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A Study on Mycotic Diversity of Tannery Effluent and Chromium Adsorption Capability

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Tannery effluents are of large-scale environmental concern because they colour and diminish the quality of water bodies and create adverse effects. The present study deals with isolation, identification and characterization of fungi isolated from tannery effluents collected from Ambur in Tamilnadu, India. Samples were studied from the period of April 2011 to March 2012 to know the occurrence and distribution of fungi. On serial dilution, it was found that a total of 21 fungal species belonging to 9 different genera were isolated. The collected data were subjected to statistical analysis like Standard error, ANOVA and species diversity index. Among the genera, *Aspergillus* was represented by 7 species followed by *Penicillium* with 6 species. The fungus, *Penicillium purpurescens* (644 CFU/ml), *Aspergillus japonicas* (461 CFU/ml), *Gliocladium roseum* (397 CFU/ml) and *Aspergillus flavus* (280 CFU/ml) showed their dominance. The average CFU/ml, percentage contribution and isolation frequency were calculated. Further, the fungal species like *Aspergillus flavus, A. japonicus, A. niger, Paecilomyces variotii* and *Penicillium citrinum* were selected based on their prevalent and dominant to study their Chromium adsorption ability. The order of adsorption showed, *Penicillium citrinum* > *Paecilomyces variotii* >*A. japonicus* > *A. niger* > *A. flavus*.

Key words: Tannery effluent, *Paecilomyces, Penicillium, Aspergillus,* ANOVA, Shannon index, Chromium.

The leather industry is one among the industries which occupies a prominent place in Indian economy due to its potency of growth, export and employment. India's share in global value added from this sector is around 1.8 percent in 2009[1]. The production capacity of leather hides and skin was around 65 and 170 million pairs per year respectively[2]. The effluent generated by this major industry pose a serious environmental threat as it is associated with the generation of large amount of polluted water during tanning process and chrome contaminated effluents[3].

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It is utmost important to find a solution to the pollution raised due to the development of these industries and microbes are one of the major bio-remedial measures to curtail the pollutants. Thus, the knowledge on the native flora of the polluted effluents is required and the studies on the microbial diversity will help in identifying the potential microbes in treatment of the pollutants like heavy metal. The microbial diversity of effluents like sugar industries[4], rubber industries[5] and dairies[6] were studied. However, scanty reports[7] are available on the study related to the diversity of microbes from the effluent of tanneries. Among the microbes, fungi have their own advantages like eukaryotic nature, better metal tolerant ability, stress tolerance, biomass and large surface area when compared to other microbes. Hence, in the present study the presence of mycotic diversity in the effluents of tannery from

Ambur, India was carried out.

Trivalent Chromium salts (Cr^{3+}), are used as tanning agents in leather industry. The oxidised trivalent becomes Hexavalent Chromium which is toxic to biological system. Conventional methods used for the removal of hexavalent Cr use chemical procedures, which are expensive and lack specificity. As an alternative, biological approaches utilizing microorganisms offer the potential for a highly selective removal of toxic metals coupled with considerable operational flexibility, hence they can be both *in situ* or *ex situ* in a range of bioreactor configurations[8]. There are certain microorganisms, which can survive in high oxygen demand, high concentrations of metals and have the potential to accumulate different metals. This is achieved by virtue of covalent interaction of metal at cell surface or within the cell by different processes[9]. In this regard, fungi are a versatile group as they can adapt and grow under various extreme conditions of pH, temperature and nutrients availability as well as high metal concentrations[10]. Considering the various mechanisms of metal resistance in fungi, it is expected that screening of metal tolerant fungi may provide strains with improved metal accumulation. In this study, an attempt was made to study the Chromium bioadsorption ability of the species isolated from leather tannery effluents of Ambur, India.

MATERIALS AND METHODS

Sampling Sites

Tannery effluent samples were collected from the final discharge point of the tannery in Ambur of Vellore district in Tamil Nadu. The samples were collected at monthly intervals from the period of April 2011 to March 2012. The effluent samples were collected in sterile polythene containers of 5 litres capacity and brought to the laboratory with due care and processed for the isolation of fungi immediately before storage.

Isolation of fungi

From the collected tannery effluent samples the fungi were isolated using serial dilution plate technique. One ml of diluted effluent sample is pipetted from 1/100 dilution into sterile petridishes and Potato Dextrose Agar (PDA) was used as an isolation media[11]. The antibiotic, Streptomycin (0.06 g/L) was used to arrest the growth of bacteria. Six replicates were maintained for each sample. The petridishes containing the effluent sample in PDA were incubated at room temperature 28 ± 2°C in a glass chamber for the isolation of fungi. The growing fungal colonies were isolated and identified after a period of 72-96 hours based on their morphological, i.e. microscopic and macroscopic characters[12-15].

