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Isolation and Identification of Heterotrophic Nitrifying Bacteria From Sewage Sludge

KEYWORDS: Heterotrophic, Nitrifying, Bacillus, Sludge, Waste water.

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ABSTRACT *Screening of heterotrophic nitrifying bacteria as an alternative source of biological removal of nitrogen compounds in polluted lake waters was carried by soil samples collected from the lake bed were subjected to primary and secondary enrichment were performed. Culture samples showing nitrate production were subjected to confirmatory test. Change of colour from pink to yellow and decrease in pH indicated the presence of nitrifying bacteria. The ammonia oxidizing bacteria isolated belonged to genus Bacillus sp. Isolation and characterization using 16s ribosomal RNA is in progress.*

Introduction

Release of sewage water containing fecal matter directly in to water stream results in increase in inorganic contents followed by oxidation, resulting in poor water quality (Velusamy and Krishnani 2013). Nitrification is a process of oxidation of ammonia (NH_3) in to nitrate (NO_3) which is a key component of nitrogen (N) cycle by nitrifying microorganisms (Lu *et al.* 2015). Removal of nitrogen elements from polluted water is one of the most important aspects in waste water treatment (Qiu *et al.*2012). Microorganisms removes nitrogen in two aspects: nitrification by autotrophs under aerobic condition and denitrification by heterotrophs under anaerobic condition (Chen and Ni 2011). There are several papers report on isolation of nitrifying bacteria (Lin *et al.* 2007; Sorokin *et al.* 2001; Elbanna *et al.*2012; Zhou *et al* 2014) which display nitrogen removal efficiency. Isolation and characterization of new nitrifying microorganisms is one of the most important step in this process (Koops and Roser 2001). Hence, in the present study, screening of heterotrophic nitrifying bacteria was carried out to isolate potent bacterial strain capable of removing ammonia from water. The resultant bacterial strain may provide alternate microbial resource for biological removal of nitrogen compounds from waste water.

Materials and methods

Soil sampling

Soil samples were collected from the beds of Kukkarahalli Lake located in Mysore city, Karnataka the soil samples were collected in air tight Polythene bag and brought to laboratory and kept in 4°C until further use.

Primary Enrichment

Isolation of Nitrifying bacteria was carried out according to procedure mentioned by Saratchandra,(1978). Soil Sludge sample collected from, Mysore, Karnataka., was used to isolate nitrifying bacteria. About 0.5 gm of fresh Soil was placed in a flask containing 100 ml of sterile growth medium to provide 0.5gm/L of Ammonium sulphate and 0.5 mg/L of Phenol red at pH 7.5-8.5. The flask was maintained at room temperature in a rotary incubator for 2-3 weeks until the colour of medium changed from pink to yellow, an indication of acidity from nitrite production. The pH was readjusted with 5% sodium carbonate solution until second colour change took place.

Secondary Enrichment

A sample (0.5ml) of the enrichment was serially diluted in tubes containing 4.5ml of growth medium to obtain dilutions up to 10^{-6} or 10^{-9}, two or three complete sets of dilution were prepared and incubated for 18-20 days at room temperature. The highest dilution showing a pH change was again serially diluted. This dilution procedure was repeated two or three times until the highest dilution tube showing a pH change containing nitrifying bacteria. Samples of 0.5 ml from the highest dilution tubes showing nitrite production were inoculated in to flasks containing 100 ml of this growth medium.

Confirmatory test

Confirmatory test was carried out according to Yan et al (2006). Pure isolates were obtained from the system by plating onto a peptone-meat extract (PM) agar; the composition was identical to the liquid medium with the addition of 2% agar. The composition of the PM liquid medium was as follows: peptone 10 g l^{-1}, beef extract 10 g l^{-1} and sodium chloride 5 g l^{-1} . The resulting isolates of bacteria were tested for their ability to produce nitrite or nitrate by inoculation into 10 ml sterile ammonium sulphate liquid medium. The composition of the medium was as follows: $(NH_4)_2SO_4$ 0.5 g l^{-1}, NaCl 0.3 g l^{-1} , KH_2PO_4 1.0 g l^{-1}, $MnSO_4 \bullet 7H_2O$ 0.3 g l^{-1}, $FeSO_4 \bullet 7H_2O$ 0.03 g l^{-1} , $CaCl_2 \bullet 2H_2O$ 0.5 g l^{-1}. Spot tests for total oxidized-N (nitrite and nitrate) were made on approximately 2ml medium using the Griess-Ilosvay method (Keeney and Nelson, 1982) every week. When the test proved positive for total oxidized-N, about 2 ml aliquot of the enrichment cultures was transferred to fresh medium. This procedure of transfer to fresh medium was repeated when the spot tests again proved positive

Results and Discussion

Isolation of Nitrifying Bacteria

0.5 gm of collected Soil (S1,S2 and S3) was inoculated into

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100ml of Ammonium medium at pH 7.0-8.0 in separate conical flasks. After two weeks of incubation the colour of the media was changed from Pink to Yellow and also decrease in pH was observed (Table-1), which indicates the possibility of presence of Nitrifying Bacteria.

SL NO.	pH (After two weeks)	pH (After three weeks)	Indication
Control	6.54	6.40	Negative
S1	7.45	6.03	Positive
S2	7.30	5.42	Positive
S3	7.08	4.64	Positive

Table-1: Initial pH and final pH of Ammonium oxidizing Media

After secondary enrichment in test tubes containing Ammonia media at initial pH-7, the change of colour was again observed from Pink to Yellow after two weeks in all three samples in all dilution (10^{-1} to 10^{-6}) .

Confirmatory test was done by inoculating the pure isolate obtained from peptone meat extract agar in to ammonium sulphate liquid medium. After two weeks, approximately 2ml of Griess-ilosvay reagent was added to the test tubes and the result observed was positive by change in colour (yellow to red) this indicates presence of Heterotrophic nitrifying bacteria

Identification of nitrifying bacteria

The Ammonia oxidizing bacteria isolated from Sandy clay soil of Kukkarahalli Lake, was gram positive, Coccobacillus and appeared in groups. Based on Bergey's manual of systematic bacteriology the isolated strain belonged to genus *Bacillus* sp. The results obtained from the primary and secondary enrichment cultures indicated the present heterotrophic nitrifying bacteria in sewage soil. The confirmatory test indicated the presence of pure isolates of ammonia oxidizing bacteria Coccobacillus in groups and the isolated strain was *Bacillus* strain. Characterization by 16s ribosomal RNA techniques in progress.

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