iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** XI **Month of publication:** November 2019

DOI: http://doi.org/10.22214/ijraset.2019.11092

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue XI, Nov 2019- Available at www.ijraset.com

Molecular Characterization of Bacillus sps. having Ability to utilize Leachate

Rashmi P A[1], Neethu Thomas[2], Dayana Joseph[3], Loretta Alvarez[4]
[1,2,3,4] *Faculties of Department of Microbiology- Presentation College of Applied Sciences, Puthenvelikkara, Ernakulam , MG University., Kerela- 683594*

Abstract: ***Solid waste is an important environmental problem in both developing and developed countries. Landfill leachate is defined as an aqueous effluent produced when water percolates through the waste in a landfill. The nature of landfill leachate depends on the type of municipal solid waste being dumped, landfill age, moisture content, seasonal weather variations, site hydrology, the stage of decomposition in the landfill and pH. The present investigation was designed to isolate bacteria from garden soil and was identified by means of morphological, cultural and some biochemical characterizations. The proteolytic, lipolytic and amylolytic properties of the bacterial isolates were evaluated. Qualitative analysis of certain components of leachate, such as starch, lipid, and protein were carried out. This work was also undertaken to detect the molecular characterization of Bacillus sps. isolated from garden soil which had well known ability to consume leachate by using universal primers 5'AGTTGATCCTGGCTCAG3' and 5' ACCTTGTTACGACTT3'.***
Keywords: Leachate, proteolytic, lipolytic, amylolytic, Bacillus sps, primers

I. INTRODUCTION

Proper waste disposal is critical due to the fact that certain types of wastes can be hazardous and can contaminate the environment. The hazardous chemicals will enter into surrounding water bodies through leachate can impact the local population's health and local wildlife. Leachate is the aqueous effluent generated as a consequence of rainwater percolation through wastes, biochemical processes in situ and the inherent water content of wastes. Land fill leachate contains dissolved organic matter, inorganic macro components, heavy metals and xenobiotic organic compounds such as halogenated organics. These contaminants play an important role in ground water and soil pollution.

Leachate can cause increase in turbidity of water, limiting the amount of light penetration which reduces photosynthesis and production of dissolved oxygen. Leachate has also been reported to increase water alkalinity and hardness [1] (Pillay *et al.*, 2011). Micro organisms in waste dump sites use waste constituents as source of nutrients there by detoxifying the materials as there digestive processes that break down complex organic molecules into simpler less toxic molecules. When bacteria consume waste, they convert the waste into safe by products and produce several metabolites to break down the complex waste into simple compounds [2] (Amrita and Subhas, 2014). Various enzymes producing micro organisms from soil has the potential to utilize leachate as source of nutrient or substrate, and converting it to useful byproduct. Organic waste is consumed by bacteria as nutrients and is no longer present to produce odors, sludge, pollution or unsightly mess. In the present work enzyme producing bacteria, having the ability to utilize leachate, were isolated and identified from the garden soil. The qualitative analysis of the leachate was done. Molecular characterization of the most efficient isolate that utilized leachate was done and a phylogenetic tree was constructed.

II. AIM AND OBJECTIVES

A. *Aim*

Our study aimed the isolation, identification and molecular characterization of *Bacillus sps*. having ability to utilize leachate

B. *Objectives*

1) To isolation and identify *Bacillus sps*.from dump soil.
2) To screen the enzymatic activity of isolates.
3) To study the substrate utilization of *Bacillus sps*.on leachate.
4) Molecular characterization of *Bacillus sps*.

©IJRASET: All Rights are Reserved

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue XI, Nov 2019- Available at www.ijraset.com

III. MATERIALS AND METHODS

A. Isolation and Identification of Enzyme Producing Bacteria

Garden soil samples were collected serially diluted and spread plate technique done using nutrient agar plate. The bacterial isolates were presumptively identified by means of morphological examination and biochemical characterization (Bergey's Manual 1992). The isolated colony obtained was screened for enzyme activity such as amylase, Proteas, Lipase (Hasan *et al*., 2005).