Data Analysis

The results obtained were analysed and presented as average CFU/ml, relative frequency and isolation frequency as follows:

The colonies isolated were converted to Colony Forming Units (CFU)/ml as follows:

$$\text{Average CFU / ml} = \frac{\text{Total CFU / ml of a species}}{\text{Total number of samplings } (12)}$$

$$\text{Percent Contribution} = \frac{\text{Total CFU / ml of an individual species}}{\text{Total number of CFU / ml of all species}} \times 100$$

$$\text{Isolation Frequency} = \frac{\text{No. of samplings in which the species was recorded}}{\text{Total number of samplings } (12)} \times 100$$

Statistical Analysis

The data were subjected to statistical analysis to find out the standard deviation, standard error, one way analysis of variance (ANOVA) and the species diversity index (Shannon and Simpson's index) using SPSS software version 13.

Chromium reduction

The species *Aspergillus japonicus, Aspergillus niger, Aspergillus flavus, Paecilomyces variotii* and *Pencillium citrinum* were selected based on their prevalence and predominance for the study on their Chromium adsorption. The species were inoculated into the conical flask containing 100 ml of Potato Dextrose broth media and grown for a period of 12 days for the fungal mycelium to grow. These mycelial mats were harvested and 1 gram each was treated with deionized water of 100 ml possessing 1000 ppm/L concentration of Chromium.

They were kept in orbit shaker for 2 days and maintained at 28± 2°C arvested and digested.

Digestion of fungal mat for the analysis of Chromium

After a period of 2 days the fungal mat were filtered and digested for the analysis of Chromium using Nitric acid. The filtered sample was subjected to metal analysis through Atomic Adsorption Spectrophotometer. The analysis was carried in Chennai Mettex Lab Private Limited, Chennai.

RESULTS

A total of 21 species belonging to 9 genera were isolated from the tannery effluent samples collected from Ambur. Among the fungi isolated, 2 species belongs to Zygomycotina and the remaining 19 species belongs to Mitosporic fungi. Among the genera isolated, the genus *Aspergillus* was represented by maximum number of species (7) followed by *Penicillium* (6 species) and *Curvularia* (2 species). All other genera were represented by single species each.

A total average of 2708 CFU/ml of fungi was isolated from the leather tannery effluents from Ambur. The order of dominance of the fungi isolated is as, *Penicillium purpurescens* (644 CFU/ml), *Aspergillus japonicus* (461 CFU/ml), *Gliocladium roseum* (397 CFU/ml), *Aspergillus flavus* (280 CFU/ml), *A. terreus* (247 CFU/ml) *Penicillium corylophylum* (175 CFU/ml) and *Penicillium citrinum* (163 CFU/ml) showed their dominance. The number of species isolated, their average CFU/ml recorded, standard error and percent occurrence are given in Table 1. The percent

Table 1. List of species isolated, average CFU, standard error and Frequency occurrence of fungi from tannery effluent of Ambur

S. No.	Species	Average CFU/ml ± SE	Frequency Occurrence
1	*Absidia corymbifera*	2.78 ± 2.78	8.33
2	*Aspergillus flavipes*	13.89 ± 2.78	16.66
3	*Aspergillus flavus*	280.56 ± 43.77	100.00
4	*Aspergillus japonicas*	461.11 ± 43.09	75.00
5	*Aspergillus nidulans*	2.78±2.78	8.33
6	*Aspergillus niger*	100±33.88	91.66
7	*Aspergillus tamarii*	38.89±15.92	58.33
8	*Aspergillus terreus*	247.22±23.90	41.66
9	*Curvularia lunta*	41.67±4.81	16.66
10	*Curvularia brachyspora*	2.78± 2.78	8.33
11	*Geotrichum candidum*	11.11±2.78	16.66
12	*Gliocladium roseum*	397.22± 19.51	16.66
13	*Paecilomyces variotii*	13.89± 8.33	25.00
14	*Penicillium citrinum*	163.89±8.33	50.00
15	*Penicillium corylophilum*	175±12.90	33.30
16	*Penicillium funiculosum*	2.78± 2.78	8.33
17	*Penicillium oxalicum*	5.56± 2.78	8.33
18	*Penicillium purpurescens*	644.44±16.97	25.00
19	*Penicillium restrictum*	38.89±2.78	8.33
20	*Rhizopus stolonifer*	2.78±2.78	8.33
21	*Trichoderma* sp.	11.11±2.78	16.66
22	Non-sporulating colonies	50±13.89	50.00

