



## **Isolation And Identification of Fungi from Paper and Pulp Industrial Wastewater**

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### **ABSTRACT**

*Industrial wastewater consists various types of pollutants, organic, and inorganic constituents. Directly discharge of industrial waste to the natural resources, water bodies, and their forms will alter their aesthetic and socioeconomic values. Industrial wastewater is hazardous to human and other aquatic life forms. It may cause serious health issues to the individuals prone to exposure to it. Many physical and microbiological methods are used to remove pollutants from industrial wastewater. Samples were collected from paper and pulp industry in paithan, MIDC, Aurangabad, Maharashtra. Then pure fungal culture were isolated by using serial dilution method and spread plate method. For screening of fungal strain biochemical tests and fungal staining were done. For confirmation of fungal species 18rRNA Sequencing was done.*

**Keywords:** *Fungi, Industrial waste, socioeconomic, 18rRNA Sequencing.*

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### **INTRODUCTION-**

In India paper industry is the also biggest concern in main reason of pollution. As per Ministry of Environment and Forest (MoEF) it is the fifth and biggest sector in the pollution causing from industrial sector. To generate and acquire mobility to produce paper, there is about 325-450 m<sup>3</sup> water is needed and generated wastewater is 70-270 m<sup>3</sup> [3]. Thus, wastewater from the industry is highly hazardous and the water quantity disturbs the economic importance of environment and social impact belonging to impact of the environment if the untreated wastewater of paper and pulp industry is directly discharge into water bodies. The operation of wastewater treatment technologies depends on a combination of physical, chemical & biological factors. Industrial wastewater represents a potential threat to natural water systems. These effluents contain many inorganic and organic substances, which are toxic to various life forms of the ecosystem. Industrial activities -generate a large number & variety of waste products. The one of the major challenges of today's civilization is pollution in India, it has been found that one-third of the total water pollution comes in the form of solid waste and other hazardous waste discharged from industrial effluents.

Microbes are an important part of the ecological system. They help in the high rate of meditative way to drag the process of bioremediation in a natural manner and to optimize, and relieve in a reliable way to indulge and carry out the process of the associative type of biological and socio-economical value, and significant type because they are beloved to be important in reformative in the improvement of life forms to perform a derivative work for waste treatment and perform activity of the direct efficiency of wastewater management. Because in India wastewater is the largest threat due to highly elementally induced and nonpalatability towards the fused and intermingled which micro compounds of paper and pulp industrial evolved wastewater. It consists of phenolic reagents, organic, inorganic compounds, dyes, and other miscellaneous compounds. The microorganism present in industrial waste / wastewater are bacteria, archaea, fungi, algae, protozoa & viruses. The aim of the presented work is microbial analysis of industrial waste [3]. High loads of fungi have been reported in industrial waste.

Microorganisms help to keep a healthy equilibrium in an environment which signifies that and maintain relationships regarding biotic and abiotic factors. That seeks all toxic compounds of which are degraded and returned back in a recyclable manner to form an ideal environment. In India wastewater treatment and recycling methods to treat wastewater are inevitably, cost effective which are not liable to be used in all industries [1]. So, waste management and treatment is necessary for maintain good environment.

## **MATERIALS AND METHODS**

### **Sample Collection –**

Wastewater collection and analysis for this present studies investigation was collected from paper and pulp industry located in Paithan, Aurangabad district in Maharashtra, India. The latitude of Paithan, Maharashtra, India is 19.475899, and the longitude is 75.385178. Paithan, Maharashtra, India is located at India country in the Towns place category. Samples were collected from outlet and inlet source of wastewater treating plant side drainage periodically by the technique, of sampling (grabbing) with help of hand gloves. Surrounding soil from industrial waste sample taken in sterile plastic pouches. Thus, after the sample collection, it is brought to the laboratory of the department of microbiology at Yashwantrao Chavan institute of science satara, of Microbiology for further aspects of the investigation. There was 2 samples were collected 1)- liquid 2) Soil. The liquid sample was filtered 0.10mm sieve to remove suspended and other waste particulate matter and stored at 5°C.

### **Performance for pure sample prototype:**

1. Then these 2 samples were serially diluted up to  $10^{-9}$  and spread on potato dextrose agar and sabouraud dextrose agar medium. To investigate microorganisms from the industrial waste sample was filtered.
2. For identification purpose Sabouraud agar and potato dextrose agar were used for isolation purpose.
3. Sample was spread on a sterile Sabouraud agar plate and potato dextrose agar plate. After that the plate we're incubated at 37°C for 2 days.
4. After incubation fungi isolation on both media plates and Fungi was also stained and seen under the microscopic field.
5. Cultural and morphological characteristics were recorded. Here liquid and soil sample were used for identification. After that Biochemical test like Sugar Fermentation was performed and Results were recorded.

