



**RESEARCH ARTICLE**

**Isolation, Identification and Characterization of Microbial Species from Sugar Mill Effluent and Affected Soil**

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**ABSTRACT**

Soil pollution caused by innumerable industrial effluents has become a thoughtful problem. The sugar industries throughout the world are discharging enormous quantities of common salts during the processing of cane juice for making sugar. These salts get deposited into the soil when the effluent comes in contact with the soil. The ingredients of the effluent react with soil clay complex leading to accumulation of salts resulting in increase in the amount of exchangeable sodium and other nutrients. Large number of microorganisms has been detected in such affected soil and sugar mill effluents. This paper gives us the types of microorganisms present in effluents and affected soil.

**Key words:** effluents, microorganisms, pollution, soil, salts.

**INTRODUCTION**

A study on the presence of type of microorganisms present in the soil of fields from nearby areas of sugar mill and sugar mill effluents has been carried out in this study.

**MATERIALS AND METHODS**

The present study is carried out on sugar mill waste of Cooperative Sugar Mills, Meham, Haryana, India and on soil of nearby areas. This sugar mill is located at 28°59'49.2"N 76°14'30.1"E. Soil samples were taken from field which is irrigated with sugar mill effluents for last few years.

**RESULTS AND DISCUSSION**

**Identification and Characterization of Fungal Isolates:**

Identification and characterization of fungal isolates were done on the basis of colony growth (diameter), presence or absence of aerial mycelium, color of the colony, presence of furrows in the medium, pigment production, spore morphology *etc.* The fungal species (lactophenol-cotton blue preparation) were identified by microscopic analysis using standard procedure (Barnett and Hunter, 1972; Ellis, 1976; Domsch *et al.*, 1980; Nelson *et al.*, 1983; Gilman, 1998).

The major cultural features used for identification included color of the colony, growth pattern, mycelial structure, spore bearing structure, and spore morphology. The identified species are:

**First Identified Fungus F1:**

First identified fungus strain was *Aspergillus sp.* F1 (Figures 1 (A) and (B), which is a filamentous fungus (mould) frequently reported from indoor environments that are used in different value added properties like food and enzyme industries. Various species of *Aspergillus* are used in the manufacturing of citric acid, gluconic acid and many other products of commercial importance. *Aspergillus sp.* can be isolated from many different ecological habitats and it is characterized by a round vesicle with extending conidial chains, appearing as white and fluffy strands. Taxonomic as well as identification points are given below:-

**Taxonomic Classification:**

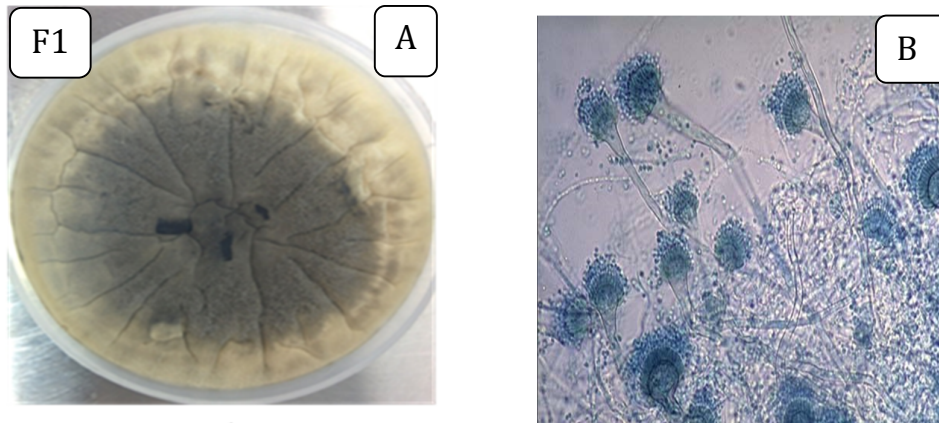
Kingdom	:	Fungi	Division	:	Ascomycota
Class	:	Eurotiomycetes	Order	:	Eurotiales
Family	:	Trichocomaceae	Genus	:	<i>Aspergillus</i>

**Identification Features:**

1. Colonies grows very quickly.
2. Colony produced with yellow to white hyphae, turning black with the formation of conidia. Hyphae are septate.
3. Conidiophores are long and globose at the tip.
4. Spores that are globose with conspicuous ridges not arranged in rows.
5. Colony diameter was found 10-12 mm in size after 4 days of incubation on PDA plates
6. Conidia slightly roughened or finely echinulate.

