Vol.15 (1), Jan. – Mar. 2017

ENVIS NEWSLETTER

MICROORGANISMS AND ENVIRONMENT MANAGEMENT

(Sponsored by Ministry of Environment, Forest & Climate Change, Government of India)

# FUNGAL DIVERSITY OF INDUSTRIAL SEWAGES

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ARTICLE INFO

***Article history:***
Received 30 April 2017
Received in revised form 14 June 2017
Accepted 04 July 2017

***Keywords:***
*Mycoflora,*
*Industrial sewage,*
*Cooum River,*
*Diversity index*

ABSTRACT

Fungi in sewages are known as one involved in degradation of lignin, cellulose, hemicellulose, fermentation of ammonia and also used in bio removal of heavy metals and dyes. Their diversity varies according to the composition of sewage. Any data on the diversity, prevalence and predominance of fungal species is of importance to identify the specific fungal species for the application as remediators. In the present study, the variation of fungal diversity in sewages of three different industrial hubs of Chennai i.e., Ambattur, Chromepet and Guindy each possessing different types of industrial units were compared with Cooum River, the drainage for city's domestic sewage. A total of 1492 colonies were isolated from the samples and classified under 33 species belonging to 19 genera along with non-sporulating colonies. *Absidia corymbifera, Aspergillus flavus, A. fumigatus, A. nidulans, A. niger, A. tamari, A. terreus* and *Trichoderma viride* were commonly recorded in all the sites studied. The data obtained were subjected to statistical analysis like Jaccard co-efficient Index and Shannon diversity index. These indices imply that the fungal diversity differ according to the environment and nature of sewage.

## Introduction

Sewages are artificial ecosystems of unknown composition that varies from time to time and place to place. The nature of the sewage is defined by the condition of environment, nature of waste and traces of other substances like odour, taste, pesticides, steroids etc. (Cooke, 1970). Based on the human settlements and establishments such as industries and their function the effluent generated differs. Fungi in these sewages are important members serving in absorption of nutrients, digesting sludges and granular formation (Subramanian *et al.,* 2008). Sewages which are largely composed of organic matter (Strachan *et al.,* 1983) from diverse source (Maki, 1954) support growth of numerous fungi and act as a continuous nutrient source (Razi and Molla, 2007). However, the viability of fungi depends on the adaptation to the absurdity caused by contaminants.

The presence of fungi either as spores or vegetative cells, on synthetic or semi-synthetic substrates in sewages represent the diversity of native flora which can potentially be used in cleaning up of the environment (Vidali, 2007). These native flora are known to be involved in the degradation of lignin, cellulose, hemicellulose (Dashtban *et al.,* 2010), fermentation of ammonia (Hayatsu *et al.,* 2008), granular formation in sewage treatment processes (Weber *et al.,* 2009), bio removal of heavy metals (Dacera and Babel, 2008) and dyes (Rani *et al.,* 2014). Fungal biomass has been successfully used as adsorbing agents for removal of heavy metals (Prasenjit and Sumathi, 2005) and degradation of hydrocarbons (Zheng and Obbard, 2002). Further, the fungal enzymes such as peroxidases, tyrosinases and laccases (Cabana *et al.,* 2007) are found to play a major role in dye removal. Thus, any data on the diversity, prevalence and predominance of fungal species are of importance to identify the specific fungal species and exploitation of the same in bioremediation.

Though the studies on the occurrence and distribution of fungi in sewage sludge were widely reported (Avasn *et al.,* 2012; Matsunaga *et al.,* 2014; Pandey *et al.,* 2014), the diversity of fungi in sewage water is scanty. It is known that sewage water induces more stress due to its low nutrient composition, agitation and flow resulting in the variation of fungal diversity. Thus, in this study, the prevalence of fungal species from the sewage water of three major industrial hubs of Chennai city with different industrial activities, i.e. leather industries (Chrompet), metal finishing and electroplating industries (Ambattur) and electronic and light engineering industries (Guindy) were studied and compared with the Cooum River, one of the major rivers comprised mostly of domestic sewage as major constituent.

## Materials and methods

### Sampling site:

The samples of sewages were collected from different industrial hubs present in Chennai which is one among the major metropolitan cities in India. The city is situated between 13.04° N and 80.17° E on the East coast of India and its altitude is around 16m

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above mean sea level. Ambattur Industrial Estate has more than 2000 manufacturing units working on auto components, machineries, tools and accessories, welding, moulding, dyes, etc. Chrompet solely has tannery industries (Ramesh and Thirumangai, 2014) while Guindy, with a spread over area of 404 acres, houses about 700 units of industries dealing with electronics, readymade garments and light engineering works. The Cooum River which runs through the city is highly polluted by discharge of untreated sewage, industrial and domestic effluents especially at the downstream end. The sewage generated from these sites were studied for their mycological diversity.

