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ISOLATION AND IDENTIFICATION OF BACTERIA FROM INDUSTRIAL WASTE

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ABSTRACT
Water pollution is a crucial problem in today's life. The industry has been identified as one of the major source of water pollution. Any industrial site discharges polluted water and contained large amounts of perilous compounds. Industrial techniques include physical and chemical treatment together with biological processes. This polluted water eliminates towards rivers, lakes, etc. This water eliminated directly into the water bodies it give risk on environment and human life.So analysis prior elimination of industrial wastewater towards water bodies is essential. Industrial wastewater and a wide range of environmental pollutants are degraded with the help of microorganisms such as bacteria and fungi present on surrounding soil region. The aim of this study was to isolate and identify various bacterial species. Out of them, three different bacterial species was isolated using Sabouraud agar medium. These bacteria tested for gram staining, glucose test, IMViCTest. Bacteria were identified by 16S rRNA sequencing.
Keywords:*Industrial waste, Biochemical Test, 16s rRNA,*

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INTRODUCTION

Due to population, the world is constantly in need of water.Earth's population has tripled in the last century while water consumption has enlarged [3]. Mostof the major challenges of today's civilization is pollution [2]. Industrial effluents are an important factor in aquatic pollution [7]. It has been detected that one-third of the total water in India is water Pollution comes in the form of industrial effluents, solid wastes, and other toxic wastes [13].Industrial waste sampling involves the collection of various physical forms of waste like solid, liquid, semi-solid, etc. Groundwater sources are contaminated due to untreated industrial effluents[15]. Many waterborne microorganisms are infectious to humans as well as animals and cause many diseases [6].Therefore the analysis is required before the disposal of industrial water to groundwater. For wastewater treatment Governments are finding for cheap, efficient, effective solutions. Therefore, in recent times biological treatment systems have become famous and also helped develop relatively efficient, cheap waste treatment[16]. Micrograms involved in industrial wastewater are bacteria, algae, fungi, archaea, and viruses. Bacteria are the main constituents of wastewater[3].Bacteria live in infectious environments because they are metabolically competent and able to use the resources available to them and hold specific places [7]. Hence, it is essential understand the bacterial species present in industrial wastewater [9].These bacteria can generate some toxins that can cause several diseases or they can produce some industrially essential bioactive molecules [9]. But some bacteria present into waste water are not harmful. Some Bacteria help to handle the wastewater [14]. Three bacterial species were isolated from industrial wastewater. Bacteria wasidentified by 16s rRNA sequencing. They give genus and species identification of given bacteria [6]. Isolated Bacteria from industrial wastewater including. Noted regarding to the isolation and identification of bacteria from industrial effluents[10]. The objective of the present work was to isolate and identify bacteria that may be present in paper and pulp industrial wastewater [1]. Microbial analysis is used to help control and growth of viruses, bacteria. Application of the microbial analysis was identification, detection, isolation, detect fecal contamination in water, identification of pathogen, as well as identify contaminants and treat them.

MATERIAL AND METHODS

Sample collection

The sample was collected from an area of industrial wastewater. The sample was collected according to the protocol in sterile vials (APHA2012). Sample was collected using clean noncontaminated bottles. The sample was preserved at the refrigerator to stop the characteristics of the water from changing.

Isolation of bacteria -

Under hygienic conditions, the sample was serially diluted from 10^{-1} to 10^{-10}.For the isolation of unknown bacteria. From dilution 10^{-3} to 10^{-7} of diluted sample was selected and 0.1 ml transferred into sabouraud agar medium. Sabouraud agar contain 1% peptone, 4% Dextrose, 3% agar, distilled water and pH was adjusted to 5.4 [7]. After transferred sample on Sabouraudagar medium plate, the plate was incubated at 37^0c for 24 hrs. After incubation isolated colonies was observed. From that single colony were picked and subculture for isolation and identification. [1]. A total 5 isolated were gained from that 3 isolate was selected and used for further studies.

Identification

Identification of bacterial species was concluded by microscopic characters. The selected colonies were subjected to gram staining as well as characterized using biochemical test [7].Grams staining was performed for gram nature of the bacteria. Morphology of bacteria was observed by their shape, size,elevation and color.

Biochemical Test –

Sugar fermentation test –

A fermentation test is used to determine whether bacteria can use a particular carbohydrate or not. Isolated bacteria inoculated into sugar fermentation tube. Incubated at 37^0c for 24hrs. The pinkcolour indicated positive test.

IMViC Test –

Identification of unknown isolated bacteria.