**Second Identified Fungus F2:**

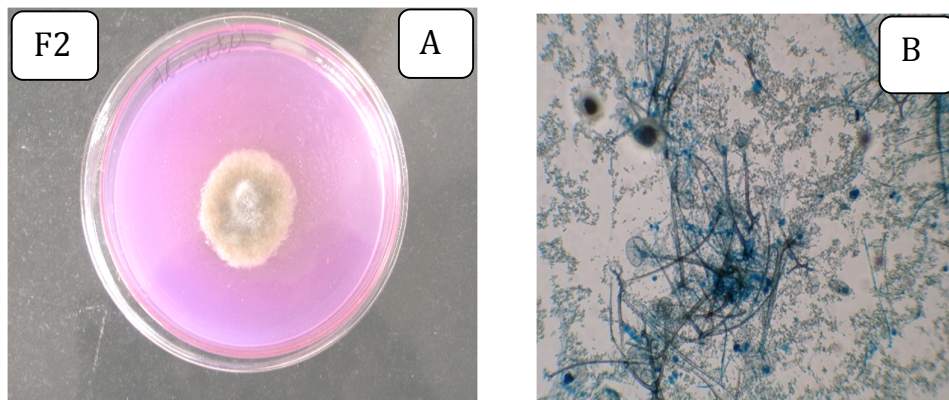
Second identified fungus was *Rhizopus* sp. F2 (Figure 2 (A) and (B)), which is a cosmopolitan filamentous fungus commonly isolated from soil, decaying plant products, animal feces, and old bread. Besides being common contaminants, *Rhizopus* species are also random causes of serious infections in humans. Certain species of *Rhizopus* are plant pathogens also.



**Fig. 1:** First identified isolated fungus *Aspergillus* sp. F1 (A): showing growth on agar plate (B): morphological features under microscope (100x)

**Taxonomic Classification:**

Kingdom	:	Fungi	Phylum	:	Zygomycota
Order	:	Mucorales	Family	:	Mucoraceae
Genus	:	<i>Rhizopus</i>			



**Fig. 2:** Second identified isolated fungus *Rhizopus* sp. F2 (A): showing growth on agar plates (B): morphological observations under microscope (100x)

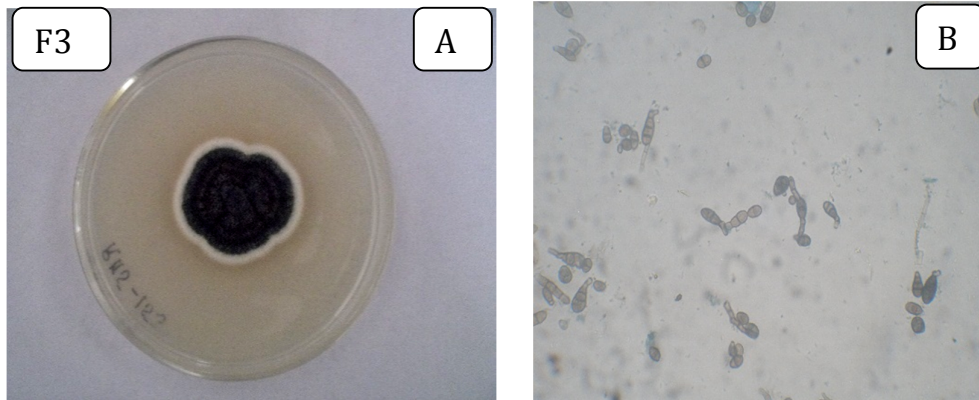
**Third Identified Fungus F3:****Identification Features:**

1. Colonies spread rapidly at 25°C, about 5-8 mm high.
2. Apophysis, rhizoids stolons is present.
3. Mycelium shows white in colour at starting stage after that dark brown or blackish brown to black in colour.