### **18s rRNA Identification:**

#### **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's microbes 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 Alignment Search Tool (BLAST) finds regions of similarity between homologous sequences. The program compares nucleotide sequences to sequence databases and calculates the statistical significance.

#### **Phylogenetic Analysis**

For the phylogenetic analysis, up to 10 closest-neighbor sequences belonging to different taxa from amongst the top 1000 hits with highest similarity in the search results were retrieved from the database and aligned using the MUSCLE aligner [22].

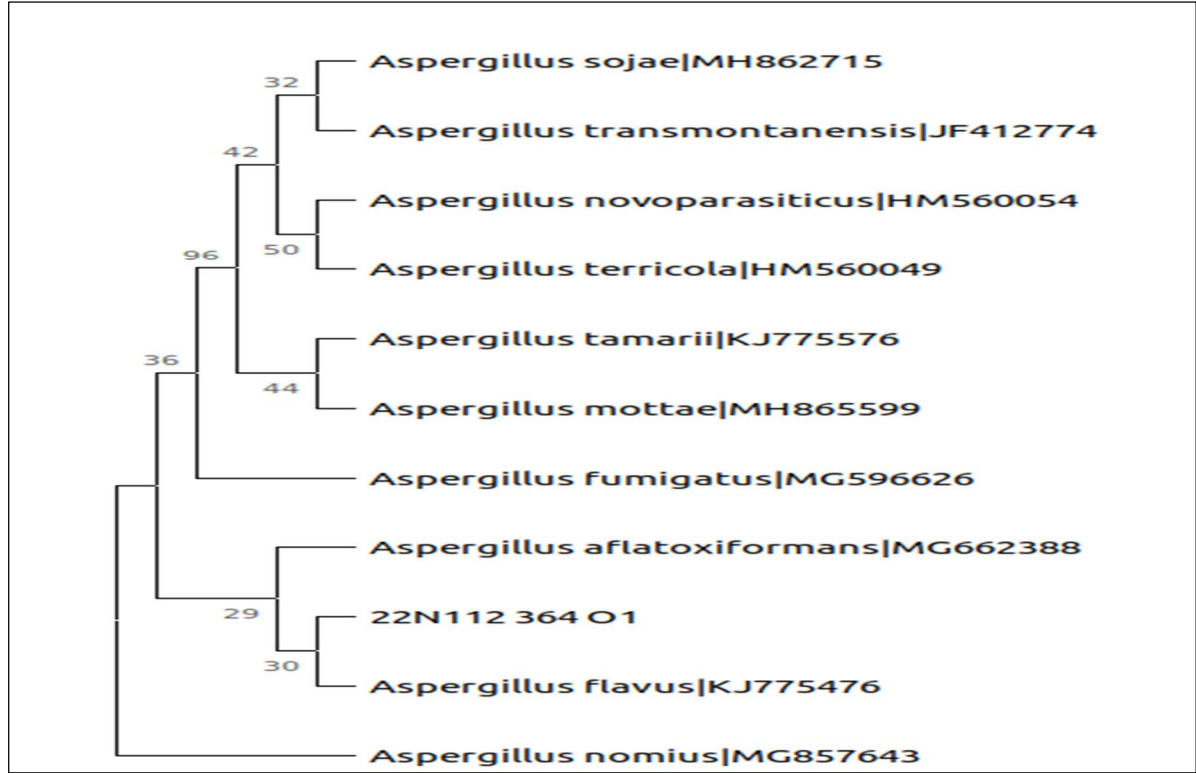
A phylogenetic tree is used to visually depict this relationship in order to investigate how closely related groups of species are to one another evolutionarily. Each branch of a phylogenetic tree indicates a taxon that is being compared within the tree. Taxa that have evolved from their common ancestor 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 bootstrap percentage values, which

