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Identification and Characterization of the microbial communities found in electronic industrial effluent and their potential for bioremediation

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ABSTRACT

Microbial communities are dynamic systems that develop depending on the ecological niche in which they survive. Electronic industry effluent, rich in heavy metals and salts is one such ecosystem where diverse heavy metal resistant microbes exist. Taxonomic identification of this microbial community would be interesting as no information on the microbial diversity from electronic industry effluent is available till date. Our paper attempts to characterize the microbial inhabitants of this niche. Culture dependent microbiological methods were used to establish and identify various microbial species from the effluent. Culture independent methods of identification involving biochemical tests and molecular biology based methods like 16 S- r DNA sequencing and lipid analyses (FAME analysis) were also carried out to confirm the identity of isolated species. Our study, first of its kind revealed the presence of a diverse group of resistant aerobic microbes and disclosed a total of ten bacterial and two fungal isolates. All these isolates were found to survive in presence of heavy metals like cadmium, lead and zinc and were resistant to antibiotics like ampicillin, tetracycline, streptomycin, penicillin and chloramphenicol as indicated by their Minimum Inhibitory Concentrations (MIC). Such resistant isolates harbor possibilities of metal adaptive/selective pathways which render them as economically beneficial bio-sorbent alternatives in bioremediation of heavy metals.

1. Introduction

The production of electric and electronic equipment is one of the fastest growing areas due to the technological innovation and market expansion that is accelerating with time and demand. The production of electric and electronic equipment (EEE) is increasing worldwide. This surge in the EEE has resulted in an increase of waste electric and electronic equipment (WEEE). Due to the existing, inefficient recycling processes, environmental pollution from this industry with toxic heavy metals is rapidly spreading throughout the globe (Gupta et al., 2001; Widmer et al., 2005). These heavy metals are persistent in nature and because of their indestructible property, cannot be removed from the environment (Sharma et al., 2009).

Microorganisms are the pioneer colonizers in the environment, and represent the richest repertoire of microbial and molecular diversity in nature. Owing to their ubiquitous nature, these organisms are able to adapt to virtually any ecological niche, be it natural or manmade. Irrespective of the nature of the environment, microorganisms survive in any given diverse habitats. The available literature on the microorganisms characterized till date represents only a tiny fraction of the natural diversity.

Reports on characterization of industrial effluents are limited. Detailed characterization of the pesticide industry effluent revealed the presence of species belonging to the genera *Alcaligenes, Bacillus, Pseudomonas, Brevundimonas, Citrobacter, Pandoraea* and *Stenotrophomonas* (Rani et al., 2008), Faryal and Hameed described the presence of fungal species like *Rhizopus, Aspergillus, Penicillium, Candida, Drechslera* and *Rhodotorula* in the effluents of a textile industry (Faryal and Hameed, 2005). Parameshwari et al. reported the isolation of metal-tolerant fungi including *Aspergillus niger, Phanerochaete chrysosporium* and *Trichoderma viride* from municipal sewage contaminated soil (Parameswari et al., 2010). Sarkar et al. isolated and characterized novel bacteria from a mineral-ore site in Andhra Pradesh, India, revealing its rich biodiversity. The cultures were identified as *Pseudomonas fluorescens*, *Janibacter anopheles*, *Bacillus licheniformis* and *Acinetobacter baumannii* (Sarkar et al., 2008). These culture isolates were screened for their potential metal accumulation and all of them were found to be resistant to a wide range of heavy metals. Characterization of the electroplating industry effluents depicted the presence of *Staphylococcus, Bacillus and Macrococcus* genera (Varadarajan and Shikha,

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2014). Majority of the organisms isolated from all of these effluents belonged to the genera *Bacillus* and *Pseudomonas*. Adverse ecological habitats like the electronic industry effluents can also act as niche to harbor heavy metal resistant microorganisms. The nature of microbial diversity of many such effluents remains uncharacterized.

Microorganisms and microbial products can be highly efficient bio accumulators of soluble and particulate forms of metals especially from dilute external solutions. Microbes have the ability to bind to metal ions present in the external environment at the cell surface or to transport them into the cell for various intracellular functions (Mishra and Young-Ha, 2010). Moreover, bacteria have developed resistance to these metals and are able to make them less toxic and innocuous (Yoshida et al., 2006). Hence, heavy metal resistant microorganisms show us possible methods to prevent metal pollution which utilize their natural heavy metal disposing abilities (Olaniran et al., 2013). Therefore, the use of heavy metal resistant microorganisms for decontaminating heavy metals has attracted growing attention owing to its low cost, environmental green procedure of metal removal and overall is aesthetically pleasing (Juwarkar et al., 2010). Thus, microorganisms with unique abilities such as metal adsorption, accumulation or resistance can be identified among naturally occurring organisms from a heavy metal contaminated habitat.