B. Biochemical Analysis of Leachate

Presence of carbohydrate, protein and lipid were estimated using Starch Iodine Test, Heat and Acetic Acid Test, Saponification Test.

C. Detection of Leachate Utilization

Sterile 50ml of leachate along with agar was used for detecting the substrate utilization by the isolated organisms from the garden soil.

D. Molecular Characterization of Bacteria

Genomic DNA was extracted from isolated *Bacillus sps (*Pitcher et al 2008). The DNA isolated from bacteria were subjected to polymerase chain reaction (PCR) technique for amplification of DNA using 16s r RNA specific primer, Forward 5'AGTTGATCCTGGCTCAG -3' and reverse primer -5'ACCTTGTTACGACTT – 3'.

IV. RESULT AND DISCUSSION

A. Isolation and Identification of Enzyme Producing Bacteria

The present study deals with the isolation and identification of leachate utilizing bacteria from garden soil. A total 6 of bacterial strains were isolated. The bacterial isolates designated as T1, T2, T3, T4, T5, T6 were identified as *Bacillus sps* based on their morphology, cultural and biochemical characteristics.

B. Screening of Enzyme Producing Bacteria

Out of 6 isolates, T1 showed the maximum zone of clearance on the starch agar medium ie, 38 mm. Protease activity was observed and T2 strain showed largest zone (32mm) of hydrolysis .Among the six isolates T6 showed maximum lipolytic activity by forming precipitation around the colonies (22 mm) and was selected for further study (Fig. 1, 2, 3).These findings are similar to the findings of Wan *et al*.

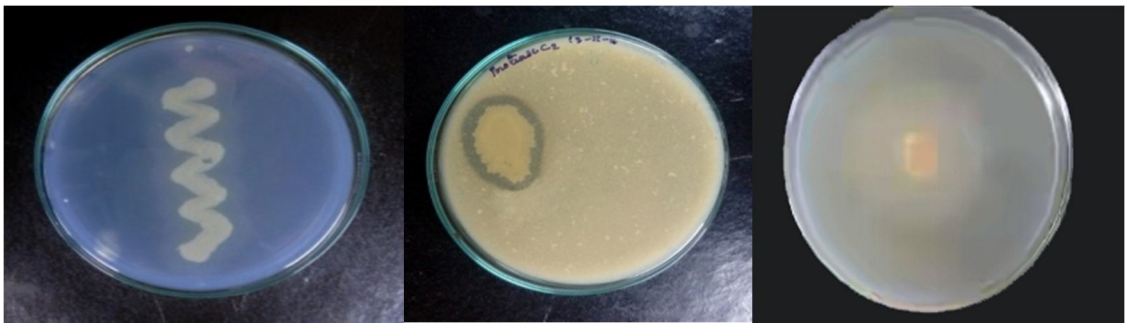


Fig. 1 Amylolytic activity Fig. 2 Proteolytic activity Fig. 3 Lipolytic activity

C. Biochemical Analysis of Leachate

The chemical composition of the leachate was tested. It contains carbohydrate, protein and lipid. The characteristics of the leachate vary with regard to its composition and volume, and the biodegradable matter present in the leachate against time (Anouzla *et al*., 2014). Presence of carbohydrate, protein and lipid were detected using Starch Iodine Test, Heat and Acetic Acid Test, Saponification Test.

D. Detection of Leachate Utilisation

Out of three *Bacillus s*ps, T1 isolate showed good growth on leachate (Fig. 4). It have amylolytic, lipolytic and proteolytic activity. Molecular characterization of T1 isolate was done.