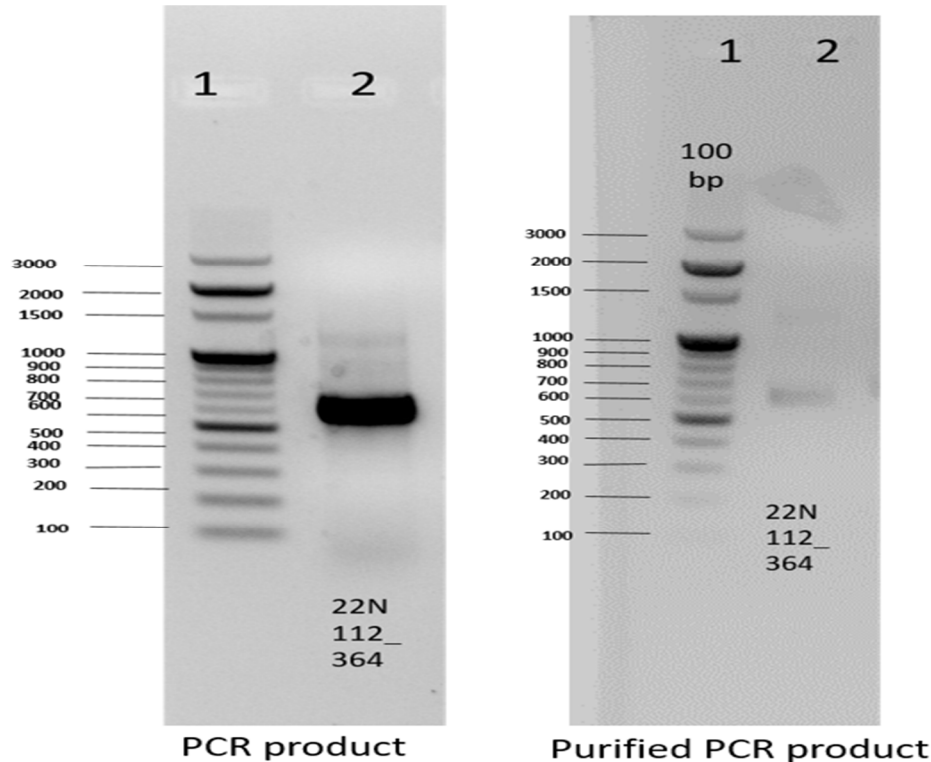
show how often the branches were repeated with the same configuration during the iterations. As a result, a higher bootstrap number indicates a higher level of confidence in the branch.

**RESULT AND DISCUSSION:**

For this study the aim was to isolate and identify most of frequently, optimally occurring fungal isolates from derived and unorganized sector of industrial wastewater. Total about 4 fungal strains isolates out of 2 samples were identified by 18 srRNA sequencing.



