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 Thioacillus, Acinetobacter, Achromobacter, Nitrosomonas, 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°C till use.

2.2 Isolation 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...
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 inorganic 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

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>No of Bacterial colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
</tr>
<tr>
<td>10^-0</td>
<td>&gt;78</td>
</tr>
<tr>
<td>10^-1</td>
<td>&gt;64</td>
</tr>
<tr>
<td>10^-2</td>
<td>&gt;52</td>
</tr>
<tr>
<td>10^-3</td>
<td>47</td>
</tr>
<tr>
<td>10^-4</td>
<td>33</td>
</tr>
<tr>
<td>10^-5</td>
<td>24</td>
</tr>
</tbody>
</table>
Table 2: An Outline of the Morphological and Physiological Characteristic of Bacteria Isolated from Different Industrial Effluents

<table>
<thead>
<tr>
<th>Bacterial genus</th>
<th>Morphology</th>
<th>Gram reaction</th>
<th>Spore</th>
<th>Motility</th>
<th>Pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Micrococcus</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Trichococcus</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Deinococcus</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Syntrophospora</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Flavobacterium</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Vagococcus</em> sp.</td>
<td>cocci</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>White</td>
</tr>
<tr>
<td><em>Microbacterium</em> sp.</td>
<td>rod</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Bordetella</em> sp.</td>
<td>cocci</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Cupriavidus</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Aureobacterium</em> sp.</td>
<td>rod</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>rod</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Yersinia</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Klebsiella</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Serratia</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter</em> sp.</td>
<td>rod</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>No. of Bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+</td>
</tr>
<tr>
<td>Lysine</td>
<td>-</td>
</tr>
<tr>
<td>Ornithine</td>
<td>-</td>
</tr>
<tr>
<td>H$_2$S</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
</tr>
<tr>
<td>ONPG</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
</tr>
<tr>
<td>V.P</td>
<td>-</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
</tr>
<tr>
<td>TDA</td>
<td>-</td>
</tr>
<tr>
<td>Gelatine</td>
<td>-</td>
</tr>
<tr>
<td>Malonate</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
</tr>
<tr>
<td>Adonitol</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 4: List of Metal Tolerant Bacterial Species

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Place Of Collection</th>
<th>Date of Collection</th>
<th>Medium Cultured On</th>
<th>Effluent pH</th>
<th>Isolated Bacterial Species</th>
</tr>
</thead>
</table>
| 1         | Textile industry, Sheikhupora | 11<sup>th</sup> 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 pertussis  
 |           |                     |                    |      | 13. Staphylococcus carnosus  
 |           |                     |                    |      | 14. Micrococcus sp.  |
| 2         | Glass industry, Sheikhupora | 16<sup>th</sup> 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 | 28<sup>th</sup> October 2008 | LBA | 8 | 21. Bacillus subtilis  
 |           |                     |                    |      | 22. Yersinia sp.  
 |           |                     |                    |      | 23. Proteus mirabilis  
 |           |                     |                    |      | 24. Klebsiella pneumoniae  
 |           |                     |                    |      | 25. Pseudomonas fluorescens  
 |           |                     |                    |      | 26. Pseudomonas malolitica  
 |           |                     |                    |      | 27. Serratia marcescens  
 |           |                     |                    |      | 28. Bacillus sp.  
 |           |                     |                    |      | 29. Acinetobacter lwoffii  
 |           |                     |                    |      | 30. Klebsiella pneumoniae  |

### References


Amna Ali has earned her M.Sc (Hons.) and B.Sc (Hons.) degree in Agriculture with Plant Pathology as her major discipline in 2007 and 2010 respectively. She is also doing Ph.D in same subject from Institute of Agricultural Sciences (IAGS), University of Punjab, Lahore, Pakistan. She is now working as Research Associate in IAGS, under the mega project of “First Fungal Culture Bank of Pakistan (FCBP)”, Pakistan. She is the author of numerous scientific publications. She has participated in several National Conferences for presenting her research papers and already published 10 papers in National/International journals.