NAME AND DESIGNATION OF THE PRINCIPAL INVESTIGATOR

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TITLE OF THE PROJECT AND FILE NUMBER:

“Isolation and Characterisation of Phenol degrading anaerobic bacteria: with special reference to Methanogens and Sulphate reducers from coconut husk retting”

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EXECUTIVE SUMMARY OF THE PROJECT

The present study reveals the biodegradation potential of five methanogenic strains when subjected to different concentrations of phenol. The growth of the cultures can be related to the Percentage of phenol degradation. Maximum reduction of phenol was seen when the growth was maximum. This shows that the cultures were utilizing phenol as a carbon source or substrate for their growth and metabolism. Most of the phenol degradation studies done earlier were pertaining to aerobic microbial cultures.

Methanogenic strains were observed to show a degradation pattern upto 91%. The residual phenol concentration was estimated and biodegradation percentage of each culture was arrived. Biodegradation of phenol by bacterial isolates increased with time period. The maximum degradation was observed at 96h for almost all the cultures. The methaogenic biodegradability of phenol indicates the aromatic ring compounds are not refractory under strict anaerobic conditions and that reductive pathways in heterotrophic bacteria exist for its decomposition. The present investigation also reveals the biodegradation potential of five strains of SRB to degrade phenol at different concentrations. The phenol degradation potential of these anaerobic cultures was not much studied earlier.

The present study emphasizes on the development of methanogenic and sulphate reducing consortia capable of degrading phenolic compounds produced as intermediates of husk retting. For this, Samples were collected from kadinamkulam retting zones and subsequent enrichment was done. Isolated bacterial isolates both under aerobic and anaerobic conditions were found in the retting grounds. Characterization of aerobic strains was conducted in the first phase of the work. Hence analysis and characterization of the anaerobic strains isolated was carried out so that a comparison can be done between isolated strains.

Assessment of the growth pattern of isolated cultures and their minimum inhibitory concentration (MIC) of phenol was also carried out using their variability in protein content which implies whether these isolated strains can survive in polluted anaerobic conditions which is a common scenario of the land and water bodies. Hydrophobicity and
emulsification activity of all the isolated strains were carried out. From this best aerobic and anaerobic strains were compared to know their efficiencies with respect to phenol degradation.

Analysis of the extent of growth of SRB cultures in various concentrations of phenol was done. Then, estimated the residual phenol concentration to know the rate of phenol degradation after which the phenol degradation potential of SRB was evaluated. The above parameters helped in the easy comparison of the biodegradation potential of different strains of SRB. Finally, strong SRB strains in phenol degrading were identified by molecular method using amplification of 18S rRNA gene region. The selection was based on uniqueness in the colony and cell morphologies of the cultures. Only one culture (out of two or more) representing similar morphological properties were selected for sequence analysis of 16S rRNA gene, which is the gene of choice for identifying bacteria.

The sequencing results showed that these isolated SRB strains belonged to Desulphovibrio genus. Partial 16S rRNA gene sequencing and analysis of the fatty acid profile demonstrated a strong similarity between the new species and members from the Citrobacter species. This was confirmed by the results obtained following purification and characterisation of the key proteins involved in the sulphate-reduction pathway. However, the position of the strain within the phylogenetic tree clearly indicates that the bacterium is closely related to Citrobacter freundii, and these four strains (S1, S2, S3, S4) form a separate cluster in the delta subdivision of the Eubacteriaceae.

Bacteria of the genus Citrobacter dominated the isolates obtained, accounting for 71.8% of the total isolates examined. Additional bacterial groups and the percentage in which they were isolated included: Bacillus sp. (10.8%), unidentified curved rods (9.5%), gram-positive nonsporing rods (5.6%), and motile gram-negative rods (1.9%). Temperature growth studies demonstrated the ability of all the isolates to grow at in situ sediment temperatures. Isolate S5 was found to be positively belonging to Bacillus tequilensis by nucleotide homology and phylogenetic study conducted in this study. Just because many of the procedures like plating, purification, and extraction with organic solvents, as well as analytical techniques like high performance liquid chromatography, nuclear magnetic resonance, and mass spectrometry, are routinely used in screening for microorganisms are lengthy, laborious, and time-consuming, we in this present study, have used PCR as a quick approach for the screening and identification. As mentioned above, the 16S rRNA gene from over 20 DNA extracts of environmental isolates in aerobic conditions and over 10 DNA extracts of isolates in anaerobic condition from sediment samples were collected from the retting regions of Kadinamkulam was sequenced.

So, this study gave a hope that when these strains are added to industrial effluents, they can grow well utilizing the nutrients available in the effluent in the anaerobic conditions. At the same time they can reduce sulphate in addition to phenol, which is very effective and economic.

**PAPERS PUBLISHED**


Reshma,J.K, Anu Mathew and V. Salom Gnana Thanga, “Screening of Enzymatic activity in different Bacterial Isolates treated with phenol” in the proceedings of the ‘International Conference on emerging frontiers and challenges in Chemistry’ organised by Department of Chemistry, All Saints' College on 17th and 18th February 2014.


FUTURE PROSPECTS

- More unidentified strains have to be isolated having greater potential to degrade phenol
- New genes or pathways have to be identified leading to phenol degradation.
- Little is known of the identities of the key players in situ or the environmental factors that affect their activity and distribution.