of atmospheric nitrogen into the nitrogenous compound, either biologically or physically. Biological Nitrogen Fixation (BNF) contributes about 90% of the process and rest 10% is carried out by the physical activities which include lightening, thunder, etc. Each and every year, approximately 17.2x10^7 tons of nitrogen are biologically fixed worldwide. The main contributors in BNF are the microorganisms which falls under two categories i.e. may be aerobic or anaerobic. Majority of the microbes do not have the potentiality to reduce nitrogen. The microorganisms having the potentiality of reducing nitrogen play a vital role in the nitrogen fixation along with nitrogen cycle in the nature. The microbes involved in BNF are usually the prokaryotes which make use of a biocatalyst or enzyme nitrogenise to bring about the catalysis of atmospheric nitrogen (N_2) to form ammonia (NH_3). These prokaryotes consists of aquatic organisms like blue green algae, free living nitrogen fixers like *Azotobacter*, *Clostridium*, symbiotic nitrogen fixers like *Rhizobium* and associative symbiotic includes *Azospirillium*. The current review paper delineates with reference to the morphological study of nitrogen fixers.

Key Words : Aerobic, Anaerobic, *Azotobacter*, Biological nitrogen fixation, Morphology, Nitrogenase, Nitrogen fixation, Prokaryotes, *Rhizobium*

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INTRODUCTION

Nitrogen fixers or the bacteria employed in nitrogen fixation process are broadly dispersed in Mother Creation, the place where the nitrogen fixers carry out their action of reducing atmospheric nitrogen in soil or in interrelation with the plants (Onyeze Rosemary *et al.*, 2013). They have been established in a vast variety of terrene as well as aquatic habitat in both the tropical and temperate zone of the world. Certain microbes possess the potentiality to utilise the reusable activation energy to amend the nitrogen gas present in the atmosphere (about78% of the atmospheric air) under favourable compassionate circumstances such as favourable environmental factors like heat, humidity and pressure

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(Qi Cheng, 2008). The process of fixation of nitrogen is an essential operation in which the molecular N_2 is converted to NH_3, that is a form of nitrogen gas that is extensively utilised by the biological system for the production of different biologically organic products (Earl and Ausubel, 1982). The nitrogen obtained biologically through the use of aerobic and anaerobic microorganisms could be instantly assimilated and absorbed by the plants and retain the surrounding completely untouched.

In agroecology and agroecosystem, Nitrogen in the atmosphere is one of the crucial nutrients hindering the extension, reproduction, expansion and maturing of plants. There is a high demand for nitrogen in the agricultural field and to fulfil the increased ultimatum of this macronutrient in farming, synthetic and chemical fertilizers have been applied heavily in the latter part of the 20^{th} century, which have open the way for several environmental challenges and disorders such as nitrate pollution, which shows the dangerous impact on the soil (Kishan Mahumud *et al.*, 2020). The process of Nitrogen Fixation carried out by biologically active microorganisms in flora is a necessary appliance for endurable and supportable crop productivity generation and diseased free ecological system development. Biologic N_2 Assimilation by the leguminosae family as well as asymbiotic, symbiotic, associative symbiotic, the mutualistic relationship in which the symbiotic microorganism lives within the body of the other organism called endosymbiotic and the mutualistic relation endophytic nitrogen assimilation in non-leguminous plants contributor vital part in minimising the application of artifical nitrogen fertilizer in crop fields which is directly proportional to the increase in plant nutrient content and health of soil (Kishan Mahmud *et al.*, 2020). More than fifty percent of the nitrogen based fertiliser applied to the crop field is utilised by the flora and the rest are exposed to wastes such as spring flow, stream flow and percolation and penetration resulting in nitrate contamination of the soil and groundwater (Jose *et al.*, 1978). However, based on energy efficiency, production of nitrogen based fertilisers need sir times more energy than that required to manufacture phosphorus and potassium based fertilisers (Kishan Mahmud *et al.*, 2020).