*Alternaria* sp. F3 (Figures 3 (A) and (B)), is an ascomycetous fungus and is known as major plant pathogen causing diseases various species of plant, common allergens in humans, grow indoors. Approximately 20 percent of agricultural spoilage is caused by *Alternaria* species and also cause many human health disorders. It is an opportunistic pathogen on numerous hosts causing leaf spots in many plant parts.

**Taxonomic Classification:**

Kingdom	:	Fungi	Phylum	:	Ascomycota
Class	:	Dothideomycetes	Order	:	Plesoporales
Family	:	Pleosporaceae	Genus	:	<i>Alternaria</i>



**Fig. 3:** Third identified isolated fungus *Alternaria* sp. F3 **(A)**: showing growth on agar plates **(B)**: morphological observations under microscope (100x)

**Identification Features:**

1. Colonies are slow growing with whitish margin and gray to light brown in color.
2. Conidiophores arises single or in groups, usually simple, erect, straight or curved.
3. Conidiophores are occasionally geniculate, cylindrical but slightly rounded at the base.
4. Conidia are pale brown to light brown, obclavate to ellipsoid, short conical beak or beakless. Conidia are septate.

**Unidentified Fungal Isolates F4 & F5:**

Two unidentified fungal isolates F4 (Figure 4 (A) and (B) and F5 (Figure 5 (A) and (B) were obtained.

**Identification and Characterization of Bacterial Isolates:**

The identification of bacterial isolates is based on many factors, including morphology of cell and colony, chemical composition, biochemical activities, colony growth (diameter), colony color, pigment production *etc.* The bacterial species were recognized by microscopic analysis using standard procedure (Barnett and Hunter, 1972; Ellis, 1976; Domsch et al., 1980; Nelson et al., 1983; Gilman, 1998). From the samples only two bacterial isolates were identified on the basis of following characteristics.

**Gram Staining:**

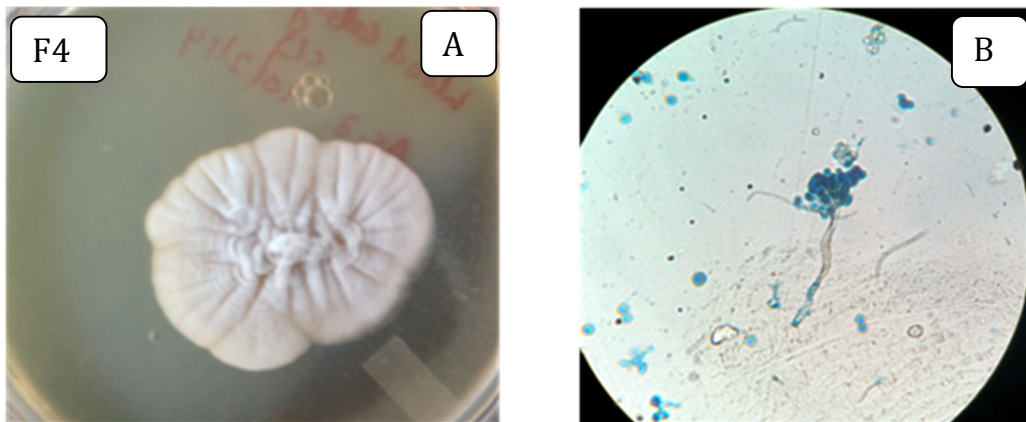
An initial step in identifying a bacterial species is determining if it is Gram-positive or Gram-negative. Gram stain is one of the most commonly used tools in the identification of bacteria. After staining, Gram-positive cells appear purple and Gram-negative cells appear pink.

