Bangladesh J Microbiol, Volume 29, Number 2, December 2012, pp 84-88

Original Article

Isolation, Characterization, and Identification of Bacterial Population from Textile and Tannery Effluents of Bangladesh

A Hakim[1], S Hoque[2] and S M Ullah[3]

[1]Dept. of Soil Sciences, Chittagong University, Bangladesh, [2,3] Dept. of Soil, Water and Environment, University of Dhaka, Bangladesh.

Ten effluent samples from two different sites located at Hazaribagh tannery belt and Dhaka EPZ, Savar were collected. This study aimed to compare the bacterial composition isolated from tannery and textile effluents and to investigate the occurrence of metal toxicity tolerant and dye degrading bacteria and to select the potential strains for the use in bioremediation. The average bacterial count of HT and DETDE varied in between 3.35×10^6 and 5.45×10^6 cfu/mL and 4.8×10^6 and 7.75×10^6cfu/mL, respectively. A total of 12 bacterial isolates were characterized as strains of *Bacillus, Staphylococcus, and Pseudomonas*. 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-positive bacillus with a yellow pigment was considered as a major group of the population. Among them three isolates were identified based on alignments of partial sequence of 16S rRNA gene. These are also being used in different wastewater and metal treatment plants all over the world.

***Key words*: Isolation, effluent, 16S rRNA.**

Introduction

The industrial effluents contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes, phenols, carbonates, alcohols, cyanide, heavy metals etc[1]. Leather industries and tanneries generate massive by-products, solid wastes, high amounts of wastewater rich in organic wastes with different loads of pollutants and emissions into the air.

Microbial survival in polluted environment depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation[2].

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[3]. In recent years identification of unknown bacterial isolates has been enhanced via PCR amplification of 16S rRNA gene sequencing and subsequent sequence analysis. Portions of the 16S rRNA gene sequences within bacteria are identical in all known bacteria[4].

Bacteria survive in contaminated habitats because they are metabolically capable of utilizing available resources and can occupy a suitable niche[5]. As well as play their role in providing basic material for the development of pharmaceutical drugs, agrochemicals, bioremediation and bio-control agents, food and/drink agents, toiletries, and products for other industries[6]. 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[7]. The majority of bacteria are present in different industrial effluents include *Thiobacillus, Acinetobacter, Achromobacter, Nitrosomonasr Nitrobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus and Pseudomonas* [8,9]. The objectives of the present work are to compare the bacterial composition isolated from tannery and textile effluents and to investigate the occurrence of metal toxicity tolerant and dye degrading bacteria and to select the potential strains for the use in bioremediation.

Materials and Methods

Collection of samples

Effluent samples were collected from ten randomly selected areas of tannery (Hazaribagh) and textile (Dhaka EPZ, Savar) industries. All samples were placed in separate sterile bottle and brought to the laboratory and stored in a refrigerator at 4°C till use.

Isolation of unknown bacterial species

The heterotrophic bacteria were counted by the pour-plate technique. Using an aspetic technique, the standard dilution methods in Luria Bertani (L.B) agar plates was used for recovery of bacteria from different samples[10]. Primary isolation was effected by streaking sample on the surface of agar plate. Such plates were then incubated at 37°C for 48 to 72 h. Single colonies were removed from these plates and sub-cultured for isolation and purification.

Corresponding Author:
Dr. Sirajul Hoque, Professor, Dept. of Soil, Water and Environment, University of Dhaka. Mobile: 01816013622. Email: sirajswedu@gmail.com

Identification of unknown bacterial species

Identification of bacterial species was done by recording morphological cultural, and microscopic characters. The purified colonies were subjected to Gram staining and characterized by using biochemical tests and consulting the pertinent literature[11, 12, 13].