**Fig.1 Phylogenetic tree of *Aspergillus flavus* (KJ775476)**



**Fig.2 Agarose gel electrophoresis of PCR product *Aspergillus flavus* (KJ775476)**

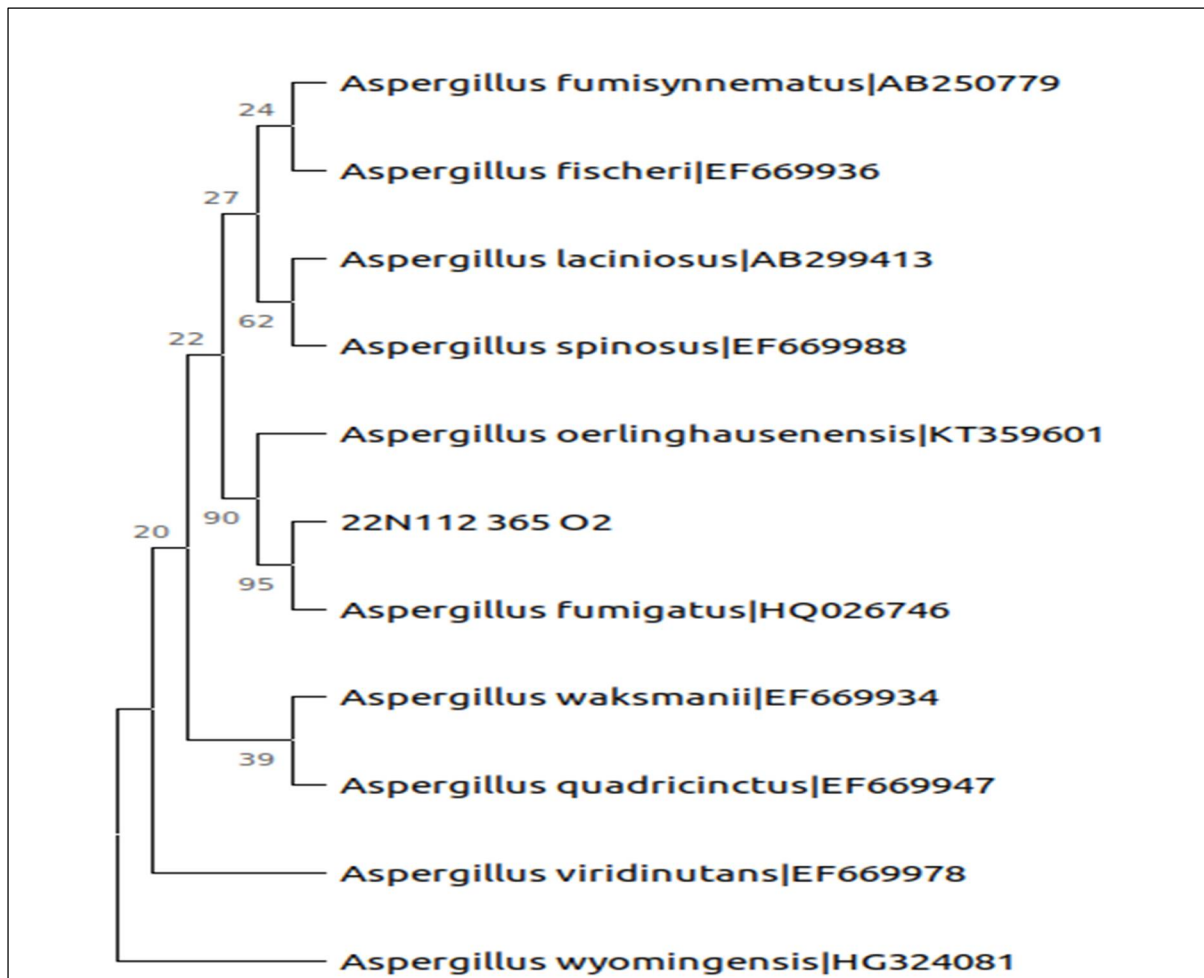


Fig.3 Phylogenetic tree of *Aspergillus fumigatus* (HQ026746)

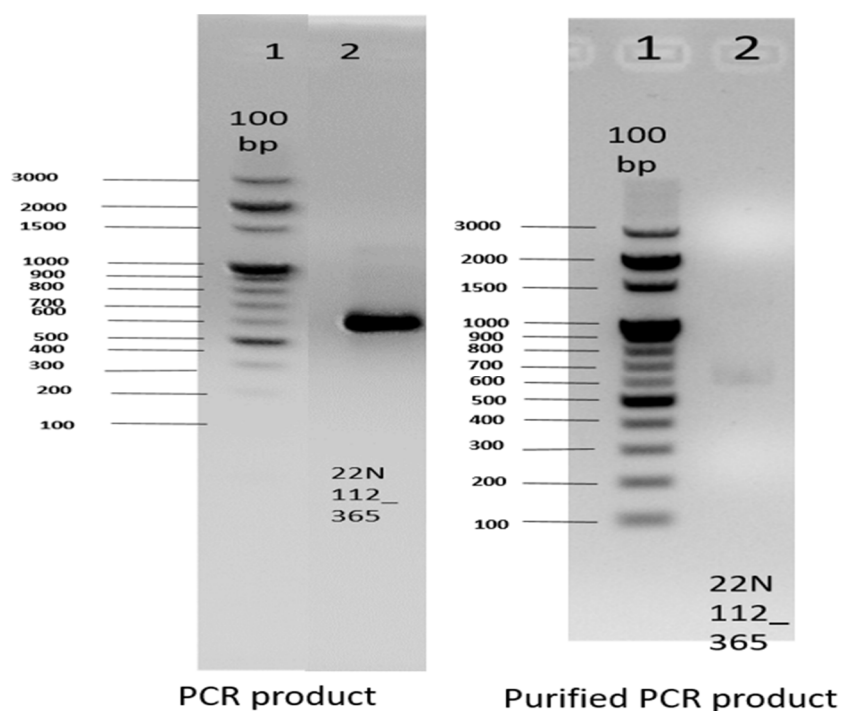


Fig. 4 Agarose gel electrophoresis of PCR product *Aspergillus fumigatus* (HQ026746)