Though, microbial characterization of some industrial effluents are available in literature, there is no data till date, on the characterization of an electronic industry effluent that has high levels of heavy metals, phosphates and sulphates as their major hazardous constituent. Hence, this study focuses on isolation of resistant strains and characterization of the microbial diversity in the electronic industry effluents. This will open new arenas for application of these isolates for the remediation of industrial effluents. Hence, the present study deals with isolation, identification and characterization of heavy metal resistant microorganisms from an industrial electronic effluent and its potential utilization in the preparation of a novel bioadsorbent.

2. Materials and methods

2.1. Collection points and transport of effluents

The electronic industrial effluent samples were collected from battery industry located near Hyderabad. They were collected from various discharge points of the industry involving the entry points of the reverse osmosis unit and the common effluent treatment plant. Samples were collected in sterile plastic falcons and transported to our laboratory for further bacteriological and chemical characterization.

2.2. Physical and chemical characterizations of the effluents

The effluent sample was analysed for a number of characteristics i.e; colour, odour, turbidity, pH, Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Chlorides and sulphates, total alkalinity and various metals (Ogunfowokan and Fakankun, 1998).

Table 2
Chemical characterization of the effluent.

Characteristic parameter	Values
pH	12.0
Zinc	4.0 ppm
Cadmium	1.0 ppm
Nickel	22.5 ppm
Aluminium	Nil
Chromium	Nil
Magnesium	5.5 ppm
Lead	2.5 ppm
Calcium	49.1 ppm
Sulphates	3.28 gm/l
Chlorides	0.16%
TDS	49,626 ppm
Alkalinity	1096 ppm
BOD_5	27.39 ppm
COD	437.21 ppm

3. Enumeration of the bacterial, fungal and actinomycetes community in the effluent

Determination of the microbial population was done using spread plate count method with serial dilutions of the effluent. Presence of bacterial, fungal and actinomycetes population in the samples was assessed by inoculating the effluent samples in Nutrient Agar (NA), Sabourauds Dextrose Agar medium (SDA) and Actinomycetes Isolation Medium (AIM) respectively (Smrithi and Usha, 2012). Isolation experiments were performed in replicates of three for the effluent samples collected twice from the points mentioned above.

4. Identification of the isolated microbes

4.1. Biochemical identification

Morphologically distinct colonies were selected from the mixed cultures of different dilutions, from different collection points, which were subsequently developed into pure cultures. Morphological identification with Gram's staining reaction and motility test was performed for the isolated strains. Biochemical identification was carried out with Catalase, IMVIC, carbohydrate fermentation and starch hydrolysis to establish the biochemical patterns of the strains. These tests helped in arriving at a genus level identification (Bergey and Holt, 1994; Pagnanelli et al., 2000) of the isolates.

4.2. Minimum inhibitory concentration (MIC)

The isolates being obtained from a heavy metal rich electronic industry effluent, were anticipated to be resistant to a range of heavy metals. To prove this, as the next step, the MIC of the isolated strains for various metals and antibiotics was performed using turbidometric analysis (Aleem et al., 2003). The minimum concentration of heavy metal/antibiotics at which the bacterial growth was inhibited was considered as MIC. Metals under consideration, in the present study were lead, cadmium, zinc and chromium. The heavy metal salt solutions were prepared using metal salts $CdCl_2$, $ZnSO_4$, $K_2Cr_2O_7$, and $PbCl_2$.

Table 1
Exposed metal ion concentration.

Metal salt	Mol wt of salt (g/mol)	At wt of metal ion (g/mol)	1000 (ppm)	50 (ppm)	100 (ppm)	150 (ppm)	200 (ppm)	250 (ppm)	300 (ppm)	350 (ppm)	400 (ppm)	450 (ppm)
$CdCl_2$	183.32	112.4	1.63	0.08	0.16	0.24	0.32	0.40	0.48	0.57	0.65	0.73
$ZnSO_4$	161.47	65.38	2.46	0.12	0.24	0.36	0.49	0.61	0.73	0.86	0.98	1.10
$K_2Cr_2O_7$	294.185	103.98	2.82	0.14	0.28	0.42	0.56	0.70	0.84	0.98	1.12	1.26
$PbCl_2$	278.1	207.2	1.34	0.06	0.13	0.20	0.26	0.33	0.40	0.46	0.53	0.60