The thorough fare for the biotic degradation of stationary nitrogen (N_2) into the nitrogenous form, ammonia (NH_3) under microbial aerobic, in presence of oxygen circumstances is given below:

$$N_2 + 8H + 8e- + 16Mg\text{-}ATP = 2NH_3 + H_2 + 16Mg\text{-}ADP + 16P$$

Asymbiotic diazotrophs harmonizing to a minor portion of the flora microbe storehouse ecological community and these are included in a class of proteobacteria (Diazotropic bacteria like rhizobium, Bradyrhizobium and Rhodobacter), Betaproteobacter (Nitrosospira), Gammaproteobacter (Pseudomonadaceae family, Xanthomonas), firmicuts and Blue Green Algae (Jeffrey Morris *et al.*, 2018). Although, their existence, habit in addition to significance may be described to be the "Black Queen" supposition which prognosticates that in asymbiotic microorganism population, hardly any helpers, that have the massive weight in relation to function, like high energy requirement for nitrogen fixation, hold up the rest of the greenery and wildlife communities or the 'inheritors' that depend on the 'co-workers' or the 'fellow workers' for nitrogen requirements (Alvaro Peix *et al.*, 2014).

The mutual cooperative association linking soil microbes, popularly called as diazotrophic bacteria rhizobia, (involving the genera Rhizobium, Bradyrhizobium, Mesorhizobium and Sinorhizobium) and radial system of leguminous plants produce nodules that have the vital role in fixing the meterological nitrogen by the activity of the biocatalyst nitrogenise (Peter *et al.*, 2003). Biological Nitrogen Assimilation by the mutual association between rhizobia and plant is umpired by internally associated symbiotic interrelation when trees evolve radial clusters commonly named as root nodules; in leguminous plants and diazotrophic bacteria rhizobium, gram negative alphaproteobacteria are the popularly involved microorganisms that interact with leguminous plants of the leguminosae family. Actinomycetes like *Parasponia* species and *Frankia* species that forms a mutual symbiotic relationship with the angiospemic groups featured by the potentiality of forming symbiotic association with actnobacteria are also involved in the nitrogen assimilation (Carole Santi *et al.*, 2013). Symbiotic Associative nitrogen assimilation and/or endosymbionts are often seen in the company of diazotrophic bacteria like *Azospirillium* species, *Azocarpus* Species etc., with a massive diversity of flora rootstock including grains. The enzyme nitrogenase is highly sensitive to the oxygen. For this characteristic of enzyme nitrogenase, restrict anaerobiotics such as *Clostridium pasteurianum* are the best suitable candidate to carry out fixation of nitrogen; however elective anaerobiotics such as *Klebsiella oxytoca* can fix the nitrogen in absence of oxygen (Yates and Jones, 1974). Obligate aerobiotics requiring oxygen for their growth such as *Azotobacter vinelandii* can also cover the enzyme nitrogenase from O_2 in addition to proceeds with N_2 assimilation process by absorbing O_2 via cytochrome oxidase (Robert and Susane, 1997).

Morphological characters of nitrogen fixers:

The organisms involved in nitrogen fixation are prokaryotes *i.e.* the single celled microorganisms devoid of distinct nucleus, nuclear membrane and any membrane bound organelle (Akkermans and Houwers, 1983). The prokaryotes involved in nitrogen fixation comprise both asymbiotic and symbiotic microorganisms. C-autotrophic (Cyanobacteria), phototropic bacteria and C-heterotropic bacteria comes under the category of free living nitrogen fixers. While Rhizobia, Frankia and cyanobacteria (Anabeana and Nostoc) are the three taxa coming under the symbiotic nitrogen fixers, living in symbiosis with higher plants.