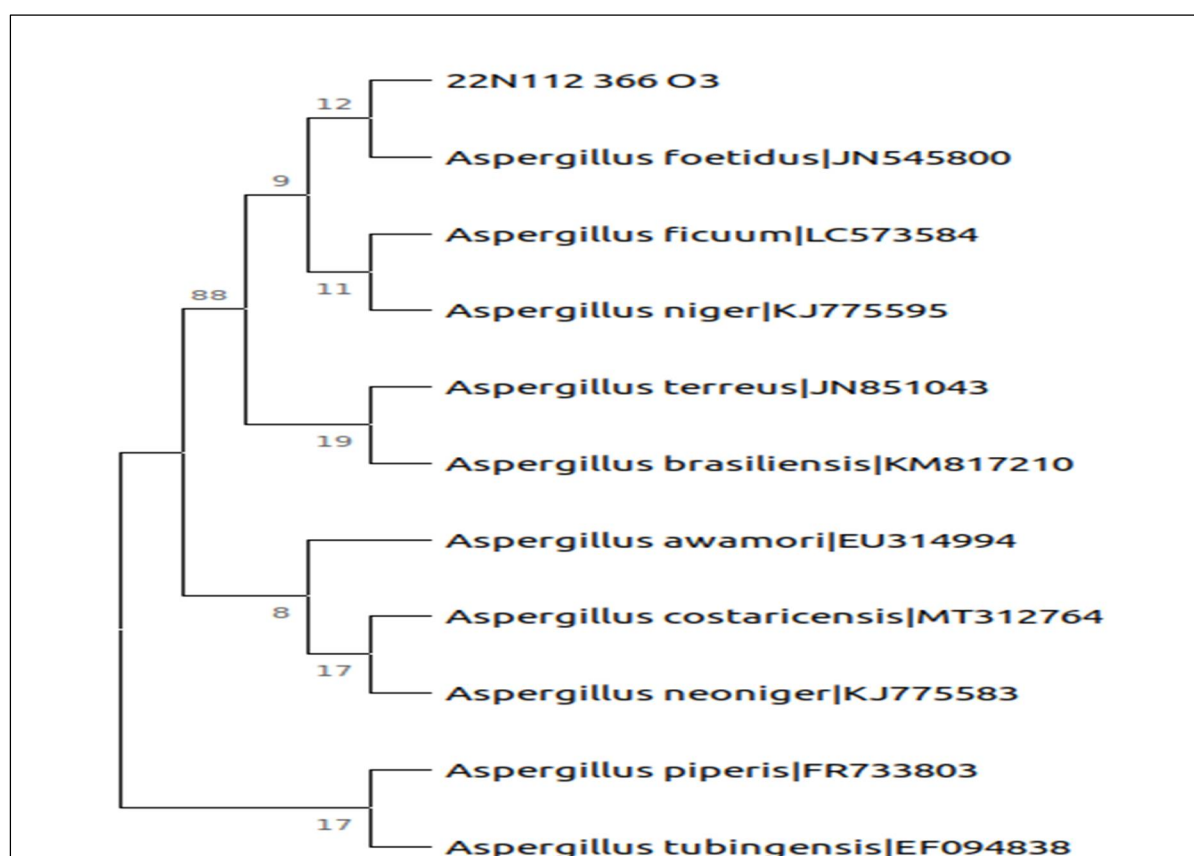


Fig.5 Phylogenetic tree of *Aspergillus foetidus* (JN545800)

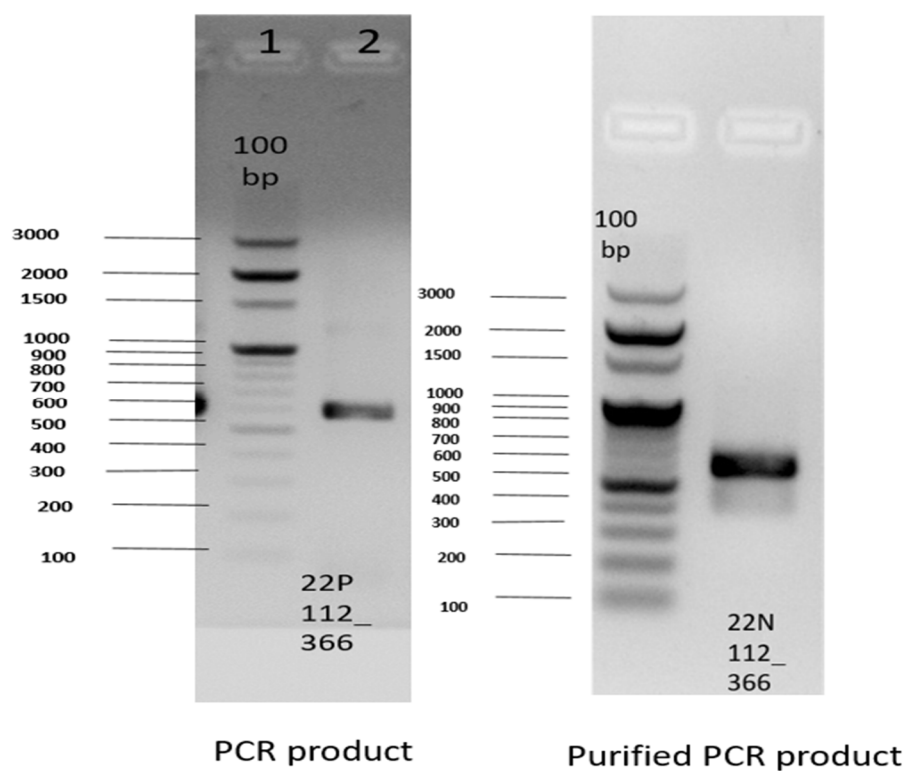