Urease Test –

Urease test used to determine the ability of organisms to split urea. Isolated bacteria streaked on Christensen's medium. The tube was incubated at 37^0C for 24 hrs. After incubation red color indicated positive test [14].

Nitrate reduction test –

To determine if an organism is capable of reducing nitrate or not. Isolated bacteria inoculated into tryptone nitrate broth. Incubated at 37^0c for 24 hrs. After incubation pink colour indicated positive test.

Catalase Test –

Catalase Test used for detection of the catalase enzyme in bacteria. Isolated bacteria colony immerse into hydrogen peroxide. Observe immediate bubbling.

Sangers Sequencing-

The gold standard approach is chain termination-based sequencing, also known as Sanger's sequencing technology, against which all other sequencing methods are measured. By employing universal primers to PCR-amplify the housekeeping gene targets, this method is frequently used to quickly identify species. Next, sequencing, database searches, and phylogenetic analysis with the nearest neighbor'smicrobes are used to deduce the identity of the organism of interest.

PCR Amplification and Sequencing

In this case, the bacterial 16S rRNA gene was used as a target and the amplification was carried out using the universal primers 16S27F (5'-CCA GAG TTT GAT CMT GGC TCA G-3') and 16S1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') [17].The amplified PCR product was further purified by salt-precipitation. Agarose gel electrophoresis was carried out to determine the quality of PCR amplicons as well as post purification of the PCR products. Purified amplicons were then subjected to cycle sequencing using BDT v3.1 chemistry and subsequently sequenced on an ABI 3500XL Genetic Analyzer. Additional internal primers were used to obtain near-full length sequence to generate good quality base reads covering the target.

BLAST

Basic Local Alignemnt Search Tool (BLAST) finds regions of similarity between homologus sequences. The program compares nucleotide sequences to sequence databases and calculates the statistical significance.

Phylogenetic Analysis

For the phylogenetic analysis, upto 10 closest-neighbor sequences belonging to different taxa from amongst the top 1000 hits with the highest similarity in the search results were retrieved from the database and aligned using the MUSCLE aligner [18].

A phylogenetic tree is used to visually depict this relationship in order to investigate how closely related groups of species are to one another evolutionarily. Each branch of a phylogenetic tree indicates a taxon that is being compared within the tree. Taxa that have evolved from their common ancestor are represented by branches that start at a single node, also known as a node representing a point of divergence. A common root to the tree reflects the most ancestral taxon from which all taxa within the tree are likely to have evolved. Trees can be rooted or unrooted.

Typically, when determining the evolutionary distance between two animals, the length of the branch is what is important. Therefore, discussing the topology of the tree in terms of branch lengths rather than their vertical arrangement is more pertinent. The horizontal lines, which are branches, show how different evolutionary lineages have evolved over time. The amount of genetic alteration increases with the length of the horizontal branch. The numbers at the nodes are the bootstarp percentage values, which show how often the branches were repeated with the same configuration during the iterations. As a result, a higher bootstarp number indicates a higher level of confidence in the branch.

RESULT

In the study the bacterial colonies were isolated from industrial wastewater. Out of them Three different Bacteria Isolated from industrial wastewater on Sabouraud agar medium [10]. Bacterial growth depends upon various conditions like temperature, pH, and incubation period. [5] Isolated bacteria subject for gram staining, motility and biochemical test. The morphological, colony character biochemical test and 16srRNA sequencing are given below the table.

Sample No. S1

Colony character –

Size	Shape	Elevation	Margin	Consistency	Opacity	Colour
1mm	Circular	Convex	Regular	Smooth	Opaque	Yellow