Table 2. Species diversity index like Shannon index and Simpson Index studied for the fungal species isolated from Tannery effluent of Ambur

Species	2011									2012		
Diversty Index	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Shannon H' Log Base 10.	0.666	0.712	0.722	0.56	0.472	0.628	0.803	0.871	0.217	0.725	0.301	0.579
Simpsons Diversity (D)	0.289	0.244	0.242	0.359	0.443	0.266	0.19	0.156	0.679	0.229	0.496	0.276

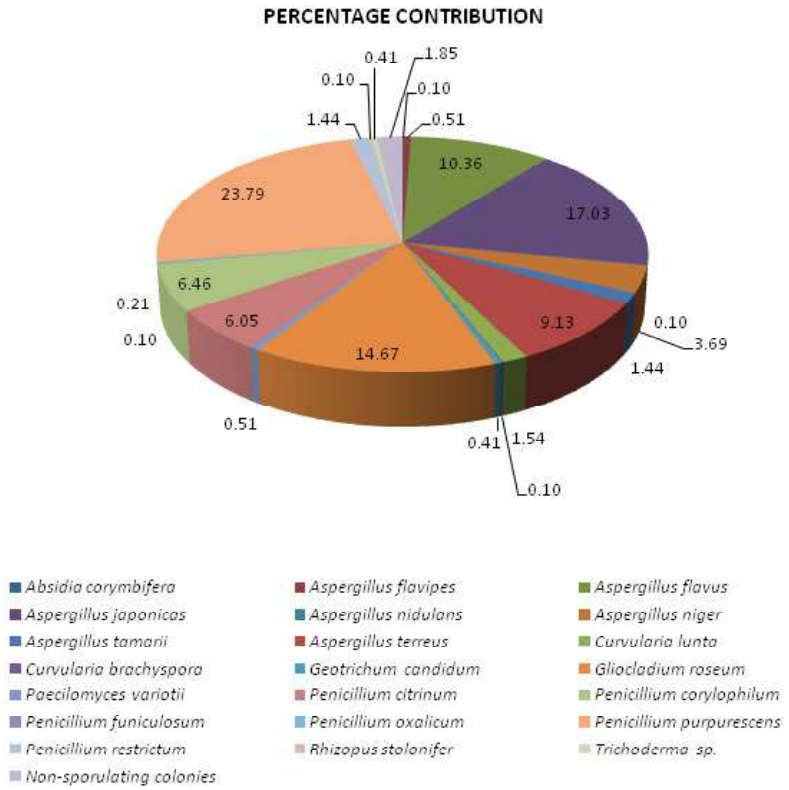

Fig. 1. Percent contribution of fungi isolated from Tannery effluent of Ambur

contribution of individual species along with the non-sporulating colonies is presented in Fig. 1.

The Shannon index and the Simpson's index on the species diversity is presented in Table 2. The analysis of variance by different month interval has been studied through SPSS software and the results give that the different species of the fungus in all the months showed significant difference at 5% level (Table 3).

The Chromium adsorption studies using Atomic Adsorption Spectrophotometer has resulted in maximum adsorption by the fungus, *Penicillium citrinum* (175 mg/L), *Paecilomyces variotii* (159 mg/L) and *Aspergillus japonicas* (132 mg/L). The other two species, *Aspergillus niger* and *Aspergillus flavus* have not recorded significant adsorption value for Chromium. The adsorption value recorded for different species of fungi is presented in Table 4.

DISCUSSION

Bioremediation is addressed as one example of an environmental biotechnology. Due to its comparatively low cost and environmental impact, bioremediation offers an attractive alternative for clean-up technologies[16]. Fungal biomasses are known to tolerate heavy metals[17, 18]. Malik[19], reported that microbial biomasses have responses to heavy metals through processes such as transport across cell membrane, biosorption through cell walls, entrapment by extracellular capsules, and precipitation along with transformation of metals.

Considering the fact that, fungi have the potency for tolerance to many factors and their advantage of biosurface and biomass, it is expected to screen the fungi of indigenous fungal strain. Only few studies have been carried to screen the fungi systematically from the individual contaminated sites for their biodiversity and biopotential towards bioadsorption[20,21].

