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Isolation of Aspergillus SPS from Different Painted Walls and Comparative Analysis of Decolorization with Wild and Mutated Strains

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Abstract: Environmental pollution due to human activities is a major challenge in the present world. Due to rapid urbanization and industrialization people are using a various of chemicals, these are polluting our environment. Around 8000 chemical products associated with the dyeing process are listed in the Colour Index. Degradation of dye is a complex process works to detoxify, decolorize, and degrade the dyes are done in lab scale only. Hence there is the need of effective complete conversion of textile effluent into useful liquid waste by using microbes is necessary. Biological processes convert organic compounds to water and carbon dioxide in a low cost, sustainable and are easy to use. Degradation of dye effluent is cheap, effective and also non toxic. Different fugal strains are isolated from painted walls and the sample plated on PDA media.Totally 8 different strains were identified through colony morphology and lactophenol cotton blue stain method.Among 8 different fugai , Aspergillus was isolate for this study. The comparative ability of dye effluent degradation by UV mutation . The results showed that highest percentage of dye effluent with mutant strain was 98.41% whereas the wild strain showed 96.95%. The enzyme estimation results showed wild type 540 µg/ml and the mutatnt strain showed 740 µg/ml.

Key words*:* Effluent degradation, Organic compounds, Lactophenol cotton blue, Mutant strain

I. Introduction

Environmental pollution is mainly caused by release of various chemicals from various industrial progress which has now become a persistent environmental contaminant. Due to rapid urbanization and industrialization a lot of chemicals including dyes, pigments and aromatic molecular structural compounds were extensively used for several industrial applications such as textiles, pharmaceuticals, printing food, toys, paper, plastic and cosmetics are manufactured and used in day-to-day life. Textile dyes were classified as five different types such as azo, diazo, cationic, basic, anthraquione and metal complex based, depending on thenature of their chemical structure. There are more than 100,000 commercially available dyes with over 7 x 105 tons of dyestuff produced annually. Around 8000 chemical products associated with the dyeing process are listed in the Colour Index [1].

In the present world Environmental pollution due to human activities is a major challenge .Textile,cosmetics, pharmaceuticals and dying industry effluents constitute a major source of water pollution. Dyes or their breakdown products are highly toxic and carcinogenic for living organisms[2]. Growing environmental pollution from rapid industrial developments is one of the major challenges confronting the modern world . The 10 to 15% of the dye releases from textile industry which finds its way in to waste water . It mainly comprised of residual dyes, auxiliary chemicals, surfactants, chlorinated compounds and salts [3].

Dyes chemical structure was resistant to fading on exposure to light, water and many chemicals . Discharge of colored effluents from dye manufacturing units and textile processing industries is an major environmental pollution . The production of high amount of effluents mixes into water leading to pollution especially the aquatic systems and represent major environmental problems. Color of the dye effluent was one of the most obvious indicators of water pollution. Discharge of highly colored synthetic dye effluents can damage the receiving water bodies. Colored wastewaters associated with the reactive azo dye constitute approximately 30% of the total dye market **[4].**

Due to the over population in india and their increased demand for textile products, the textile industry and its waste water have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide. Approximately 100000 commercial dyes and dyestuff are used in the coloring of different industries like textile, cosmetic and leather around 10-15% of all dyestuff are directly lost to wastewater. Particularly, azo dyes are the most commonly used synthetic dyes in textile, food, paper-making and cosmetic industries **[5] .**

However, release of residual azo dye into industrial effluents mainly affect the water quality not only because of their color which result in aesthetic problems and affects aquaticplants photosynthesis, but also because many azo dyes from wastewater and their breakdown products are toxic and/or mutagenic to various forms of life and especially they can cause a significant impact on human health due to their mutagenic and carcinogenic effects. In addition to the environmental problem, the textile industry consumes large amounts of potable water. In many countries where potable water is scarce, this large water consumption has become intolerable and wastewater recycling has been recommended in order to decrease the water requirements and also recycling of dyes to be used again. Without adequate treatment, these dyes are stable and can remain in the environment for an extended period of time. Therefore, this effluent must be treated before discharge into natural water streams.**[6].** Biological treatment of dye effluent is very cheap, effective and non toxic . Therefore this study evaluates the dye effluent degradation by isolating potent fungal strain. Isolate Aspergillus strain and check the ability to degrading dye effluent and their protease enzyme production.

II. Materials and Methods

2.1 Collection of sample

Sample collected from different painted walls in various places. The sample was scraped from painted walls in various places. The sample was serially diluted and inoculated in Potato Dextrose Agar medium plate, and was identified as per the standard procedure.