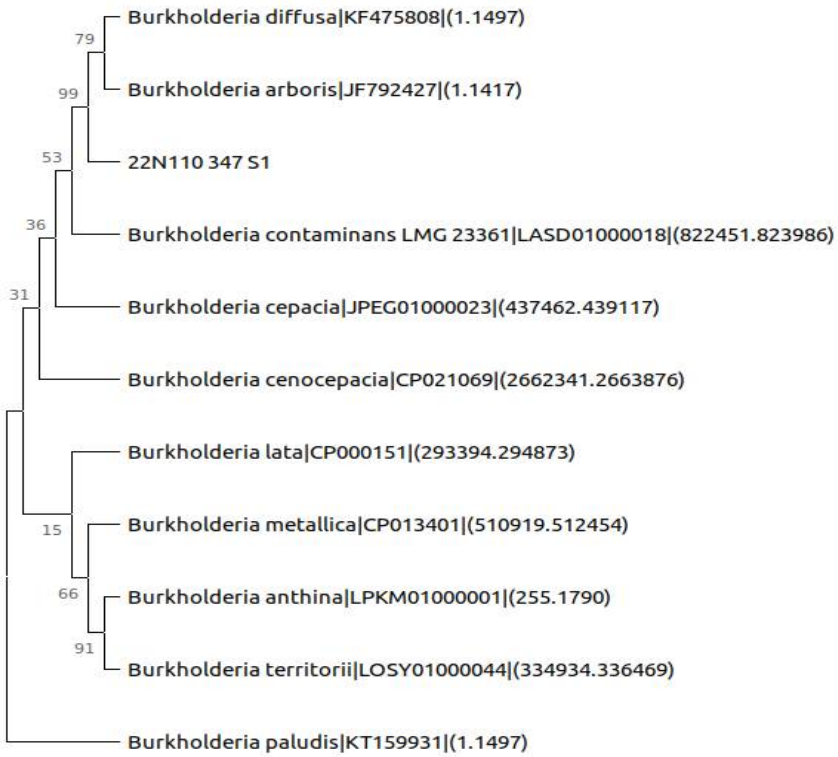


Fig. 1. Phylogenetic tree of S1 sample.

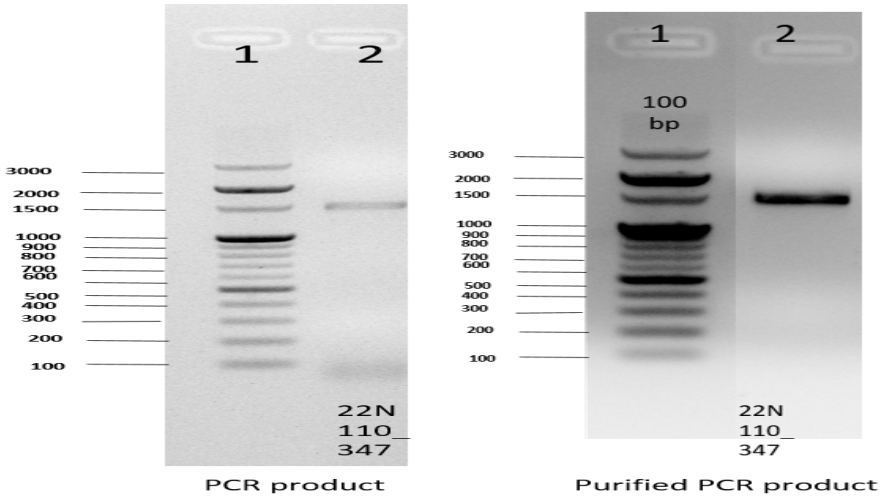


Fig 2. The agarose gel electrophoresis is carried out with PCR product and purified PCR product.

Sample No. S2
Colony character -

Size	Shape	Elevation	Margin	Consistency	Opacity	Colour
1mm	Circular	Convex	Regular	Smooth	Opaque	White

Fig.3. Phylogenetic tree of S2 sample.

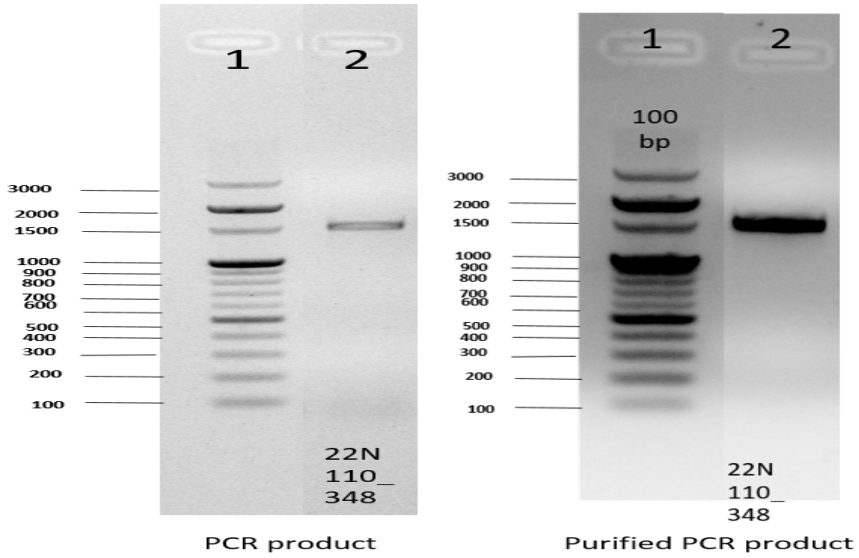


Fig. 4. The agarose gel electrophoresis is carried out with PCR product and purified PCR product.