Mostly the asymbiotic, C-heterotropic nitrogen fixers are gram negative, rod shaped bacteria. Not all species in all genera are able to reduce the atmospheric nitrogen, with the exception to genera *Azotobacter* in which all the species have the potentiality to fix atmospheric nitrogen (Graham and Vance, 2000). In the genus *Clostridium*, neither the pathogenic ones nor the proteolytic ones are able to carry out nitrogen fixation, but this task can be carried out by saccharolytic clostridia. *Thiobacillus ferro-oxydans*, under the genus *Thiobacillus* can carry out the process of nitrogen fixation. Within the gram negative, non spore forming bacteria *i.e.* Enterobacteria, *Escherichia coli*, *Erwinia herbicola* and *Enterobacter aerogenes* are the few strains which are involved in the nitrogen fixation process (Dreyfus *et al.*, 1988). All associates of heterocystous cyanobacteria e.g. *Anabeana* and *Nostoc* are able to fix nitrogen. On the other hand only a few strains e.g. *Oscillatoria* of non-heterocystous cyanobacteria can fix atmospheric nitrogen (Stephen, 2011).

Azospirillium:

Members of facultative endophytic diazotroph group which establish their colony on the surface and in the internal portion of the radical is regarded as the initial point of BNF with the non-leguminous crops. *Azospirillium* are the gram negative, devoid of spore formation and rod shaped bacteria. They have their motility organ in form of flagella which is mostly single and rarely many. The favourable temperature and pH for their growth falls under 5 degree Celsius and 42 degree Celsius and 5 to 9, respectively, with optimum growth occurring around 3o degree Celsius and 7pH. *Azospirillium* is beneficial to the plants in terms of the fact that they improve the plumule and radical expansion and raise the speed of aqua and nutrient absorption by the radical system (Gonzalez *et al.*, 2005).

Azotobacter:

Azotobacter is a free living nitrogen fixer which requires the oxygen for its growth (*i.e.* obligate aerobe) (Shridhar, 2012). Cells of azotobacter ranges from 2-4 micrometer in diameter, usually oval sometimes rod shaped to spherical shape. The *Azotobacter* colony shape and size can be regarded as flat on the top, domed, conical, regular and sometimes irregular. Colour varies from white to milky white (Shridhar, 2012).

Rhizobia :

Rhizobium is the popular symbiotic nitrogen fixer that forms the mutual symbiosis with the legumes by the production and establishment of the rhizome nodule, which is the site of nitrogen fixation (Galal, 1997). *Rhizobium* do not retain the crystal violet stain on staining procedure *i.e.,* it is gram negative, bacilliform bacteria, with the size ranging from 0.5-0.9 x 1.2-3.0 micrometer. Colony is spherical, bulging, partially translucent, raised and glutinous. Movement occurs by one polar or mid polar locomotory organ or 2 to 6 multiple flagella projecting in all directions (Jogree *et al.*, 1999).

Advantageous association between microbes and flora:

Agronomical enhancer is a term which refers to the biotic or abiotic agents which decreases the period of development, enhances the yield and/or standard of the agricultural generates along with the period of flowering and fruiting. The involvement of the microbial population as enhancers is due to the presence of enzyme are their role. Taking the example of ability of some of the prokaryotes to fix the atmospheric nitrogen. During the development, the flora interacts closely with the soil microbes. The microbial populations beneficial to the plants belong to three groups as: (a) Microbial populations that can enhance the supplementation of macro and micronutrients needed for the expansion and extension of plants. (b) Microbes that provoke the development of the flora at an unintended form through the repression of the pathogens. (c) Microbes that manage the biotic growth and development of the plants, by plant hormones.

Ecological and environmental problems:

Industrialization and urbanization is the major factor in making the imbalancement in the nitrogen and phosphorus regulation as a result of huge utilization of synthetic fertilizer. Huge utilization of fertilizers, pesticides, fungicides, weedicides etc has deteriorated the quality of ecosystem. N_2 is regarded to be the most crucial macronutrient in rice generation. One of the greatest demerits of extreme utilization of synthetic fertilizers is the environmental pollution on one hand and increase in pollutant concentration in the ecosystem on the other hand. Nitrogen manure not utilized by the poaceae may be the major contributors of environmental pollutants. Out the total nitrogen assimilation, some fractions are waste through percolation and denitrification process. The enhancement in the application of nitrogen fertilizer is directly proportional to the leaching nitrate concentration which acts as the major factor for groundwater pollution. To prevent the N_2 losses in rice fields, eyes on nitrogen fixation by biological microorganisms are increasing (Hurst *et al.*, 2000).