2.2 Collection of dye effluent

The dye effluents were collected from nearby places where the dye effluents were discharged from the small and medium scale textile dyeing units around Salem district, Tamilnadu. The highly coloured effluent from dye house utilizing dying as the raw material was collected in airtight brown bottle and stored for experiments.

2.3 Screening , Isolation and Identification of textile dye effluent decolorizing Fungi

1gram of the collected sample was added to 10 ml of sterile distilled water and mixed well. This suspension was serially diluted from 10^{-1} to 10^{-7}. Later, 0.1 ml of each dilution was spread on the surface of PDA which is containing 0.01% Chloramphenicol and 1% Benomyl by using an L Shaped glass rod and incubated at 28°C for 7days. Here the Benomyl used is autoclaved together with the media whereas Chloramphenicol was added to the media after autoclaving. The well grown fungal colonies were identified by lacto phenol cotton blue staining method. Isolate the aspergillus sps and identified by lactophenol cotton blue stain.

2.4 Srain Improvement

For the comparative study the strain was improved by UV mutation technique . After UV treatment the wild and mutated strain both are compared for its decolorizing capacity.

2.5 Cell Suspension

For preparation of cell suspension, 7 days old PDA slant culture was used. Through 100 ml of distilled water 3.9 g of PDA agar was used. Then PDA slant was prepared. Through this PDA slant the culture was inoculated. Then the tubes were incubated at 37˚C for 7 days. After incubation 10 ml of saline and 10 ml of tween 80 was added through the PDA slant. This solution was mixed with culture.

2.6 UV mutation

Cell suspension was used for UV mutation. 4 ml of cell suspension was poured in 5 Petri plates. These plates were placed in UV light at distance of 30 cm away from UV lamp. The different time exposures are 5, 10, 15, 20 &25 minutes. Each plate was stored in dark over night. The strain growth was better in 5 minutes exposed plate than the other plates .Hence the strain from the 5 minute exposed plate was taken for decoolurisation study. This was followed by Bhargavi Moturi, *et al.*, 2010.

2.7. Decolourisation study using wild and mutant strain

In 100 ml of Potato Dextrose Broth 10 ml of Aspergillus broth culture of both wild and mutant strain was added individually in two different flasks and 10 ml of textile effluent was added and incubated at 37˚C. The 5ml treated textile dye effluent was centrifuged at 10,000 rpm for 10 min and decolorisation was assessed by measuring the absorbance of the supernatant at 520nm using spectrophotometer. The percentage of decolorisation was calculated using the following formula

% of Decolorisation = Initial OD-Final OD x 100/Initial OD.

This was according to Manikandan.N, *et al.*, 2012.

2.8 Decolourisation Determination

The decolourisation rate was studied in different time intervals for both wild and mutant strain. The 5ml treated textile dye effluent was centrifuged at 10,000 rpm for 10 min and decolorisation was assessed by

measuring the absorbance of the supernatant at 520nm using spectrophotometer. The wild type strain shows 96.95% decolourisation on 10 th day and mutant type shows 98.41% of decolourisation.

III. Results

3.1 Screening and Isolation of fungi

A total of 8 different fugal strains were isolated from painted walls collected from different places.These strains were identified by colony morphology and also lacto phenol cotton blue staining method.

Fig:1 Sample collected from painted wall

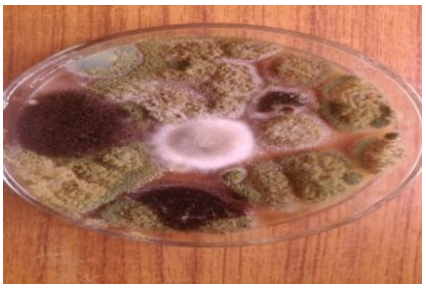

fig:2 plate showing different fungi

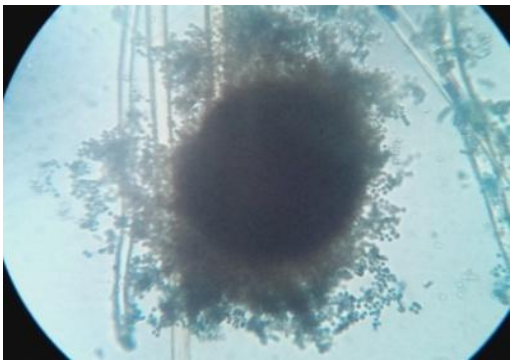

Fig:3 Microscopic view of *Aspergilus sps*

3.2 Strain Improvement

The strain was improved for better decolourisation using physical mutagen (UVexposure) to different time intervals and the strain from the 5 minutes mutant plate was taken for decolourisation study.

