Possible Sources of Assimilatory and Dissimilatory Nitrate Reducing Bacteria in Jaffna Peninsula, Sri Lanka

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Abstract
Nitrate contamination in the ground water is a common problem worldwide including Jaffna peninsula, Sri Lanka. Such contamination of drinking water is regulated by World Health Organization all around the world (11mg/L NO_3^--N) due to the health effects of over consumption of nitrate. Microbial denitrification is an environmentally sound and economically feasible method for remediation of nitrate contaminated water. The aim of the present study was to isolate nitrate reducing bacteria from different environmental samples such as pond sediments, paddy soil, manures and different wastes in Jaffna district. Spread plate and streak plate methods were used to isolate the strains. Around 90 morphologically different bacterial strains were isolated from environmental samples tested. Initial screening was carried out on BTB (Bromothymol Blue) agar plates with nitrate. Among them 70 strains were selected as nitrate reducers from the BTB agar plates. Among the nitrate reducers 38 strains were selected as dissimilatory nitrate reducers and rest of them had assimilatory nitrate reduction characteristic. Since most of the nitrate reducers are facultative heterotrophs, the highest number of strains were isolated from submerged condition namely paddy water and paddy soil. Therefore, submerged condition of paddy land could be the possible source for isolation of more number of assimilatory and dissimilatory nitrate reducing bacteria.

Keywords: Assimilatory, Dissimilatory, Heterotrophs, Nitrate contamination, Submerged condition
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Introduction

Nitrate contamination is one of the major upcoming issues of groundwater worldwide including Sri Lanka. The World Health Organization has set a limit of 11 mg/L NO_3^--N for human consumption. Consumption of nitrates can have several detrimental health effects, like methemoglobinemia (WHO, 2004). Another adverse effect of nitrate consumption in drinking water is the increased risk of certain types of cancer. Several studies have shown positive correlations between nitrate ingestion and gastric, esophageal, and stomach cancer (Ward, 2005). Jaffna district of Sri Lanka is reported to have very high levels of nitrate (Navaratnarajah, 1994). The water samples in wells where there is agricultural activities reported to have NO_3^--N levels between 20 to 50 ppm (Jeyaruba and Thushyanthy, 2013). The agricultural villages of Kondavil and Urumpiraihave very high nitrate - nitrogen levels of 30 ppm (Navaratnarajah, 1994). As it is the major natural water resource in the Jaffna Peninsula, Sri Lanka, population is entirely dependent on the groundwater resources for almost all the purposes (Jeyaruba and Thushyanthy, 2013).Though nitrate contamination of ground water has been reported in Sri Lanka, there has been little research reported on remediation of such pollution except few phytoremediation studies (Sundaralingam and Gnanavelrajah, 2014). Although the methods such as ion exchange, reverse osmosis, electrodialysis, photocatalyic reduction and adsorption have been developed for nitrate removal from water, the utility of these processes has been limited due to their expensive operation and subsequent disposal of generated waste brain. Biological denitrification is an emerging technology for drinking water treatment. In biological denitrification process, microorganisms first reduce nitrates to nitrites and then produce nitric oxide, nitrous oxide and nitrogen gas (dissimilatory nitrate reduction). Few microbes are able to reduce nitrate to ammonium (assimilatory nitrate reduction). Since most of the nitrate reducing bacteria are heterotrophs, source of organic carbon is an important component of the denitrification process. For the removal of nitrate from water, it is essential to identify potential microorganisms which could perform denitrification. Hence, the objective of this study was to isolate potential nitrate reducing bacteria from different possible sources in the Jaffna Peninsula.

Materials and Methods

Sample collection, Isolation and cultivation

Environmental samples for isolation of nitrate reducing bacteria were collected from municipal compost, municipal solid waste dumping place, pond soil, swine and poultry manure, paddy soil and Paddy water, compost and unutilized well water of Jaffna District. Spread plate and streak

plate techniques were used to get isolated colonies. Cultures were cultivated on medium described by Takaya et al., (2003) with Glucose as sole carbon source. Plate assay with KNO_3 and the pH indicator BTB was developed to isolate bacterial strains that reduce nitrate under aerobic conditions. The pH of the medium was initially adjusted to between 7.0. Plates inoculated with a bacterial strains were incubated for few days at the 37°C temperature, and then bacterial NO_3^- consumption was monitored by examining blue colonies due to the increasing pH of the medium. Positive strains obtained from this initial screening analysis were screened further.

