Isolation of Denitrifying Bacteria and Cyanobacteria and their Potential Use in Nitrate Removal of Well Water in Jaffna District

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ABSTRACT

Nitrate pollution of groundwater in the Jaffna Peninsula and its potential health hazards to the local population has been reported long past. This study aimed to identify bacterial and cyanobacterial strains suitable for bioremediation of nitrate contaminated water. Bacterial strains were isolated from various soil and water samples in the study area. Primary screening was carried out on Bromothymol blue (BTB) agar plate supplemented with KNO_3. Assimilatory and dissimilatory nitrate reduction test was performed with Nessler's reagent and nitrite reagent (sulfanilic acid and α-naphthylamine) respectively, in nutrient broth with KNO_3. Quantitative screening was carried out in same nutrient broth with KNO_3 and $NaNO_2$ substrate separately. Suitability of carbon sources on bacterial nitrate removal was investigated using mineral salt medium containing either glucose or starch as the carbon sources and KNO_3 as the substrate. Carbon sources were optimized at three levels (0.25, 0.5 and 1.0 %) for efficient nitrate removal. Liberated gases during nitrate reduction were analyzed using Shimadzu GC-9 AM gas chromatography. Selected strains were identified by 16S rRNA homology analysis. Moreover, 28 cyanobacterial strains were also isolated using BG 11 medium and screened for nitrate reduction in synthetic medium. Ten well water samples having NO_3^--N concentration within the range of 15 mg/L to 57.37 mg/L were selected for treatment with selected strains. Treatment means were compared using Duncan's multiple-range test at a significance level of 0.05. Out of 128 morphologically different bacterial strains 70 strains were identified as nitrate reducers. Among them, 38 strains were identified as dissimilatory nitrate reducers, out of which 21 strains showed more than 50% reduction of either nitrate or nitrite on nutrient broth with KNO_3 and $NaNO_2$ substrates respectively. Out of which, five strains showed greater nitrate reduction (above 70 %) either with glucose or starch. Among two carbon sources 0.5 % starch was found to be the best carbon source for efficient nitrate reduction with most of bacterial strains tested. The five strains were identified as *Paracoccuspantotrophus, Paenibacilluspolymyxa, Enterococcus casseliflavus, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Two non-pathogenic strains namely, *Paracoccuspantotrophus* and *Paenibacilluspolymyxa*were used for water treatment. Five efficient nitrate reducing cyanobacterial strains were selected and tested with one water sample having 57.37 mg/L NO_3^--N. Cyanobacterial strains were identified as *Limnothrixredekei* (T8), *Alkalinemapantanalense* (T11), *Filamentous cyanobacterium* (T15), *Limnothrixplanktonica* (T20) and *Geitlerinema* sp. Both bacterial strains reduced NO_3^--N below safe level within72 hours of treatment. Nitrate reduction percentage of strains *Paracoccuspantotrophus* and *Paenibacilluspolymyxa* were within the range of (59.63 – 100 %) and (86.67-100 %) respectively in different well water samples. However, co-culturing of these two strains was not successful in this study. Gas chromatography results indicated, the strains liberated higher percentage of N_2 (68 - 90%) gas compared to other gases (N_2O (5-13%) and trace of CO_2) while reducing nitrate. All tested cyanobacterial strains also reduced the nitrate below safe level at 14th day of culturing. Thus, the selected bacterial and cyanobacterial strains could be potentially used for removal of nitrate from water.

Keywords: Nitrate pollution, Denitrification, Bioremediation, Bacteria, Cyanobacteria

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