## Sampling method:

The samples were collected from running sewages from respective industrial hubs, i.e. Ambattur, Chromepet and Guindy along with Cooum River as control using 250ml volume sterile bottles (Schott Duran, Germany). Around 100ml of sewage from 10 different spots of each industrial hub totaling 40 samples were collected and brought to the laboratory. The collected samples were stored in a refrigerator and samples were processed within a day interval.

## Isolation method:

The fungal colonies were isolated using serial dilution method. One ml of each sample was diluted in 10ml of sterile water and serially diluted to achieve $10^2$ concentrations. One ml from the diluted sample was directly plated to Petridishes with Potato Dextrose Agar (PDA) (Himedia, Mumbai) supplemented with Streptomycin (0.06 g/L) to arrest the bacterial growth for the isolation of fungi and maintained in triplicates at room temperature (28 °C ± 2 °C) (Prashanth *et al.,* 2012).

## Identification of Fungi:

The isolated colonies were identified after 4-5 days based on their morphological, macroscopic and microscopic features using the standard manuals up to the species level wherever possible (Udayaprakash, 2004). The fungi observed in their asexual stages in the laboratory were classified under Mitosporic fungi. Identification through direct microscopy is adapted in this study as this is widely used in industries to reduce the time and cost expenditure (Udayaprakash *et al.,* 2014).

## Presentation of Data:

The **average colony forming units** (CFU /ml) of sewage sample was calculated as follows:

$$\text{Average}(CFU/ml) = \frac{No.\ of\ colonies\ counted\ (Avg.of\ 3\ replicates)}{Volume\ of\ sewage\ sample\ (1ml)} * dilution\ factor(100)$$

The **percent contribution** is the ratio of individual fungal species to the total number of CFU of all species isolated which was calculated as,

$$\%\ \text{Contribution} = \frac{CFU\ of\ an\ individual\ fungal\ species}{\text{Total No. of CFU of all species}} * 100$$

## Jaccard Coefficient:

To describe the taxonomic affinity of mycotic flora among the various sewages of Chennai, Jaccard's coefficient (JI), was used to measure the similarity between pairs of samples (Arnold *et al.,* 2000).

$$\text{JI} = \frac{a}{\text{a} + \text{b} + \text{c}}$$

Where *a* represents the number of species occurring in both samples, *b* represents the number of species restricted to sample 1, and *c* represents the number of species restricted to sample 2. JI ranges from 0 (no taxa shared) to 1 (all taxa shared).

## Shannon Diversity index:

Shannon Diversity index represents the species diversity.

$$\text{Shannon index (HS)} = -\ \Sigma_j\,(p_j \ln p_j),\ j = 1 \ldots\ldots\ Np,$$

Where, Ni is the total number of individuals, Np is the number of species identified among these isolates, And $p_j$ is the proportion of individuals in the $j^{th}$ species

# Results and discussion

The study on mycofloral diversity from different industrial sewage waters resulted in an isolation of 1492 colonies classified under 33 species belonging to 19 genera. Among the species isolated 4 belong to Zygomycotina, 1 to Ascomycotina and remaining 28 species belonged to Mitosporic fungi. Among the genera recorded, the genus *Aspergillus* was represented by maximum number of species (10 species) followed by *Penicillium* (5 species). All other genera are represented by single species except that of *Fusarium* where it was represented by 2 species. The list of fungi isolated, their percent contribution recorded in respective sites is presented in Table 1.

Of the sewage samples collected from three different industrial hubs, Chrompet recorded with highest colony forming units (612 CFU/ml), followed by Ambattur (316 CFU/ml) and Guindy (197 CFU/ml) as the least. On contrary, diversity richness was found maximum at Guindy (18), followed by Ambattur (17) and least at Chrompet (15) (Fig 1). The pollutants present in the sewage are known to reduce the diversity of sensitive fungi, while increasing the diversity of less sensitive organisms (Cooke, 1970). The presence of organic pollutants generated from leather tanneries in Chrompet might have selected and made the adapted fungal species to thrive and proliferate when compared with other two industrial hubs. The samples of Cooum River showed 367 CFU/ml with 18 different species with high diversity richness as of Guindy and Ambattur.