3.3 Decolourisation Determination of wild and mutated strain

The decolourisation rate was studied in different time intervals for both wild and mutant strain. The 5ml treated textile dye effluent was centrifuged at 10,000 rpm for 10 min and decolorisation was assessed by measuring the absorbance of the supernatant at 520nm using spectrophotometer. The wild type strain shows 96.95% decolourisation on 10 th day and mutant type shows 98.41% of decolourisation .

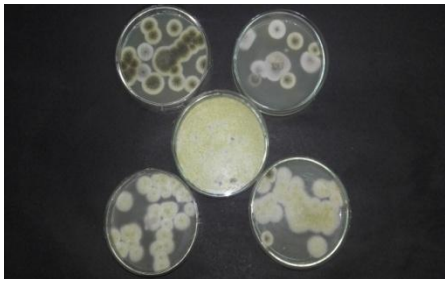

Fig: 4 Plate showing mutant strain

Fig:5 Decolourisation using wild strain

fig:6 Decolourisation by mutant strain.

Table :1 Decolourisation Rate Using Wild Strain

Colour of the effluent and quantity taken	Microorganism and its quantity	Incubation in days	Control	Sample	% of decolourisation
10 ml of brown colour effluent taken in 100 ml of PD broth	10 ml of Wild strain	0	0.134	1.973	0
		2	0.366	1.760	10.79
		4	0.701	1.233	37.60
		6	0.901	0.780	60.46
		8	1.325	0.160	91.85
		10	1.403	0.061	96.95

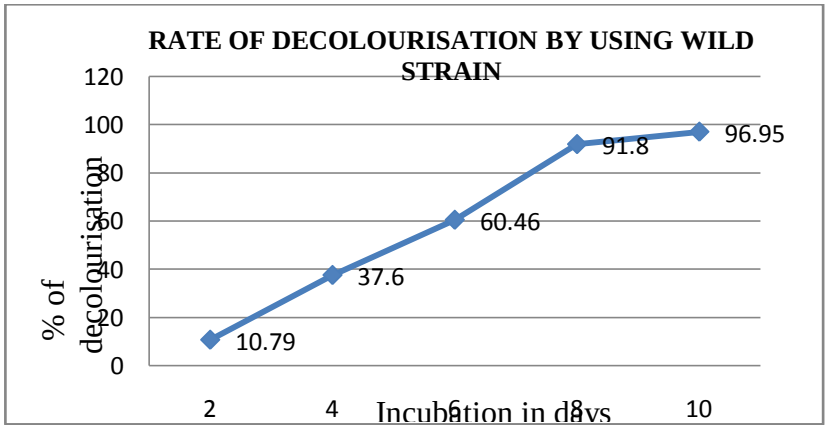

Fig:7 Decolourisation Rate Using Wild Strain

Table:2 Decolourisation Rate Using Mutant Strain

Colour of the effluent and quantity taken	Microorganism and its quantity	Incubation in days	Control	Sample	% of decolourisation
10 ml of brown colour effluent taken in 100 ml of PD broth	10 ml of Mutant strain	0	0.143	2.520	0
		2	0.295	1.621	35.67
		4	0.315	0.520	79.36
		6	0.405	0.170	93.25
		8	0.765	0.132	94.76
		10	1.328	0.040	98.41

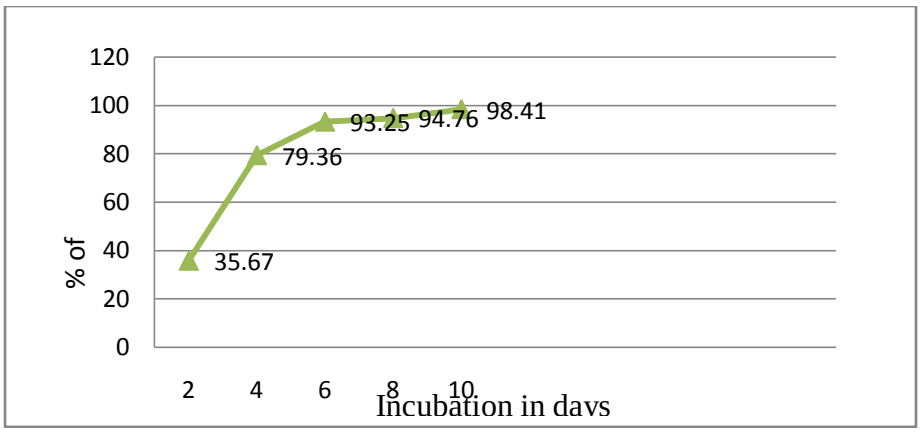

Fig: 8 Decolourisation Rate Using Mutant Strain

3.4 Enzyme Estimation:

The enzyme production was also estimated by using Lowry's method .It was found that mutant strain decolorize the effluent in higher rate when compared to wild type. The concentration of Enzyme produced by wild type was540 µg/ml whereas the mutant strain was740 µg/ml.