Sample No. 3

Size	Shape	Elevation	Margin	Consistency	Opacity	Colour
1.2mm	Circular	Flat	Irregular	Mucoid	Opaque	Lightpink

Colony Character-

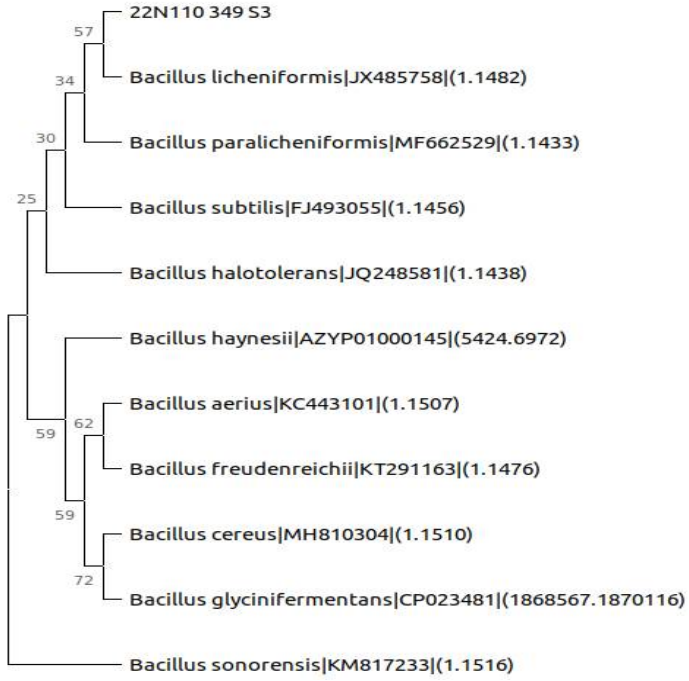


Fig.5. Phylogenetic tree of S3 sample.

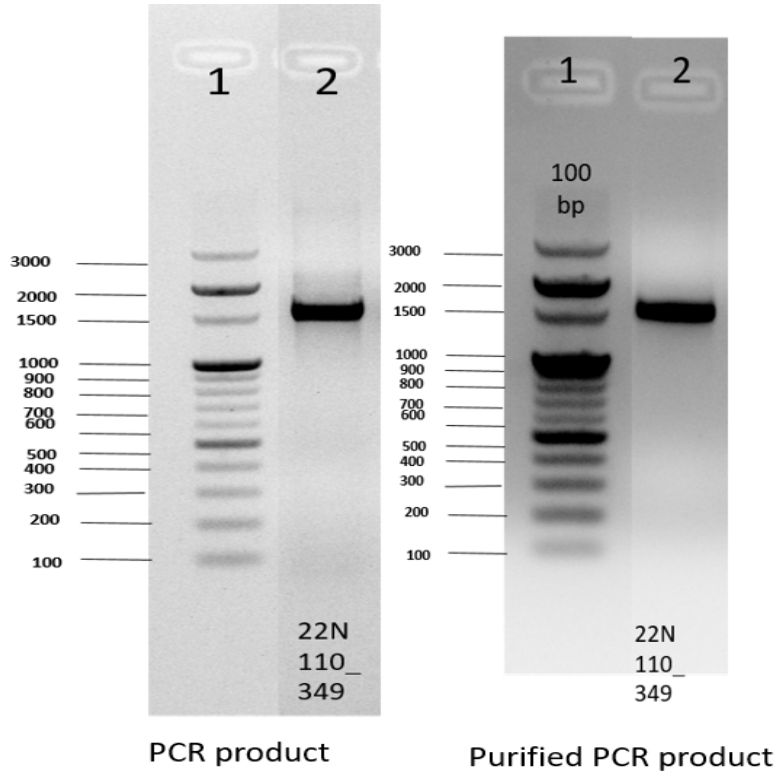


Fig. 6. The agarose gel electrophoresis is carried out with PCR product and purified PCR product.