The most dominant species isolated was *Aspergillus niger* (26.55%) followed by *Trichoderma viride* (22.26%), while *Aspergillus fumigatus* (13.21%), *Aspergillus terreus* (7.73%), *Aspergillus flavus* (5.63%) and *Absidia corymbyfera* (5.23%) were the other notable contributors. Difference in dominance of species was observed among the sites. The following species, *Trichoderma viride* and *Aspergillus niger* were recorded as dominant species from the sewages of Chrompet and Guindy. However third dominant species found to differ, it was *Aspergillus fumigatus* in Chrompet and *Aureobasidium pullulans* in Guindy. Similarly, in Ambattur and Cooum, it was *Aspergillus niger* recorded as most dominant species and the second dominant species found to differ. The difference

**Table1:** Average CFU/ml and Percent contribution of fungal species isolated from sewages of different industrial hubs in Chennai

| No. | Species | Cooum River (Control) | | Chrompet | | Guindy | | Ambattur | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average CFU ($x 10^2$) | % | Average CFU ($x 10^2$) | % | Average CFU ($x 10^2$) | % | Average CFU ($x 10^2$) | % |
| 1 | *Absidia corymbifera* | 0.06 | 0.540 | 2.30 | 11.18 | 0.2 | 2.96 | 0.03 | 0.28 |
| 2 | *Alternaria alternata* | - | - | 0.1 | 0.48 | - | - | - | - |
| 3 | *Aspergillus flavipes* | - | - | - | - | 0.03 | 0.44 | - | - |
| 4 | *A. flavus* | 0.23 | 1.91 | 1.26 | 6.16 | 0.4 | 5.93 | 0.9 | 8.41 |
| 5 | *A. fumigatus* | 0.4 | 3.26 | 3.66 | 17.83 | 0.33 | 4.89 | 2.16 | 20.1 |
| 6 | *A. glaucus* | - | - | 0.43 | 2.10 | - | - | - | - |
| 7 | *A. japonicas* | - | - | 2.1 | 10.21 | - | - | - | - |
| 8 | *A. nidulans* | 0.2 | 1.63 | 0.33 | 1.58 | 0.23 | 3.41 | 0.3 | 2.80 |
| 9 | *A. niger* | 5.03 | 41.14 | 4.03 | 19.61 | 1.1 | 16.32 | 3.03 | 28.3 |
| 10 | *A. ochraceus* | 0.03 | 0.27 | - | - | - | - | 0.03 | 0.28 |
| 11 | *A. tamari* | 0.03 | 0.27 | - | - | 0.03 | 0.44 | 0.26 | 2.42 |
| 12 | *A. terreus* | 0.93 | 7.62 | 1.03 | 5.02 | 0.13 | 1.92 | 1.76 | 16.44 |
| 13 | *Aureobasidium pullulans* | - | - | - | - | 0.46 | 6.82 | - | - |
| 14 | *Chrysosporium* sp. | - | - | 0.13 | 0.64 | - | - | - | - |
| 15 | *Curvularia lunata* | 0.03 | 0.27 | 0.13 | 0.64 | - | - | 0.23 | 2.14 |
| 16 | *Emericella nidulans* | - | - | - | - | 0.16 | 2.37 | - | - |
| 17 | *Fusarium oxysporum* | - | - | - | - | 0.03 | 0.44 | - | - |
| 18 | *F. moniliforme* | 0.06 | 0.54 | - | - | - | - | - | - |
| 19 | *Geotrichum candidum* | 0.36 | 2.99 | - | - | - | - | - | - |
| 20 | *Humicola grisea* | 0.26 | 2.17 | - | - | - | - | - | - |
| 21 | *Lasiodiplodia theobromae* | - | - | - | - | 0.06 | 0.89 | - | - |
| 22 | *Monilia sitophila* | - | - | - | - | 0.06 | 0.89 | 0.03 | 0.28 |
| 23 | *Mucor racemosus* | 0.1 | 0.81 | | | - | - | 0.06 | 0.56 |
| 24 | *Paecilomyces variotti* | - | - | 0.1 | 0.48 | 0.4 | 5.93 | 0.3 | 2.80 |
| 25 | *Penicillium citrinum* | - | - | - | - | - | - | 0.26 | 2.42 |
| 26 | *P. corylophilum* | 0.03 | 0.27 | - | - | - | - | - | - |
| 27 | *P. frequentans* | 0.03 | 0.27 | | | 0.16 | 2.37 | 0.13 | 1.21 |
| 28 | *P. funiculosum* | - | - | 0.06 | 0.32 | 0.03 | 0.44 | - | - |
| 29 | *P. oxalicum* | 0.03 | 0.27 | - | - | - | - | - | - |
| 30 | *Phialophora* sp. | - | - | - | - | 0.1 | 1.48 | - | - |
| 31 | *Rhizopus stolonifer* | 0.06 | 0.54 | - | - | 0.2 | 2.96 | 0.06 | 0.56 |
| 32 | *Syncephalastrum racemosum* | - | - | 0.4 | 1.94 | - | - | 0.03 | 0.28 |
| 33 | *Trichoderma viride* | 3.83 | 31.06 | 4.23 | 20.58 | 2.23 | 33.08 | 0.83 | 7.75 |
| | Non sporulating colonies | 0.5 | 4.08 | 0.16 | 0.81 | 0.4 | 5.93 | 0.3 | 0.28 |