Table :3 Amount of enzyme produced using wild and mutant strain

Enzyme	Enzyme produced in concentration of µg/ml by wild type	Enzyme produced in concentration of µg/ml by mutant type
Protease	540	740

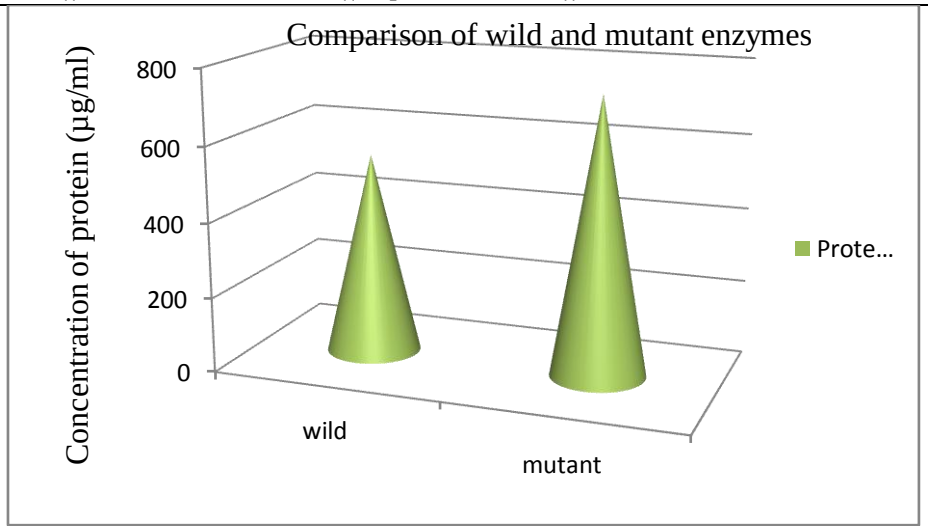

Fig :9 Comparison of wild and mutant enzymes

IV. Discussion

In the present study painted wall sample was collected from various places . The collected sample was cultured on potato dextrose agar media supplemented with Benomyl and Chloramphenicol. Particularly Benomyl was used to select wood decay fungi and Chloramphenicol is used to inhibit the unwanted bacterial growth. Benomyl and Chloramphenicol on PDA media for selecting wall paint. Among wall paint fungi particularly Aspergillus niger was identified through direct morphological identification [8].

Then the Aspergillus niger were screened for protease production on mineral salt medium supplemented with casein .If the isolated white rot fungi produce protease a clear halo around the colonies will be observed and confirms that Aspergillus niger are capable of producing protease enzyme[9].They observed a halo around the colonies with the presence of casein and confirmed the production of hydrolytic enzyme. Guiacol and Syringaldazine for screening laccase producers. They observed reddish brown colour zone around the colonies in the presence of Guiacol on PDA media and pale yellow zone in the presence of Syringaladazine[10]. The laccase enzyme was assayed by using 7 days incubated culture filtrate used as enzyme source. An amount of 0.5 ml of enzyme was added to 1.0 ml of 0.1 M phosphate buffer (pH 4.5) followed by 0.5 ml guaiacol (0.4 M). The activity gave a brownish red colour.[7].Similar brownish red colour was formed indicating the laccase enzyme.The laccase and protease enzyme were produced by using particular media components[11].

Aspergillus niger were assayed for protease assay by using casein as substrate and Tris chloro acetic acid. Folin and ciocalteau reagent is added were after the incubation period it shows that the blue colour development. Hence similar blue colour was developed in present study assay using Aspergillus niger. The strains were then mutated by exposing to UV at different periods of 5,10,15,20 and 25 minutes. The mutants that were exposed to expensive period of 10 to 25 minutes radiation showed reduced growth rate. Only in 5 minutes exposed plate shows mild increase in growth rate. The strains that were exposed to UV radiation for a time period of 3, 6 and 9 min did not vary much from the wild strain in mycelial morphology as well as in the growth pattern, instead they showed mild increase in growth rate, but the mutants that were exposed to extensive period of time such as 12 and 15 min to UV radiation showed reduced growth rate as well as reduction in extension and branching of hyphae[7].