Screening of Dissimilatory Nitrate Reducing Bacteria

Potential for nitrate reduction of the bacterial species were evaluated by using nitrate reduction broth containing (g/L): beef extract 3, yeast extract 3, peptone 5, sodium chloride 5, potassium nitrate 5, and pH 7. Loop full of bacterial isolates were inoculated and incubated at 37°C for 2 days. After incubation, the nitrite reduction was examined by the addition of 0.5mL of nitrite test solutions A (sulfanilic acid) and B (N, N-dimethyl-a-naphthylamine) respectively. The appearance of purple color in broth after 2min revealed that nitrate had been reduced to nitrite. In the absence of purple color, further testing was needed to detect nitrate reduction. A small amount of zinc dust was added and shaken vigorously. The tubes were allowed to stand for 10 to 15min. The colorless medium indicates positive (i.e., the nitrate was used up and transformed to gaseous compounds) and the pink color changes indicate negative reaction.

Screening of Aissimilatory Nitrate Reducing Bacteria

The Aissimilatory nitrate reduction of the bacterial isolates was evaluated by using nitrate reduction broth. Loop full of isolates were inoculated under aseptic condition and incubated at 37°C for 24hr. After incubation, the reduction of nitrate to ammonia was tested by the addition of 0.5mL of Nessler's reagent (Tiedje, 1994). The appearance of reddish orange color showed that nitrate had been reduced to ammonia.

Results and Discussion

The plate assay method is based on the changes in the pH of a medium due to NO_3^- depletion by denitrification. Altogether 70 bacterial strains were isolated as nitrate reducers from different sources. Number of nitrate reducers isolated from different sources are summarized in Table1.

Table 1: Nitrate reducers isolated from different sources in Jaffna Peninsula

Sources of isolation	Number of nitrate reducers	Dissimilatory reducers	Assimilatory reducers
Municipal compost	08	07	01
Pond soil	01	-	01
Fish waste	01	-	01
Municipal solid waste dumping place	04	03	01
Manure	07	05	02
Paddy soil	15	10	05
Paddy water	15	06	09
Unutilized well water	11	03	08
Compost	08	04	04
Total	70	38	32

The bacterial species reduced the nitrate effectively through either assimilatory or dissimilatory pathways. In the present study, the screening of nitrate-reducing bacterial species was undergone for both assimilatory and dissimilatory methods according to the intermediate production and emission of gaseous molecules. Out of 70 bacterial isolates, nearly 38 isolates were able to convert nitrate to nitrite form and among them 21 strains were formed gas bubbles in the Durham's tube (due to denitrification). In case of strains variability with different sources; pond soil and fish meal had only 1 strain because of less nitrate content compared to paddy soil. In contrast, paddy soils are fertilized with nitrogen fertilizers and kept under submerged condition, hence they recorded more number of denitrifiers.

Conclusion

This study screened the potential heterotrophic bacterial denitrifiers from the different sources in Jaffna district. Along with that, the potential denitrifier was also able to convert the nitrate into nitrogen gas through the dissimilatory test and also the nitrate could be rehabilitated to nitrite formation. Then the transformation of ammonia was done by the assimilatory reduction test. Hence the results of this study illustrated that the nitrate-reducing organisms could be present in natural environment and through the assimilatory and dissimilatory

reduction techniques that can be identified. Altogether 70 nitrate reducing strains were isolated. Highest number of strains were isolated from paddy water and paddy soil. Least number of strains were isolated from pond soil and fish waste. The isolated stains could be used to remediate nitrate polluted water if appropriate conditions are provided.

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References

JEYARUBA, T. & THUSHYANTHY, M. 2013. Health hazardous: nitrate-N in groundwater and soil in intensified agricultural areas.

NAVARATNARAJAH, V. 1994, Water problems in the Jaffna peninsula. 20th WEDC Conference in Affordable Water Supply and Sanitation, 1994. 160-170.

World Health Organization (WHO. (2004). Guidelines for drinking-water quality (Vol. 1). World Health Organization.

SUNDARALINGAM, T. & GNANAVELRAJAH, N. 2014. Phytoremediation potential of selected plants for Nitrate and Phosphorus from ground water. International journal of phytoremediation, 16, 275-284.

TAKAYA, N., CATALAN-SAKAIRI, M. A. B., SAKAGUCHI, Y., KATO, I., ZHOU, Z. & SHOUN, H. 2003. Aerobic denitrifying bacteria that produce low levels of nitrous oxide. Applied and environmental microbiology, 69, 3152-3157.

TIEDJE, J. M. 1994. Denitrifiers. Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties, 245-267.

WARD, M. H. 2005. Workgroup report: drinking-water nitrate and health—recent findings and research needs. Environmental health perspectives, 113, 1607.