

RESEARCH PAPER

# Deciphering soil diazotrophic diversity in the wheat-maize cropping system of Punjab using morphological, biochemical and molecular techniques

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Soil is the critical resource as well as a basic medium for the growth of natural micro flora present in it. The soil fertility depends on its physicochemical properties and microbial population diversity. Diazotrophs are the nitrogen fixing bacteria which possess *nifH* gene that is responsible for coding the nitrogenase enzyme involved in the reduction of atmospheric nitrogen to ammonia. These nitrogen fixing bacteria play an imperative role, function and significance in the soil. Soil samples were collected from the different wheat-maize cropping system of Punjab and analyzed for physicochemical properties as pH, electrical conductivity, organic carbon, soil texture, ammoniacal as well as nitrate nitrogen. Eighty diazotrophic bacteria were isolated on eight different nitrogen free media and characterized culturally, morphologically, biochemically, functionally and using molecular techniques. The diazotrophic nature of the isolates was confirmed by the amplification of *nifH* gene using two *nifH* primers viz. *nifH1* and *nifH2*. The *nifH* positive isolates were further used for 16S rDNA restriction analysis using different enzymes such as *Taq*I, *Rsa* I and *Hae* III. Based on UPGMA clustering, the representative strains were sequenced and identified as *Bacillus amyloliquifaciens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Paenibacillus* sp., *Azotobacter vinelandii*, *Stenotrophomonas maltophilia*, *Rhizobium larrymorrei*, *Flavobacterium anhuiense*, *Sphingomonas paucimobilis*, *Paenibacillus panacisoli*, *Azospirillum* sp., *Pseudomonas putida*, *Paenibacillus amylophilus*, *Bacillus circulans*, *Paenibacillus polymyxa* and *Xanthomonas oryzae*.

**Key words :** Diazotrophs, *nifH*, 16S rDNA sequencing, Physico-chemical properties, Soil

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