The present study resulted in the isolation of more number of species, i.e. 21 species belonging to 9 different genera where they can be potentially used for bioremediation. However, their

Table 3. ANOVA table for the fungi recorded from tannery effluent of Ambur during different months

Months	Groups	Sum of Squares	Df	Mean Square	F
APR'11	Between Groups	41547878.79	21	1978470.418	768.112
	Within Groups	113333.333	44	2575.758	
	Total	41661212.12	65		
MAY'11	Between Groups	37741969.7	21	1797236.652	312.152
	Within Groups	253333.333	44	5757.576	
	Total	37995303.03	65		
JUN'11	Between Groups	26690909.09	21	1270995.671	226.718
	Within Groups	246666.667	44	5606.061	
	Total	26937575.76	65		
JUL'11	Between Groups	3397727.273	21	161796.537	39.55
	Within Groups	180000	44	4090.909	
	Total	3577727.273	65		
AUG'11	Between Groups	2124242.424	21	101154.401	83.452
	Within Groups	53333.333	44	1212.121	
	Total	2177575.758	65		
SEP'11	Between Groups	8592727.273	21	409177.489	75.016
	Within Groups	240000	44	5454.545	
	Total	8832727.273	65		
OCT'11	Between Groups	157575.758	21	7503.608	6.19
	Within Groups	53333.333	44	1212.121	
	Total	210909.091	65		
NOV'11	Between Groups	509242.424	21	24249.639	14.55
	Within Groups	73333.333	44	1666.667	
	Total	582575.758	65		
DEC'11	Between Groups	211515.152	21	10072.15	33.238
	Within Groups	13333.333	44	303.03	
	Total	224848.485	65		
JAN'12	Between Groups	515909.091	21	24567.1	16.214
	Within Groups	66666.667	44	1515.152	
	Total	582575.758	65		
FEB'12	Between Groups	24242.424	21	1154.401	3.81
	Within Groups	13333.333	44	303.03	
	Total	37575.758	65		
MAR'12	Between Groups	19545.455	21	930.736	1.536
	Within Groups	26666.667	44	606.061	
	Total	46212.121	65		

Table 4. Adsorption value of Chromium by different species of fungi isolated from Tannery effluent

S. No.	Species	Chromium adsorption mg/l
1	*A.flavus*	34.4
2	*A.japonicus*	132
3	*A.niger*	41.9
4	*Paecilomyces variotii*	159
5	*Penicillium citrinum*	175

prevalence is required to identify the potential metal removers. Few of the fungi although recorded as dominant species in this study were isolated only during specific months and they are not prevalent throughout the year in tannery effluent. The species like *Penicillium purpurescens, Gliocladium roseum, Aspergillus terreus* and *Penicillium corylophilum* are found to be seasonal in their occurrence. Hence, *Aspergillus japonicus, A. flavus, A. niger, Paecilomyces variotti* and *Penicillium citrinum* were selected for the study

on their bioadsorption of Chromium.

Biosorption can be defined as adsorption of pollutants on microbial biomass which are not dependent on the metabolism[22]. Recently microbes like fungi, algae and bacteria were successfully used as adsorbing agents[23]. Prasenjit and Sumathi[24] stated that microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant.

The high surface to volume ratio of fungi and their ability to detoxify metals are major reason that they are considered as potential agent for remediation. Different species of *Aspergillus*, *Phanerochaete*, etc., have been reported as efficient Chromium and nickel reducers[25,26]. Fungal cells, both living and dead such as *Aspergillus*, *Penicillium*, *Rhizopus,* and *Saccharomyces* have been applied to metal removal from aqueous streams using either batch or continuous modes[27,28,29]. Fourest and Roux[30] have used fungal mycelial biomass to remove heavy metals from water. Biosorption of hexavalent Chromium from aqueous solution by a tropical basidiomycete BDT-14 was reported by Trivedi *et al.,*[31]. The present study was aimed at investigating the growth of indigenous fungal strains in the tannery effluents from which they were isolated and identified and used for Chromium adsorption.

CONCLUSION

The present study resulted in isolation of 21 different fungal species from the tannery effluent. Among them, 5 different species were identified as most prevalent and predominant and were taken for further studies on Chromium adsorption. Among the fungal species studied, *Penicillium citrinum* showed maximum adsorption capacity followed by *Paecilomyces variotii.* These species can be further exploited for industrial remediation of effluents.

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