

Nitrogen fixing bacterial populations associated with biological soil crusts and reclaimed precipitates from an acid mine drainage site and their potential for mine land reclamation
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Very few studies have described the presence of *nifH* genes in degraded mine lands, and the use of nitrogen fixing microorganisms such as cyanobacteria in mine land reclamation has received little consideration especially in the Philippines. In a collaborative work between the University of the Philippines Baguio and The Pennsylvania State University, we aimed to analyze the unculturable diazotrophic bacterial community of bryophyte dominated biological crusts and reclaimed mine lands from an acid mine drainage (AMD) site in Pennsylvania, USA. Amplification of *nifH* genes using a degenerate primer was used to assess the diazotrophic community from samples collected from the acidic precipitates (pH 2.5-2.7) rich in Fe(III)oxy(hydr)oxide and were compared to untreated control areas. BLAST and phylogenetic analyses of *nifH* cloned sequences revealed that they belong to the Proteobacteria group, (94%), Cyanobacteria (2%) Actinobacteria (2%) and 2% did not match any cloned sequences in the database. The majority of the samples, both from reclaimed and control biological crusts, contained sequences that were similar to a methanotrophic bacteria, *Methylocystis bryophila*, which was originally described to be isolated from an acidic sphagnum peat at pH 4.3. Symbiotic nitrogen fixers *Bradyrhizopium* sp, and *Bradyrhizobium japonicum* were also found in both samples which were also reported to be tolerant of acidic soils. Cloned sequences with similarities to *Methyloferulla stellata*, *Halorhodospira halophila* and *Methylocella tundrae* were found only in the amended or reclaimed precipitates, while cloned sequences with similarities to *Nostoc punctiforme*, *Streptomyces*, *Mesorhizobium shangrilense* and *Desulfonatronum thioautotrophicum* were found only in the biological crusts. Presence of these nitrogen fixing bacterial strains assures the capability of nitrogen fixation in the biological crusts and rhizosphere of plants in the reclaimed mine lands that maybe explored in future studies as plant growth promoters in these reclaimed mine lands. This work paves the way to investigate the application of nitrogen fixers like cyanobacteria as well as methanotrophs to mine lands in the Philippines as a means to enrich soils with fixed nitrogen, protect newly forming soils from erosion, and condition soils for seed germination.

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(3) Rojas et al. (2016) Appl. Soil Ecol. 105 (2016) 57–66.

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This work was funded by the Fulbright-Philippine Agriculture Scholarship Program (FPASP), The University of the Philippines Baguio and The Pennsylvania State University.