Sequence based identification of isolates

The isolates can be identified based on alignment of partial sequence of 16S rRNA gene with the existing sequences available in the database. In the present experiment three different isolates were used to amplify their 16S rRNA gene. Polymerase chain reaction (PCR) amplified DNA of the 3 isolates was gel purified using phenol freeze method and sent for automated sequencing (Applied Biosystem 3130). The sequence generated from automated sequencing of PCR amplified DNA was analyzed through NCBI BLAST (http://blast.ncbi.nlm.nih.gov/) program to find out possible similar organism through alignment of homologous sequences.

Results and Discussion

The average bacterial count of Hazaribagh tannery (HT) and Dhaka EPZ textile dyeing effluent (DETDE) varied in between 3.35×10^6 and 5.45×10^6 cfu/mL, 4.8×10^6 cfu/mL and 7.75×10^6cfu/mL, respectively (Table 1). In a study Saha *et al*. (2006) reported the average bacterial load in the textile effluent as in the range of 6.1×10^7 to 19.4×10^7 cfu/ml.

A total of 12 bacterial species were identified from tannery and textile effluent samples and among these three were identified by 16s rRNA gene sequencing. They were isolated in pure culture on Luria-Bertani (LB) agar media. The quantitative estimates of the microbial population are shown in Table 1. The morphological and biochemical characteristics of the cultures and bacterial isolates are given in Table 2 and 3. On the basis of Gram reaction 11 isolates were found as Gram positive and the remaining 1 isolate was Gram negative. Most of the strains produce yellow pigment on nutrient agar plates. Further examinations were carried out on the cultures. The comparison of results with Bergey's Manual showed that six strains of Gram positive bacilli isolates were identified as *Bacillus megaterium, B. thuringiensis, B. glabisporus, B. firmus, B. lentus, B. cereus, B. pumilus,* other two Gram positive cocci were identified as genus *Staphylococcus* and only one strain was identified as *Pseudomonas pseudoalcaligenes*.

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 metabolic functions, reproduction, and growth. Many research reported that a large number of bacterial species were 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[14].

Sequence analysis of 16S ribosomal RNA (16S rRNA) was performed of the isolates by amplifying the 16S rRNA genes by PCR using the bacterial universal primers 27f and 1492r. The PCR products purified through alcohol precipitation were sequenced directly using a DNA auto sequencer (Applied Biosystem 3130). The most closely related sequences were found using the BLAST programs. In order to identify the bacterial isolates, their 16S rRNA genes were amplified and sequenced. Strain TA-1 was affiliated to *Bacillus megaterium* strain H2 (99% similarity), strain TX-1 to *Staphylococcus saprophyticus* strain AUCASVE3 (99%), and strain TX-3 to *Staphylococcus saprophyticus* strain A20 (92%) (Table 4).

The results of present work also indicated that Gram-positive bacillus bacteria constituted the majority of species in the industrial effluents. In this study the majority of the isolated Gram-positive bacteria belonged to the genus *Bacillus*, while two of the isolates belonged to genus *Staphylococcus* and remaining one is *Pseudomanas*. These bacterial strains are novel addition to the micro-diversity of industrial effluents of textile and tannery and can be used for treatment of industrial wastewater. The bacterial isolates described here are potentially useful for removing contaminating compounds in effluents. So, further studies are needed to optimize the conditions for evaluation of metal removal and detoxifying capacities of isolated bacterial species for large scale operations.

Staphylococcus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. *Staphylococcus* can cause severe food poisoning. It has been identified as the causative agent in many food poisoning outbreaks and is probably responsible for even more cases in individuals and family groups than the records show.

It is evident from the present study that the effluents are highly contaminated with bacteria. High bacterial load indicates that the water bodies contain huge amount of organic pollutants. Bacterial load and presence of pathogenic organisms are comparable in both of textile and tannery effluent.