©IJRASET: All Rights are Reserved

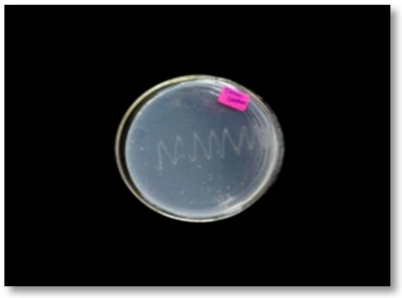


Fig. 4 Growth of bacteria (T1) on Leachate

E. Molecular Characterization of Bacteria

The genomic DNA from Bacillus sps were isolated, amplified and sequenced. Sequencing was performed at SCI genomics Cochin.

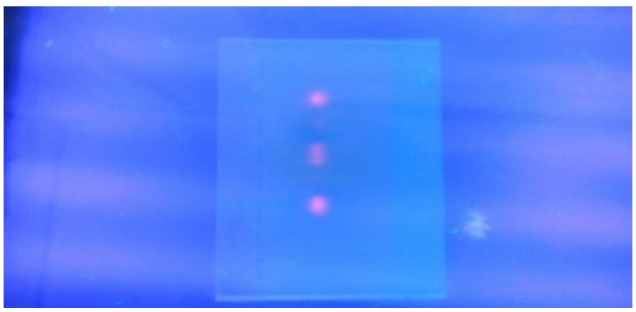


Fig: 5 Genomic DNA isolated from T1

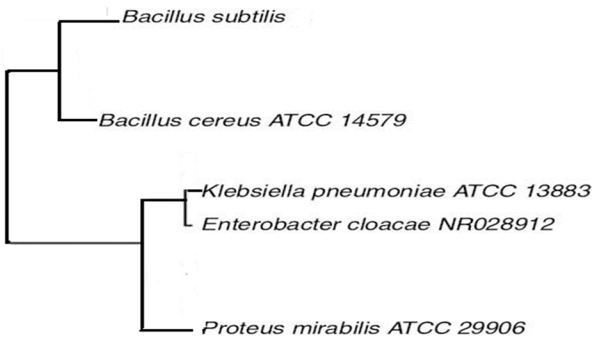


Fig : 6 Phylogenetic tree representing the strain under study.

On the basis of gene sequencing the isolate T1 was confirmed as Bacillus subtilis

V. CONCLUSION

Organisms isolated were tested for enzymatic activity such as amylolytic, proteolytic and lipolytic.

The isolates that showed enzymatic activity were tested for substrate utilization of leachate (can grow on agar incorporated with 50% and 100% leachate).

The molecular study confirmed that the isolate was Bacillus subtilis, having high ability to utilize leachate.

The conventional treatments of leachate using chemicals are a great threat to the surrounding soil, ground water and even surface water. To avoid these disadvantages, in the present study an innovative method using micro-organisms was applied to treat leachate.

©IJRASET: All Rights are Reserved

VI. ACKNOWLEDGEMENT

The authors are thankful to the authorities of Presentation College of Applied Sciences, Puthenvelikara, Ernakulam, Kerala for providing the facilities.