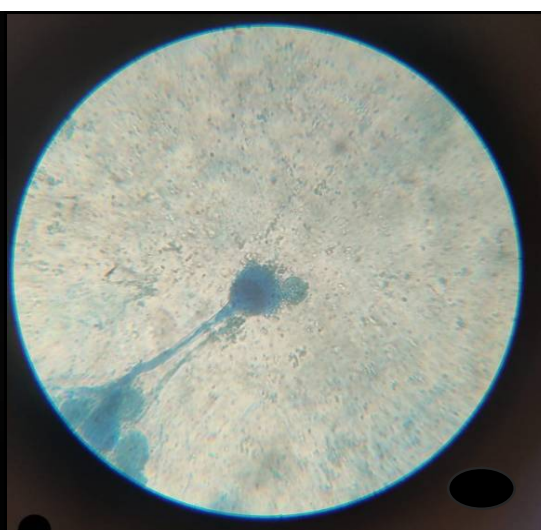
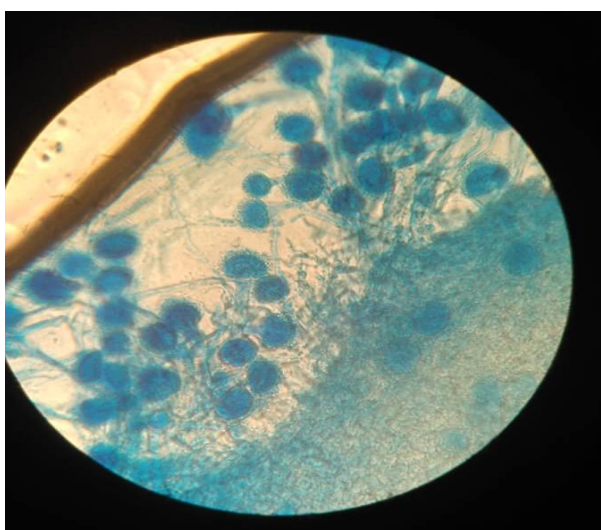
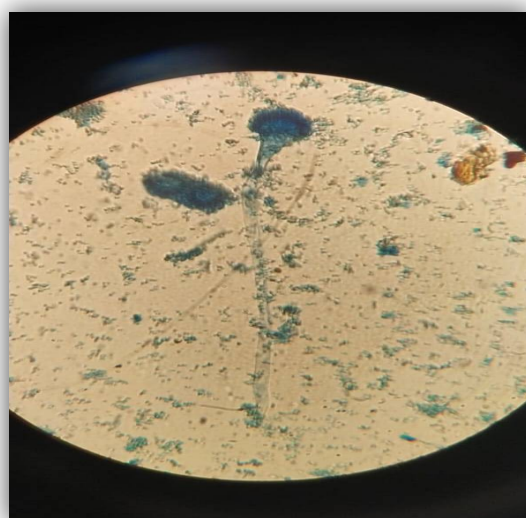
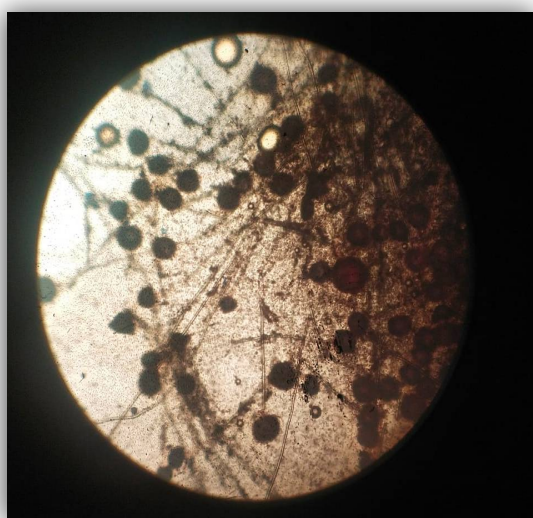