Table 1: *Bacterial counts (cfu/mL) of Hazaribagh tannery and Dhaka EPZ textile dyeing effluent samples grown on two isolating media under aerobic condition.*

Sample No.	Nutrient Agar (cfu/ml)		Modified Nutrient Agar (cfu/ml)		Average (cfu/ml)	
	HT	DETDE	HT	DETDE	HT	DETDE
S-1	4×10^6	6×10^6	4.2×10^6	6.9×10^6	4.1×10^6	6.45×10^6
S-2	3.2×10^6	7.5×10^6	3.5×10^6	8×10^6	3.35×10^6	7.75×10^6
S-3	4.5×10^6	5.5×10^6	4×10^6	5.7×10^6	4.25×10^6	5.6×10^6
S-4	5.2×10^6	6×10^6	5.7×10^6	6.2×10^6	5.45×10^6	6.1×10^6
S-5	4×10^6	4×10^6	4.3×10^6	5.6×10^6	4.15×10^6	4.8×10^6

* HT (Hazaribagh tannery); **DETDE (Dhaka EPZ textile dyeing effluent)

Table 2. *Morphological and biochemical characteristics of the Tannery isolates*

Morphological and Biochemical Characteristics	Strain NO.				
	TA-1	TA-2	TA-3	TA-4	TA-5
Morphological Characteristics					
Form	Circular	Circular	Circular	Circular	Circular
Elevation	Effuse	Umbonate	Umbonate	Effuse	Umbonate
Margin	Entire	Entire	Undulate	Erose	Undulate
Surface	Smooth	Smooth	Rough	Smooth	Smooth
Color	White	Offwhite	Offwhite	Orange	white
Shape and arrangement of cells	Rods, rounded end, occur in chain	Short rod, rounded end, occur in singly	Rods, rounded end, occur in chain	Short rod, rounded end, occur in singly	Rods, rounded end, occur in chain
Motility	-	-	+	+	+
Biochemical Charactertics					
Gram reaction	+	-	+	+	+
Oxidase test	-	+	-	+	-
Catalase test	+	+	+	+	+
Oxygen requirement	Facultative anaerobes	Strictly aerobes	Facultative anaerobes	Not done	Strictly aerobes
Starch hydrolysis	+	-	-	-	-
Gelatin liquefaction	+	+	+	+	+
VP test	_	_	_	_	_
MR test	_	_	_	+	_
Deamination of Phenylalanine	_	_	_	_	_
Utilization of Citrate	-	_	_	+	_
Acid production from D- Glucose	+	_	+	+	+
Gas production from D- Glucose	_	_	_	_	_
Name of the isolates	*Bacillus megaterium*	*Pseudomonas pseudoalcaligenes*	*Bacillus thuringiensis*	*Bacillus glabisporus*	*Bacillus firmus*

Table 3. *Morphological and biochemical characteristics of the Textile isolates*

Morphological and Biochemical Characteristics	Strain No.						
	TX-1	TX-2	TX-3	TX-4	TX-5	TX-6	TX-7
Morphological Characteristics							
Form	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Elevation	raised	convex	raised	Effuse	Effuse	Convex	Convex
Margin	Entire	Undulate	Entire	Erose	Undulate	Undulate	Erose
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Concentric
Color	OffWhite	OffWhite	Slightly yellow	OffWhite	OffWhite	OffWhite	White
Shape and Arrangement of cells	Cocci, occur in singly, and tetrads	Rods, rounded end, occur in chain	Cocci, occur in singly, and tetrads	Rods, rounde-d end, occur in chain	Rods, rounded end, occur in chain	Rods, rounded end, occur in chain	Rods, rounde-d end, occur in chain
Motility	-	-	-	_	+	_	+
Biochemical Charactertics							
Gram reaction	+	+	+	+	+	+	+
Oxidase test	-	+	-	-	-	-	+
Catalase test	+	+	+	+	+	+	+
Oxygen requirement	Facultative anaerobes	Facultati-ve anaerobes	Facultative anaerobes	Faculta-tive anaerobes	Facultative anaerobes	Strictly aerobes	Facultative anaerobes
Starch hydrolysis	-	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+
VP test	+	-	+	-	+	-	-
MR test	+	+	+	+	+	-	+
Deamination of Phenylalanine	-	-	-	-	-	-	-
Utilization of Citrate	-	-	+	-	-	+	-
Acid production from D- Glucose	+	+	+	+	+	+	+
Gas production from D- Glucose	+	_	+	_	_	_	_
Name of the isolates	*Staphylococ-cus* saprophyticus	*Bacillus* firmus	*Staphylococ-cus* saprophytic-us	*B. lentus*	*B. cereus*	*B. pumilus*	*B. lentus*