REFERENCES

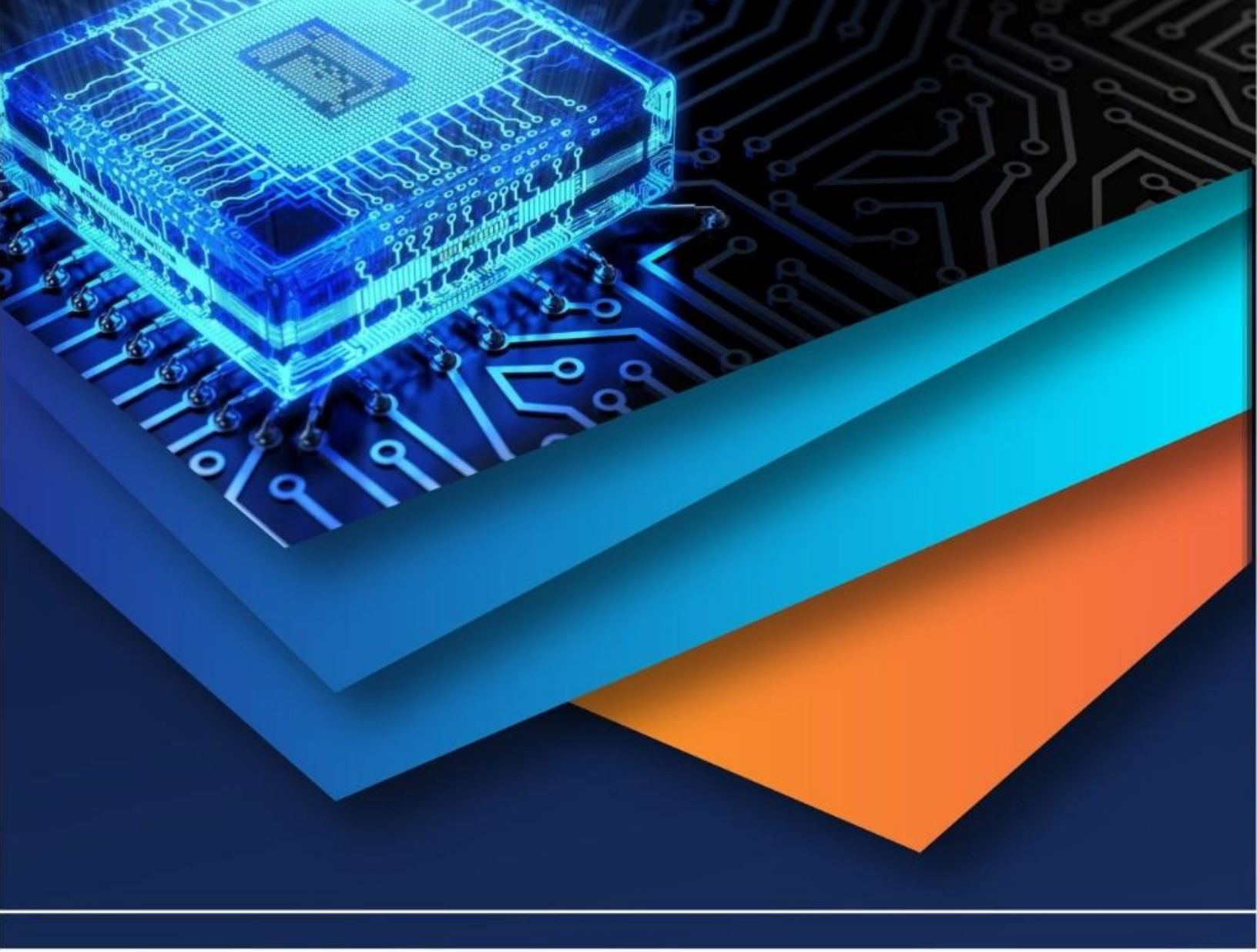
[1] Abdelkader Anouzla , Salah Souabi, Mohamed Safi, Younes Abrouki, Hayat Loukili, and Hicham Rhbal,"Waste to treat waste of landfill leachates", International Journal of Environmental Protection and Policy 2014; 2(2): 50-53.

[2] Saba Hasan, Anis Ahmad, Abhinav Purwar, Nausheen Khan, Rishi Kundan, and Garima Gupta, "Production of extracellular enzymes in the entomopathogenic fungus Verticillium lecanii", Bioinformation. 2013; 9(5): 238–242.

[3] WMF Wan Ishak,S.Jamek, NA Jalanni, NF Mohd Jamaludin. International conference on Biology, " Isolation and identification of bacteria from activated sludge and compost for municipal solid waste treatment system", Environment and Chemistry2011; 24(2011); 450 – 454.

[4] John .G. Holt,Noel R. Kreig, Peter H.A. Sneath, James T.Staley ,Stanley T. Williams, "Bergey's Manual of Determinative Bacteriology",9th Edition 1992

©IJRASET: All Rights are Reserved



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 (24*7 Support on Whatsapp)