Regulation of flora-soil-microorganism symbiosis has been experimented as an outstanding plan to enhance the regulation of nutrients, thus, resulting in more utilization of the land wealth by decreasing the utilization of synthetic fertilizers. The use of biological fertilizer is a substitute to enhance the quality of the fields and as it is devoid of contamination, aids in producing healthy products.

Ecosystem impacts and ecological controls :

– The process of nitrogen fixation is extremely energy concentrated.

– Nitrogenase enzyme is sensitive to oxygen. The biological organisms manage an intricate stability allying the efficacy of utilization of oxygen as an acceptance of electron and deactivation of enzyme nitrogenase.

– Majority of the nitrogenase enzyme require molybdenum as the cofactor.

– In most the organisms involved in nitrogen fixation, the synthesis or the functioning of the nitrogenase is hindered by huge amount of integrated N_2.

– The nitrogen fixing microorganisms are affluent in N_2 than the organisms which do not fix nitrogen.

Applications of nitrogen fixers:

Azotobacter :

The presence of Azotobacter species in soils has good and fruitful outcomes on flora, but the plethora of these microorganisms is corelated to numerous features, topsoil physico-chemical (e.g. bio-organic matter, potential of hydrogen, heat, soil moisture) and bacteriological assets (Kizikaya, 2009). Being profitable and ecological friendly, biological fertilizers can be aided in crop generation for better yield (Nagananda *et al.,* 2010). Seed dressing with bacteria helps to enhance flora development and to improve topsoil N_2 through N_2 assimilation by employing carbon for its consumption (Monib *et al.*, 1979). The *Azotobacter* injected harvest show better expansion rate. In the course of rosette phase of canola the crop growth rate (CGR) found maximum *i.e.* ten-twelve per cent increment (Yasari and Patwardhan, 2007). Biochemical examination of green pigment, macro nutrients and protein content were found to be maximum in *Azotobacter* injected plants as compared to non-inoculated control flora (Lopez *et al.*, 2005).

Rhizobium:

For plant - microbe interlinkages, the plant rhizosphere is a major soil ecological environment which requires annexation from variety of microbes in the circumference the root that may outcome in symbiotic, associative links inside the plants which depends on variety of microbes, soil environment, soil nutrient level etc. (Verma *et al.*, 2010). N_2 fixation process is related to anatomical state of the plant in *Rhizobium* legume symbiosis.

Azosperillum:

For the use in sustainable agriculture *Azosperillum* belongs to soil microbes. In the Plant Growth Promoting Rhizobacteria (PGPR) these are gram-negative free-living bacteria (Okon, 1994; Okon and Vanderleyden, 1997 and Zawoznika and Groppa, 2019). The most primitive report is the increasing number of studies of *Azosperilllum* has the capacity of nitrogen fixation that imply increase in growth.

Conclusion:

We found remarkable morphological differences among the three cell types and genera analysed. The process of N_2 fixation is essential which convert N_2 into NH_3 which is a form of N_2 that is utilized for the production of different bio- organic product by living system. N_2 fixing bacteria of the genera are present in the soil such as *Azosperillum*, *Azotobacter*, *Rhizobium*. For the soil sample by using nitrogen free media these organisms were isolated. On the basis of morphological and biological properties the species were isolated and characterized in this work. Various carbohydrate was used by these organisms such as galactose, mannitols etc.

Future work :

Selection of strain is the most vital work because an structured strain of the nitrogen fixers plays an important role in biofertiliser generation same as the seeds important in crop development. To search out substitute is to employ the strains from authentic fount in addition to all scientific stipulations for huge quantity generation of N_2 fixing microbes.

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