Table 4. *Bioinformatics of the strains able to grow well on and use azo-dye as carbon source and also reduce chromium*

Name of the isolates	Name and Accession number of the Bacterial strain having highest similarity		Basis of the bioinformatics parameters					
	Accession Number	Strain Name	Score	Bits	Query coverage	Expect value	Identities	Gaps
TX-1	JQ043188.1	*Staphylococcus saprophyticus* strain AUCASVE3	1000	541	89%	0.0	99%	2/551 (0%)
TX-3	HQ323432.1	*Staphylococcus saprophyticus* strain A20	451	244	55%	2e-123	92%	10/333 (3%)
TA-1	JQ579631.1	*Bacillus megaterium* strain H2	1002	542	90%	0.0	99%	3/562 (1%)

References

1. Cooper P. 1995. Colour in dyehouse effluent. Society of dyers and colourists, The Alden Press, Oxford.
2. Bahig AE, Aly EA, Khaled AA and Amel KA. 2008. Isolation, characterization and application of bacterial population from agric soil at Sohag Province, Egypt. *Malaysian Journal of Microbiology* **4**(2): 42- 50.
3. Yazdi MKS, Azimi C and Khalili MB. Isolation and identification of bacteria present in the activated sludge unit, in the treatment of industrial waste water. *Iranian J. Publ. Health*. **30** (3-4): 91-94.
4. Pepper IL, and Gerba CP. 2005. *Environmental Microbiology,* 2nd edn. Elsevier Academic Press publications. Burlington, MA 01803, USA.
5. Ilyina A, Castillo MI, Villarreal JA, Ramirez G and Candelas J. 2003. Isolation of soil bacteria for bioremediation of hydrocarbon contamination. *Appl. Environ. Microbiol*. **64**: 4180.
6. Kate CH. 1995. COSHH and the Microbiologist. *Letters in Applied Microbiology* **10**:109-112.
7. Autry AR and Ellis GM. 1992. Bioremediation: An effective remedial alternative for petrolium hydrocarbon-contaminated soil. *Environmental Progress*. **11**(4): 318-321.
8. Lightharst B and Oglesby RT. 1989. Bacteriology of an activated sludge wastewater treatment plant. A guide to methodology. *J. Wat. Pollut. Control Fed*. **41**: 267-81.
9. Pick EB. 1995. Aerobic bacteria. In *Ecological Aspects of Used-water Treatment*. CR Cruds and HA Awkes (eds.). Academic Press, London.
10. Kamil Z, Rizk M, Saleh M, and Moustafa S. 2007. Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol. *Global Journal of Molecular Sciences* **2**(2): 57-66.
11. Holt JG, Krieg NR, Sneath PHA, Staley JT and Stanley TW. 2000. *Bergey's manual of determinative bacteriology*, 9th edn. Lippincott Williams and Wilkins, New York, USA.
12. Konem EW, Allen SD, Janda WM, Schreckenberger PC and Winn Jr. 1997. *Atlas and textbook of diagonistic microbiology*, 5th edn. Lippincott Williams & Wilkins, 530 Walnut Street, Philadelphia, PA 19106-3621 USA.
13. Benson HJ. 1996. *Microbiological applications, laboratory manual in general microbiology*. W. M. C. Brown Publishers, Dubuque, USA.
14. Lin J, Reddy M, and Qoma BE. 2008. Bacterial removal of toxic phenols from an industrial effluent. *African Journal of Biotechnology* **7**(13): 2232-2238.