between the dominant species is evident due to different nature of sewages emanating from different industrial hubs. Shannon diversity index and Shannon evenness index provided the difference between the species at different sites (Table 2).

**Fig. 1.** The average CFU and number of species recorded in sewages of different industrial hubs

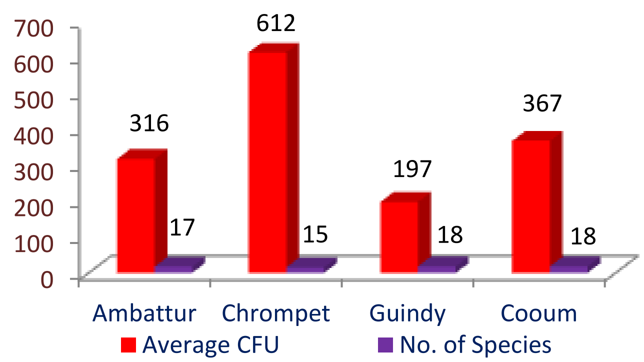


**Table 2:** Shannon diversity and evenness index recorded among the fungal species from sewages of different industrial hubs

| | Cooum River | Chrompet | Guindy | Ambattur |
|---|---|---|---|---|
| Shannon Diversity index | 4.185603 | 4.236164 | 3.018438 | 3.664098 |
| Shannon's Evenness index | 0.232533 | 0.282411 | 0.167691 | 0.215535 |

Out of 33 species isolated, nearly 16 species were reported as common inhabitants of sewage (Kacprzak *et al.,* 2005; Fleury, 2007). *Absidia corymbifera, Aspergillus flavus, A. fumigatus, A. nidulans, A. niger, A. tamari, A. terreus* and *Trichoderma viride* were commonly recorded in all the sites studied. The most dominant genera recorded was *Aspergillus* (10 species) followed by *Penicillium* (5 species), and *Fusarium* (2 species) which were in accordance with Zubeiry (2005) from Yemen. However, this was found to differ in sewage sludges where *Penicillium* was reported as dominant genus (Cooke and Pipes, 1970).

Jaccard similarity index showed chrompet had the least similarity index than the other industrial hubs. As inferred from similarity index, Chrompet had 38.46% and 45.45% species in common with species isolated from Guindy and Ambattur respectively, while Ambattur and Guindy shared 52% of their total species diversity (Table 3). However, compared with control Cooum River, Chrompet had 34.61% similarity, Guindy with 40.74% and Ambattur had 68.18% species similarity.

Few species reported in this study are widely identified as a potential fungal species for bioremediation. This includes, *Aspergillus niger* (Jamal *et al.,* 2005), Penicillium sp. and *Paecilomyces* sp. (Subramanian *et al.,* 2006 ), *Trichoderma* sp., (Verma *et al.,* 2007). Among these, *Aspergillus niger* and *Trichoderma viride* were found to be one of the dominant species in all industrial sewage types studied. Therefore, data on the diversity, prevalence and predominance of fungal species are of importance and further studies from different geographical zones with different sewage types are highly recommended.

**Table 3:** Jaccard's Similarity Coefficient among the fungal species recorded from sewages of different industrial hubs

| | Cooum River | Chrompet | Guindy | Ambattur |
|---|---|---|---|---|
| Cooum River | 1 | | | |
| Chrompet | 0.3461 | 1 | | |
| Guindy | 0.4074 | 0.3846 | 1 | |
| Ambattur | 0.6818 | 0.4545 | 0.52 | 1 |

## Conclusion

The present study has revealed the diversity of fungi in sewages of different industrial hubs, i.e. Ambattur, Chromepet and Guindy of Chennai. The fungal diversity was found to differ according to the environment and nature of sewage when compared with Cooum River as control. A total of 33 species belonging to 19 genera were recorded from the sewages among which *Aspergillus niger, Trichoderma viride* and *Aspergillus terreus* are found to be the dominant species. The isolated species from sewage water can be potentially used in bioremediation for the removal of heavy metals, toxic compounds and dyes after monitoring their epidemiology.

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