Effluent treatment methods are classified3 methoda includes into physical, chemical and biological methods. While some dyes are difficult to biodegrade few, particularly the hydrolyzed reactive and certain acidic dyes are not readily absorbed by active sludge; hence they escape treatment. Combination of various effluent treatment methods can be removes more than 85% of unwanted matter. The resulting effluent is generally high in color. A complimentary treatment process is needed to remove color and if possible residual impurities. The textile industry has been condemned to be the world's worst environment polluters. It requires large amounts of chemicals and water at every step of the textile manufacturing and finishing process. Water is needed to convey the chemicals into the fabric and to wash it at the beginning and end of every step. It becomes full of chemical additives and is then expelled as wastewater; which in turn pollutes the environment [12]. Despite the existence of a variety of chemical and physical treatment processes, biological processes have the potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. The biological treatment methods are more desirable as they are eco friendly, do not produce secondary pollutants

and have a higher possibility of wider application. Synthetic dyes causes aesthetic pollution of waterways caused by the presence of dyes leached from textile factories since they are visible even in minute amounts. The presence of dyes could also potentially reduce the amount of sunlight reaching the bottom of rivers and lakes and thus affects the ability of water plants to carry out photosynthesis [13]. Many physicochemical treatment methods, including coagulation, flocculation, precipitation, oxidation, irradiation, incineration, and membrane adsorption, have been used for the treatment of dye-contaminated effluents [14].

The application of *Aspergillus niger* in large-scale waste treatment, however, has been impeded owing to the lack of an appropriate reactor system capable of coping with rather slow fungal degradation, loss of the extracellular enzymes and mediators with discharged water, and excessive growth of fungi. In this context, a feasible system may be envisaged by coupling the excellent degradation capability of the *Aspergillus niger* with the inherent advantages of a membrane bioreactor (MBR), yielding reduced excess sludge production. Three sulphonated phenylazonaphthol dyes with similar molecular structures, Orange 7, Acid Orange 8 and Mordant Violet 5 were selected and degraded by the *Aspergillus niger*. The live pellets of the fungus *Phanerochaete chrysosporium* were found to remove more than 95% of the color of this wastewater within 24 hours. The dye-removal capacity was a dependent on time and was proportional to the agitation rate. The optimum temperature was 30 degree C. The decolorization performance of live pellets remained high and stable for 5 days and they showed twice to thrice higher decolorization capacity than dead pellets[15].

The enzymes produced by *Aspergillus niger* are assayed qualitatively. Qualitative assays are powerful tools used in screening fungi for lignocellulose degrading enzyme production. Such tests give a positive or negative indication of enzyme production. They are particularly useful in screening large numbers of fungal isolates for several classes of enzyme, where definitive quantitative data are not required. The reagents required are all commonly available and relatively inexpensive. Improvement of biomethane production was achieved by subjecting the methanogens to mutagenic changes by physical (irradiation) and chemical (colchicine, acridine orange) mutagens [16].

Proteolytic enzymes are a large group of enzymes that cleave the peptide bonds of proteins to small fragments and amino acids. Fungi cause proteolysis of collagen which is dependent on many factors such as storage and environmental conditions, in addition to presence of certain substances that reside on the mummy's skin [17].

In our results, only one fungal isolate was able to produce proteolytic activity and showed that mutant strain have the potential to degrade dye effluent than wild strain.

V. Conclusion

Degradation of dye is a complex process works to detoxify, decolorize, and degrade the dyes are done in lab scale only. Hence the need of effective complete conversion of textile effluent into useful liquid waste by using microbes is required. Recent fundamental research works have revealed the existence of wide variety of microorganisms capable of decolorizing wide range of dyes. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages this process was relatively inexpensive, running costs were low and the end products were completely mineralized with no toxicity. Biological methods of removal involve the use of microorganisms such as fungi, bacteria, algae and actinomycetes to convert the pollutants into non-toxic harmless substances. Biological processes convert organic compounds to water and carbon dioxide in a low cost, sustainable and are easy to use. Treatment of textile dye effluent by physical and chemical methods have a high cost potential and a high sludge problem, whereas biological process convert organic compounds completely into water and carbon dioxide, have low cost and are easy to use. In this study Aspergillus niger, have the ability to degrade the dye effluent in significant manner. This study showed the Aspergillus sps have a potential to treat coloured wastewaters. From our study reported thatmutated strain of aspergillus sps is an ideal candidate for degradation of dye effluent**.**

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