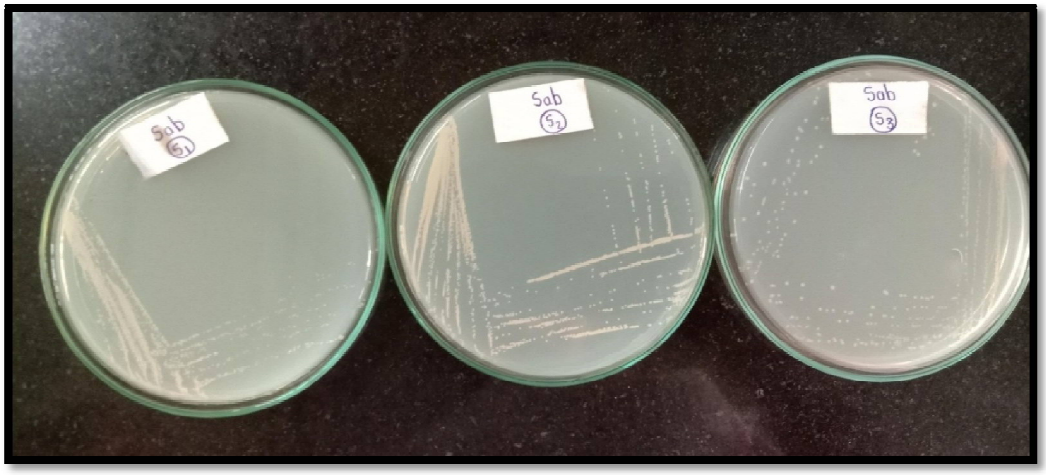
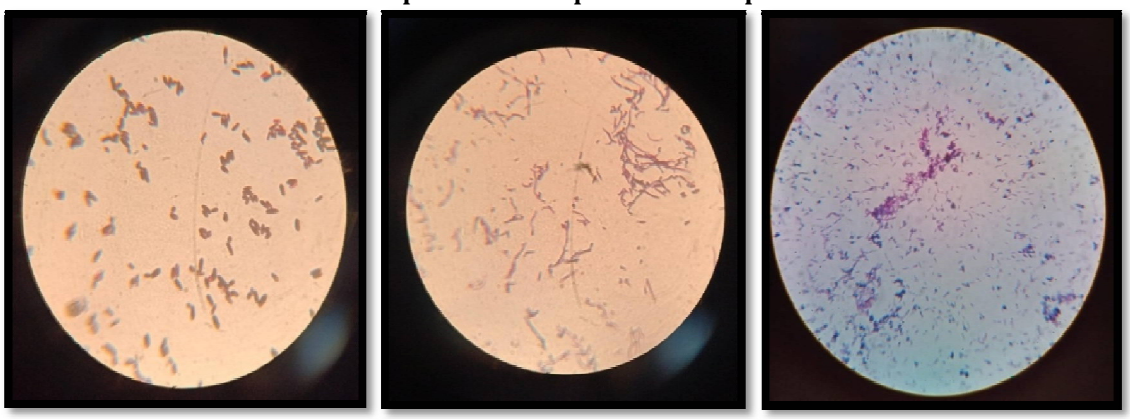


Image-1. Isolated colony of S1, S2 and S3 sample
Sample No. S1Sample No. S2Sample No. S3



Morphological Characters-
Sample No. S1

Gram staining	Motility
Gram positive	Non-motile

Sample No. S2

Gram staining	Motility
Gram positive	Non- motile

Sample No. S3

Gram staining	Motility
Gram positive	Non-motile

Biochemical Test –
Sugar fermentation Test

Sample No.	Dextrose	Fructose	Lactose	Sucrose	Xylose
S1	**+**	**+**	**-**	**+**	**+**
S2	**+**	**+**	**-**	**+**	**+**
S3	**+**	**+**	**-**	**+**	**-**

IMViC Test –

Sample No.	Indole	Methyl red	Voges proskauer	Citrate
S1	**+**	**-**	**+**	**+**
S2	**+**	**-**	**+**	**-**
S3	**+**	**-**	**-**	**-**

Catalase Test, Nitrate test and urease hydrolysis -

Sample No.	Catalase	Nitrate reduction	Urea hydrolysis
S1	**-**	**-**	**-**
S2	**-**	**-**	**-**
S3	**-**	**+**	**-**

CONCLUSION

For the growth of bacteria industrial wastewater be comes a good source. Bacterial species were isolated from surrounding soil of wastewater are. S1-*Burkholderiacontaminans* (LASD01000018), S2-*Burkholderiadiffusa* (KF475808) and S3- *Bacillus licheniformis (JX485758)*.Industrial wastewater is polluted water that contains high amounts of perilous compounds. Therefore, there is a need to treat industrial wastewater before released into water bodies. Bacteria lives in waste could have the ability to degrade toxic chemicals, phenolic compounds, and colored compounds.

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