**Morphological Characteristics:**

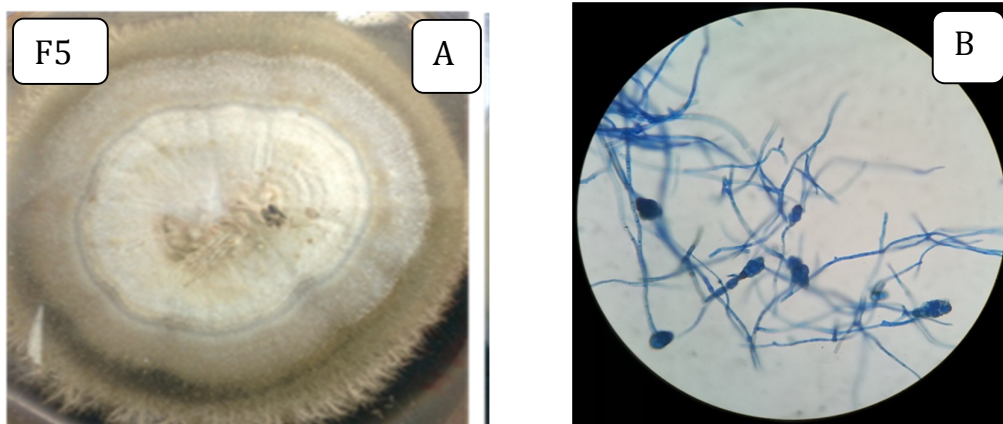
After gram staining procedure, microorganisms are also classified according to morphological features of colony and cell. Bacterial colonies have different characteristics like size, shape, texture color *etc.* These characteristics describe the morphology of a single colony and are useful in the primary identification of a bacterial species.

**Identification of Samples:**

On the basis of gram staining two isolates were found: one was Gram-positive and other was Gram-negative.



**Fig.4:** Unidentified fungus F4 (A): showing growth on agar plates (B): morphological observations under microscope (100x)



**Fig. 5:** Unidentified fungus F5 (A): showing growth on agar plates (B): observations under microscope (100x)

**First Identified Bacterial Isolate B1:**

As indicated in Figure 6 (A) and (B), first isolated bacterial strain was identified as *Bacillus* sp. B1.

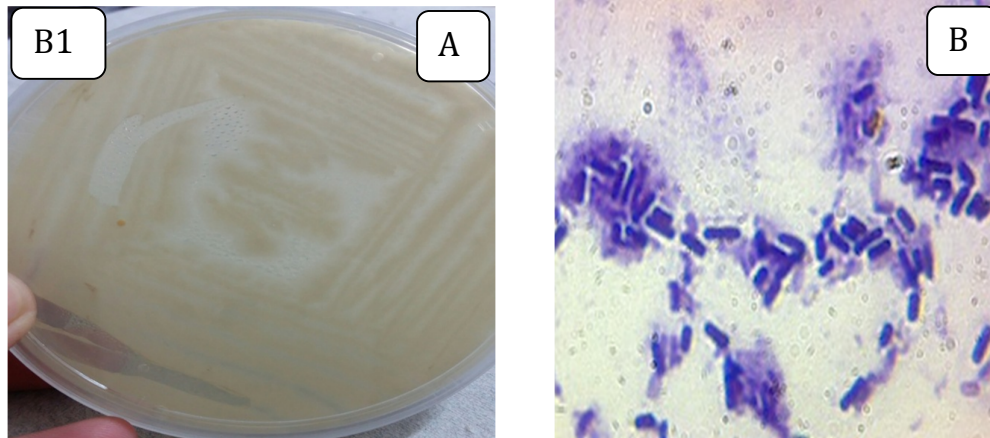
**Taxonomic Classification:**

Domain	:	Bacteria	Division	:	Firmicutes
Class	:	Bacilli	Order	:	Bacillales
Family	:	Bacillaceae	Genus	:	<i>Bacillus</i>