Fig. 6 Agarose gel electrophoresis of PCR product *Aspergillus foetidus* (JN545800)



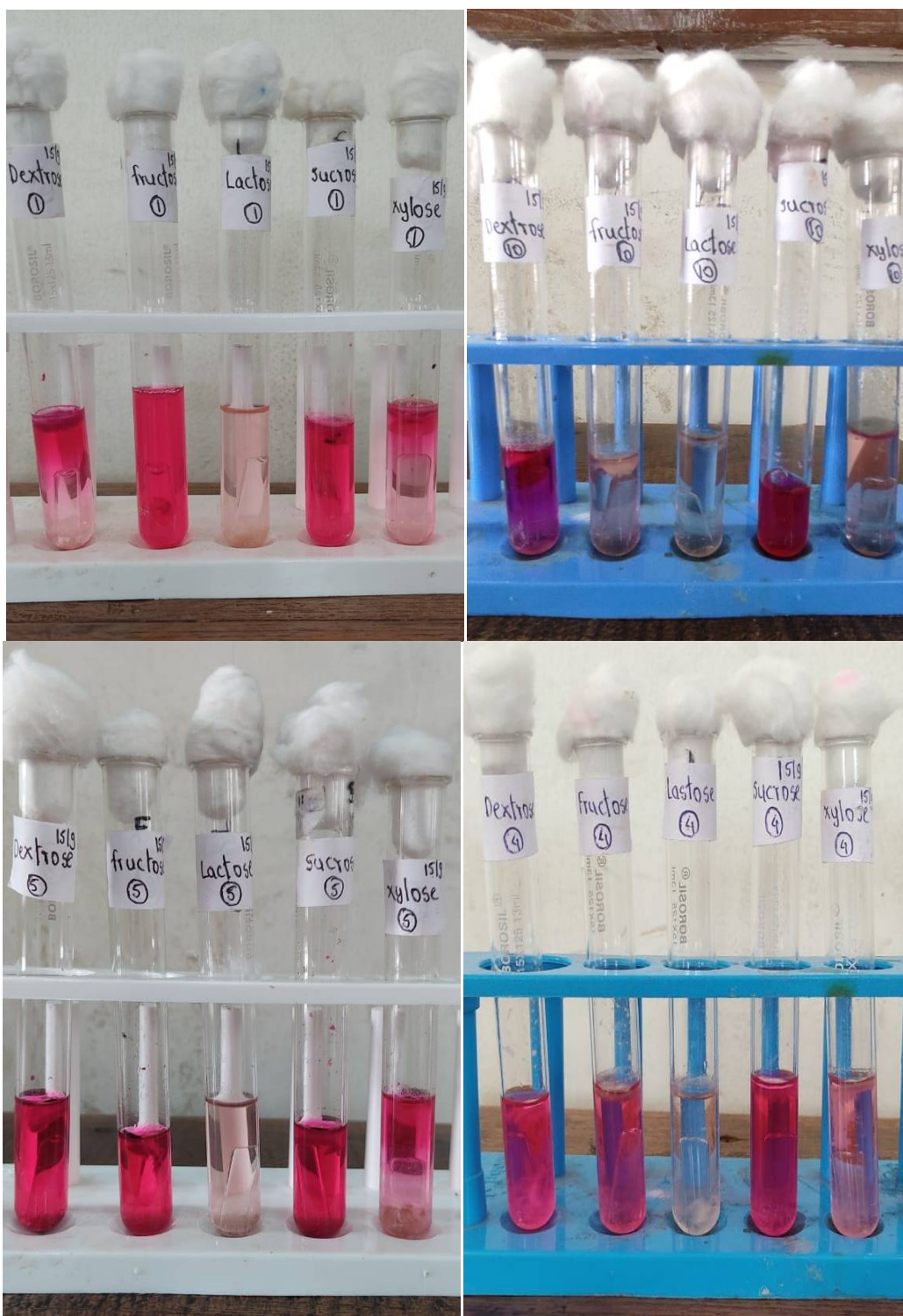


**Fig.7 - Isolates of Fungal Strains**



### Sugar Fermentation Test:

Sample	Dextrose	Fructose	Lactose	Sucrose	Xylose
Sample 1	+	+	-	+	+
Sample 2	+	+	-	+	+
Sample 3	+	+	-	+	+
Sample 4	+	+	-	+	+



Industrial wastewater also affects on different microbial community and these kind of pollution can destroy these community [6]. So, Microbial population found higher number in industrial waste as compare to tap water [12]. Microorganism and fungi can use organic matter as a nutrient. Composition of industrial waste work as good nutrient for fungal growth and those organisms lows the contamination level to the acceptable quality level for discharge easily[7]. Microbes can convert organic and inorganic elements to the energy form, so it is necessary to found such kind of organism so it can utilize as an energy source [9].

Microorganisms can use for treatment of industrial waste it will make effective in future and also it is environment friendly and less cost require as compare with other wastewater treatment[10]. Thus it itself has great significance in environmental sustainability and maintain ecology of the soil.

From paper and pulp industrial wastewater we isolate 3 fungi and also perform 18SrRNA. Following fungi were identified.

*Aspergillus flavus* (KJ775476), *Aspergillus fumigatus* (HQ026746), *Aspergillus foetidus* (JN545800)

## CONCLUSION:

1. Study concludes that identified fungal species must have serious and optimal potential for degrading industrial wastewater constituents.
2. It also aims that it has the high capacity of proliferation and performance of fungus species from industrial wastewater. Thus, industrial waste had positive effect on fungal growth.
3. It indicates that raw form wastewater of industrial wastewater consist and filled with pollutants which seeks to be unfavorable for desirable organisms.
4. Hence, above fungal species can be used for treatment industrial wastewater.
5. Future aspects shows that fungal species must be recommended and quantified for the process of bioremediation of soil and environment.

