FREQUENCY DISTRIBUTION OF BACTERIA ISOLATED FROM DIFFERENT INDUSTRIAL EFFLUENTS

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Abstract: *Industrial system involves physical and chemical treatment as well as biological processes. Therefore, waste treatment systems such as the industrial effluents depend on the activities of communities of living organism. In this study, an attempt was made on the identification of the bacterial population involved in different industrial effluents. A total of thirty bacterial strains were isolated from glass, textile and pharmaceutical effluent samples on L.B. agar plates. A few, however, were re-cultured on other recommended media for verification of diagnostic characteristics. Maximum numbers of bacterial species were isolated from textile effluent. The results showed that a gram-negative bacillus with a yellow pigment was considered as a major group of the population. These are also being used in different waste water and metal treatment plants all over the world.*

Keywords: *Bacterial identification, industrial effluents, gram negative.*

1. Introduction

The identification of microorganisms involves comparison of an unknown microbe with similar microbes that are already known, thus eventually the former unknown is named. Both processes depend on adequate information for characterizing the known unit. Many methods have been proposed for making such information readily available, some are based on the use of dichotomous keys and others on diagnostic keys and tables [1].

Bacteria survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche [2]. As well as play their role in providing basic material for the development of pharmaceutical drugs, agrochemicals, bioremediation and biocontrol agents, food/drink agents, toiletries and products for other industries[3]. Bioremediation, a process that exploits the catalytic abilities of living organisms to enhance the rate or extent of pollutant destruction, is an important tool in attempts to mitigate environmental contamination [4,5]. The majority of bacteria are present in different industrial effluents include *Thiobacillus, Acinetobacter, Achromobacter, Nitrosomonasr, Nitrobacter, Achromobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus* and *Pseudomonas* [6,7,8].

The goal of the present work was to isolate and to identify the bacteria which may be present in different industrial effluents.

2. Materials and Methods

2.1 Collection of Samples

Effluent water samples were taken from ten randomly selected areas of glass, textile and pharmaceutical industries. All samples were placed in separate sterile bottle and stored in a refrigerator at 4^{o}C till use.

2.2 Isolation of Unknown Bacterial Species

Using an aspetic technique, the standard dilution methods in Luria Bertani (L.B) agar plates was used for recovery of bacteria from different samples [9]. Primary isolation was effected by streaking sample on the surface of a dry L.B agar plate. Such plates were then incubated at 37^{o}C for 48 to 72 h. Single colonies were removed from these plates and sub-cultured for isolation and purification.

2.3 Identification of Unknown Bacterial Species

Identification of bacterial species was done by recording macroscopic and microscopic characters. The purified colonies were subjected to gram staining and characterized using

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biochemical tests and consulting the pertinent literature. [10,11,12].

3. Results

A total of 30 bacterial species were identified from glass, textile and pharmaceutical effluent samples (Table 4). They were isolated in pure culture on L.B. agar media. The quantitative estimates of the microbial population are shown in Table 1. The morphological and physiological characteristics of the cultures are given in Table 2. As it shown out of thirty isolates, nine of them were gram-positive and ten were gram-negative. Most of the strains produce yellow pigment on L.B. agar plates. Further examinations were carried out on the cultures (Table 3). The comparison of results with Bergey Manual showed that nine gram-negative bacilli isolates were identified as genus of *Flavobacterium, Cupriavidus, Enterobacter, Pseudomonas, Yersinia, Proteus, Klebsiella, Serratia* and *Acinetobacter*. The other gram-negative coccus was identified as genus *Bordetella*. Six strains of gram-positive cocci were identified as genus of *Staphylococcus, Micrococcus, Trichococcus, Deinococcus, Syntrophospora* and *Vagococcus.* The percentage of bacterial species in sample 1 was high as compared to other samples (Fig. 1).

4. Discussions

The bacteria predominant in different industrial are largely derived from water courses. Basically the bacteria are responsible for the degradation of organic and inorganic compounds. They derive their nutritional requirement from the compounds presented to them in the influent waste. They are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the feed, together with other elements. They derive their energy from oxidizing either organic compounds (chemoorganotrophic metabolism), or inoraganic compounds (chemolithotrophic metabolism), such as reduced sulfur or nitrogen compounds. They use the energy for their bodily functions, reproduction and growth. Many research reported that a large number of bacterial species isolated from different industrial effluents [1]. *Pseudomonas* species are regarded as one of the most common species of bacteria degrading phenolic compounds isolated from contaminated sites of different industries [13]. Six bacterial strains, two *Pseudomonas*, *Pantoea* sp, *Chryseomonas luteola*, *Proteus peenneri* and *Serratia* sp, were capable of growing in the presence of wastewater of petrochemical industry [14]. *Pseudomonas fluorescens putida* is also a promising bacterium, found in water and soil and also recognized in the degradation of non conventional compounds and useful in environmental bioremediation [15]. In another study, sixty bacterial strains were screened for hydrocarbon degradation in 2004 [16].

The results of present work also indicated that gram-negative bacillus bacteria constituted the majority of species in the industrial effluents. In this study the majority of the isolated gram-negative bacteria belonged to the genus *Pseudomonas,* while two of isolated belonged to genus *Bacillus.* The presence of gram-positive bacteria has been reported by some worker. In this study common gram-positive found from each effluent belonged to the genus *Micrococcous.* These bacterial strains are novel addition in the micro-diversity of industrial effluents of Pakistan and can be studied for treatment of industrial waste water. The bacterial isolates described here are potentially useful for removing contaminating compounds in effluents. So, additional investigations are needed to optimize the conditions for evaluation metal removal capacities of isolated bacterial species for large scale operations.