**Identification Features:**

1. Gram staining: Positive bacteria showing purple colour cells after staining
2. Shape: Rod shaped cells

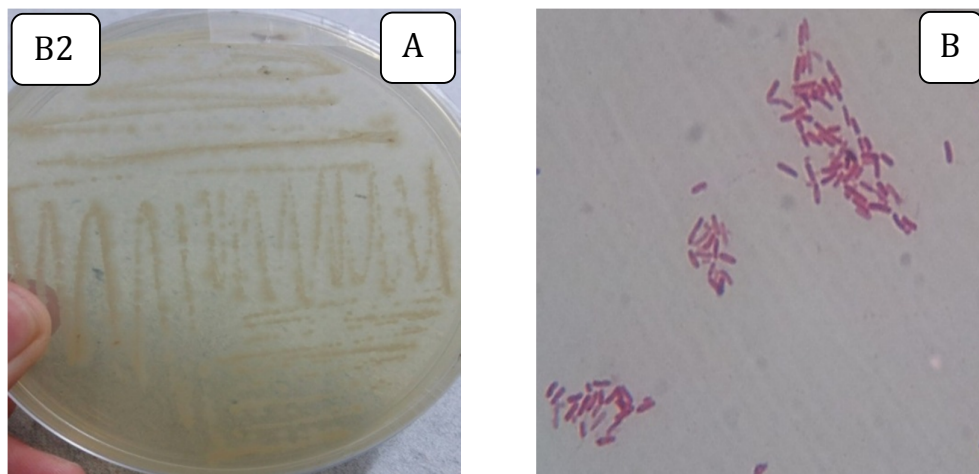




**Fig. 6:** First identified bacterial isolate *Bacillus* sp. B1 **(A)**: showing growth on agar plates **(B)**: morphological features under microscope (100x)

#### Second Identified Bacterial Isolate:

The second identified bacterial strain was *Escherichia coli* as shown in Figures 7 (A) and (B). These are Gram-negative and rod-shaped bacteria.



**Fig. 7:** Second identified isolated bacterial strain *Escherichia coli* sp. B2 **(A)**: showing growth on agar plates **(B)**: morphological features under microscope (100x)

#### Taxonomic Classification:

Domain	:	Bacteria	Phylum	:	Proteobacteria
Class	:	Gammaproteobacteria	Order	:	Enterobacteriales
Family	:	Enterobacteriaceae	Genus	:	<i>Escherichia</i>
Species	:	<i>E. coli</i>			

#### Identification Features:

1. Gram-negative bacteria
2. Slow growing colonies
3. Conidiophores arising singly or in groups
4. Colonies are growing in cottony appearance
5. Colonies are light brown in color.

#### Isolated Microorganisms:

Three fungal (*Aspergillus* sp., *Alternaria* sp., *Rhizopus* sp.) and two bacterial (*Bacillus* sp. and *Escherichia coli* sp.) cultures have been identified on the basis of colony morphology observed

on agar plates followed by microscopic features in both effluent as well as affected soils. However, two fungal isolates remain unidentified. Moreover, all these microorganisms can be further recognized on the basis of other features including biochemical and molecular.

#### REFERENCES

1. Barnett H.L. and Hunter B.B. (1972): Illustrated genera of imperfect fungi. 3<sup>rd</sup> edition, Burgess Publishing Co., pp 273.
2. Domsch K.H., Games W. and Anderson T.H. (1980): Compendium of soil fungi, Academic press, London.
3. Ellis M.B. (1976): More Dematiaceous Hyphomycetes. Common Wealth Mycological Institute, England.
4. Gilman J.C. (1998): A manual of soil fungi, Biotech books, New Delhi.
5. Nelson P.E., Toussoun T.A. and Marasas W.F.O. (1983): Fusarium Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, Pennsylvania, USA.