## REFERENCES:

1. Mukhtar, I., bajwa, R., khokhar, I., &M ushtaq, s. (2011). Isolation and identification of filamentous fungi from different industrial effluents. *Plant products research journal*, 14(1). <https://doi.org/10.4314/pprj.v14i1.70028>
2. Garcha, s., verma, n., &brar, s. (2016). Isolation, characterization and identification of microorganisms from unorganized dairy sector wastewater and sludge samples and evaluation of their biodegradability. *Water resources and industry*, 16, 19–28. <https://doi.org/10.1016/j.wri.2016.10.002>
3. Dhanushree, m., &kousar, h. (2022). Bioremediation of phenol from pulp and paper mill effluent. *Pollution research*, 241–243. <https://doi.org/10.53550/pr.2022.v41i01.035>
4. Kakade, v. B. (2022). Study of physico- chemical parameters of sugar industry effluent. *International journal of environment, agriculture and biotechnology*, 7(4), 172–177. <https://doi.org/10.22161/ijeab.74.19>
5. Hosseini, z., taghighaneian, m., jafarinadoushan, a., ehrrampoush, m. H., namayandeh, s. M., &ghafourzadeh, m. (2019). Isolation and identification of native degrading fungi from wastewater's pollutants in cardboard recycling industry. *The journal of tolooebehdasht*. <https://doi.org/10.18502/tbj.v17i6.494>
6. Li, d., jiang, x., wang, j., wang, k., &zheng, b. (2017). Effect of sewage and industrial effluents on bacterial and archaeal communities of creek sediments in the taihu basin. *Water*, 9(6), 373. <https://doi.org/10.3390/w9060373>
7. Surti, h. (2016). Physico-chemical and microbial analysis of waste water from different industry and cod reduction treatment of industrial waste water by using selective microorganisms. *International journal of current microbiology and applied sciences*, 5(6), 707–717. <https://doi.org/10.20546/ijcmas.2016.506.077>
8. Mishra, j., prakash, j., &arora, n. K. (2016). Role of beneficial soil microbes in sustainable agriculture and environmental management. *Climate change and environmental sustainability*, 4(2), 137. <https://doi.org/10.5958/2320-642x.2016.00015.6>
9. Selvarajan, r., sibanda, t., venkatachalam, s., kamika, i., &nel, w. A. J. (2018). Industrial wastewaters harbor a unique diversity of bacterial communities revealed by high-throughput amplicon analysis. *Annals of microbiology*, 68(7), 445–458. <https://doi.org/10.1007/s13213-018-1349-8>
10. Ashok kumarsingh, gaurisingh, &rajendra prasad bhatt. (2015). Effects of salt stress on cell surface properties and symbiotic performance of root nodulating bacteria. *Pharmaceutical and biosciences journal*, 23–29. <https://doi.org/10.20510/ukipb/3/i1/89222>



11. Kabler, p. W. (1960). Selection and adaptation of microorganisms in waste treatment. *American journal of public health and the nations health*, 50(2), 215-219. <https://doi.org/10.2105/ajph.50.2.215>
12. Arshia naaz, saloniswaroop and a. K. Choudhary (2016). Physico-chemical parameters and microbial identification of flavour effluent. *The asian journal of animal science*, 11(2), 115-120. <https://www.ijserp.org/>
13. Dias, m., lacerda, i., pimentel, p., de castro, h., & rosa, c. (2002). Removal of heavy metals by an aspergillus terreus strain immobilized in a polyurethane matrix. *Letters in applied microbiology*, 34(1), 46-50. <https://doi.org/10.1046/j.1472-765x.2002.01040.x>
14. Elinbaum, s., ferreyra, h., ellenrieder, g., & cuevas, c. (2002). Production of aspergillus terreus beta-l-rhamnosidase by solid state fermentation. *Letters in applied microbiology*, 34(1), 67-71. <https://doi.org/10.1046/j.1472-765x.2002.01039.x>
15. Ju, n., & wang, s. (1986). Continuous production of itaconic acid by aspergillus terreus immobilized in a porous disk bioreactor. *Applied microbiology and biotechnology*, 23(5). <https://doi.org/10.1007/bf00257025>
16. Behnood, m., nasernejad, b., & nikazar, m. (2014). Biodegradation of crude oil from saline waste water using white rot fungus phanerochaete chrysosporium. *Journal of industrial and engineering chemistry*, 20(4), 1879-1885. <https://doi.org/10.1016/j.jiec.2013.09.007>
17. Alfonsín, v., maceiras, r., & gutiérrez, c. (2019). Bioethanol production from industrial algae waste. *Waste management*, 87, 791-797. <https://doi.org/10.1016/j.wasman.2019.03.019>
18. Rosén, b., & eriksson, h. (2019). Anaerobic treatment of sludge, industrial waste water and industrial waste from food industry. *Linnaeus eco-tech*, 243-252. <https://doi.org/10.15626/eco-tech.1997.028>
19. Controlling volatile organic compound emissions from industrial wastewater. (1992). *Waste management*, 12(1), 100. [https://doi.org/10.1016/0956-053x\(92\)90021-a](https://doi.org/10.1016/0956-053x(92)90021-a)
20. Isolation of fungus from roots of water hyacinth and cellulase production by the microorganism from cardboard waste. (2020). *International journal of environment and health sciences*, 2(2). <https://doi.org/10.47062/1190.0202.03>
21. Freitas, l., & magrini, a. (2017). Waste management in industrial construction: investigating contributions from industrial ecology. *Sustainability*, 9(7), 1251. <https://doi.org/10.3390/su9071251>
22. Edgar, robert c. "muscle: multiple sequence alignment with high accuracy and high throughput." *nucleic acids research* 32.5 (2004): 1792-1797.

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