Table 1: Number of Bacterial Colonies from Different Industrial Effluents

Dilutions	No of Bacterial colonies		
	Sample	Sample 2	Sample 3
10^{-0}	>78	>56	>69
10^{-1}	>64	>43	>55
10^{-2}	>52	>34	>46
10^{-3}	47	26	32
10^{-4}	33	17	23
10^{-5}	24	09	12

Table 2: An Outline of the Morphological and Physiological Characteristic of Bacteria Isolated from Different Industrial Effluents

Bacterial genus	Morphology	Gram reaction	Spore	Motility	Pigment
Staphylococcus sp.	cocci	+	-	-	Yellow
Micrococcus sp.	cocci	+	-	+	Yellow
Trichococcus sp.	cocci	+	-	-	Yellow
Deinococcus sp.	cocci	+	-	-	Yellow
Syntrophospora sp.	cocci	+	+	-	-
Flavobacterium sp.	rod	-	-	-	Yellow
Vagococcus sp.	cocci	+	-	+	White
Microbacterium sp.	rod	+	-	-	Yellow
Bordetella sp.	cocci	-	-	-	Yellow
Cupriavidus sp.	rod	-	-	+	-
Enterobacter sp.	rod	-	-	+	-
Aureobacterium sp.	rod	+	-	-	Yellow
Pseudomonas sp.	rod	-	-	+	-
Bacillus sp.	rod	+	+	+	Yellow
Yersinia sp.	rod	-	-	+	-
Proteus sp.	rod	-	-	+	Yellow
Klebsiella sp.	rod	-	-	-	Yellow
Serratia sp.	rod	-	-	+	-
Acinetobacter sp.	rod	-	-	-	Yellow

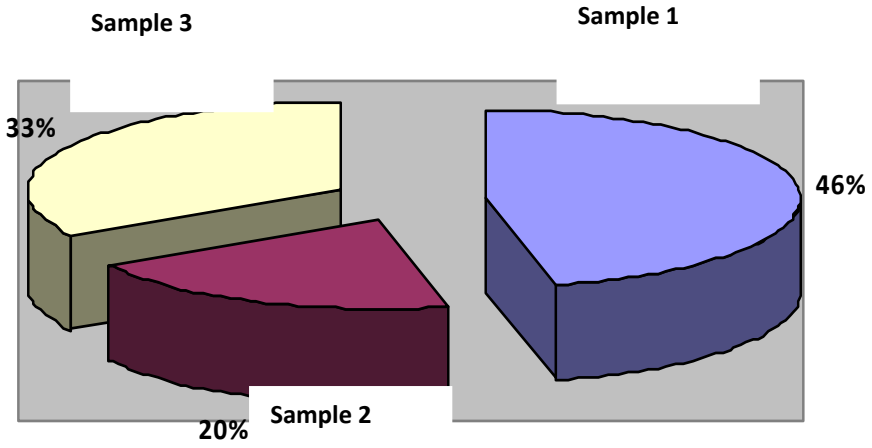

Fig. 1: Percentage of Bacterial Species Isolated from each Sample

Table 3: An Outline of Biochemical Tests Used in Classifying the Isolated Bacterial Species from Different Industrial Effluent

Biochemical tests	No. of Bacterial species																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Oxidase	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+
Catalase	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	+	-	-	-	-
H_2S	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Glucose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
Mannitol	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
ONPG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V.P	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Gelatine	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Malonate	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
Inositol	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-	+	+	+
Arabinose	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+
Raffinose	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Arginine	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+_

Table 4: List of Metal Tolerant Bacterial Species

Sample no	Place Of Collection	Date of Collection	Medium Cultured On	Effluent pH	Isolated Bacterial Species
1	Textile industry, Sheikhupora	11th November 2009	LBA	6	*1. Staphylococcus intermedius* *2. Micrococcus sedentarius* *3. Trichococcus* sp. *4. Micrococcus dentarius* *5. Staphylococcus caprae* *6. Deinococcus proteolyticus* 7. *Syntrophospora* sp. *8. Micrococcus varians* *9. Flavobacterium aquatile* *10.Vagococcus* sp. *11.Microbacterium lacticum* *12.Bordetella pertusis* *13.Staphylococcus carnosus* *14.Micrococcus* sp
2	Glass industry, Sheikhupora	16th September 2009	LBA	6	*15.Cupriavidus necator* *16.Synthophosphora sp.* *17.Enterobacter aerogenes* *18.Aureobacterium flavescense* 19.*Micrococcus* sp. 20.*Pseudomonas* sp.
3	Pharmaceutical industry, Lahore	28th October 2008	LBA	8	*21.Bacillus subtilus* *22.Yersinia* sp. *23.Proteus merabillis* *24.Klebsiella pneumoniae* *25.Pseudomonas fluorescens* *26.Pseudomonas malophilia* *27.Serratia marcescens* *28.Bacillus* sp. *29.Acinetobacter lwoffii* *30.Klebsiella pneumoniae*

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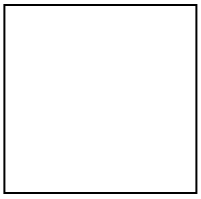

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