Extraction of Microbial Pigments from *Acinetobacter Baumannii* Isolated from Cow Dung

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ABSTRACT- Pigment extraction from cow dung via animal biotechnology is a sustainable approach that utilizes biological principles, tools, and techniques to manage and utilize waste from animal agriculture, minimizing environmental pollution and disease transmission. Cow dung, rich in nutrients like nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium, as well as micro flora including bacteria, protozoa, yeasts, and fungi is used to produce valuable pigments. These pigments have various applications in natural dyes for textiles, leather, and paper, cosmetics like skincare and haircare, food colouring after proper safety testing, paints and coatings, and biomedical applications such as wound healing. This innovative approach integrates animal biotechnology with circular economy principles, offering opportunities for resource recovery, energy self-sufficiency, and sustainable waste management, while also showcasing the potential of animal waste as a valuable resource. UV characterization of cow dung extraction is Analysing pigments from cow dung using ultraviolet (UV) light to understand their properties, identify specific pigments, and explore potential uses in various fields. The process of identifying and quantifying bioactive compounds in cow dung extracts using various biochemical techniques to understand their properties, potential applications, and commercial value.

KEYWORDS- Animal Biotechnology, Cow Dung, Ultraviolet (Uv), Biochemical Techniques, Bacteria, Protozoa, Yeasts, And Fungi.

I. INTRODUCTION:

Pigments are substance having properties that are significant to numerous industries. They serve as antioxidants color intensifiers, additives and more in the food industry. There are many different hues of pigments some of which dissolve in water. The main sources of natural colors include Microoranganisms, plants, insects and mineral ores[1]. It has been mentioned that many groups of bacteria, especially those which are the most important in hospital-acquired infections, are not controlled by routine anti-microbial agents and they pose the greatest threat for the human community. Most of the bacteria and fungi are widely studied for their potential as a source of food colorants [2]. Natural pigments possess anticancer activity, contain provitamin A and have some desirable properties like stability to light, heat and pH Thus, natural colors in addition to being environment friendly, can also serve the dual need for visually appealing colors and probiotic health benefits in food products.

Acinetobacter is a non-fermenting gram-negative *coccobacilli*, and one of the most common causes of nosocomial infections, especially in patients admitted to the ICU and patients with impaired immune systems. Acinetobacter baumannii is the most important clinical species of *Acinetobacter* [3]. At the first detection of *Acinetobacter baumannii* in hospitals, the choice of drug for treatment was carbapenems, while this bacterium is extensively resistant to this group of anti-microbial compound. Overall, it can be stated that pigments produced by microorganisms have medical properties including anti-oxidant, cytotoxic, antileishmanial, anti-ulcer, anti-viral, anti-tumor and anti-bacterial effects [4]. *Acinetobacter baumannii* (Ab) has been listed among "ESKAPE" pathogens which resists diverse spectrum of antimicrobials by virtue of heritable and transmissible genomic alterations and can also persist as dormant cells with in biofilms. *A. baumannii* strains are usually multidrug drug resistant, which makes it a hard-to-treat pathogen that causes high morbidity and mortality [5].

Pigment extraction from cow dung via animal biotechnology is a sustainable approach that utilizes biological principles, tools, and techniques to manage and utilize waste from animal agriculture, minimizing environmental pollution and disease transmission.

Cow dung, rich in nutrients like nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium, as well as microflora including bacteria, protozoa, yeasts, and fungi is used to produce valuable pigments [6]. Cow dung is the undigested residue of herbivorous matter which has passed through the animal's gut. The resultant faucal matter is rich in minerals. Color range from greenish to blackish, often darkening in color soon after exposure to air. Long term use of inorganic fertilizers without organic supplement damages the soil physical, chemical and biological properties and causes environmental pollution [7]. Organic manures not only act as a source of nutrients and organic matter, but also increase size, biodiversity and activity of the microbial population in the soil, influence structure, nutrients turnover and many other related physical, chemical and biochemical parameters of the soil.

II. MATERIALS AND METHODOLOGY

A. Materials

The fresh cow dung was collected from the farm as shown in the Figure 1.

Figure 1: Sample collection

B. Media preparation

Nutrient agar was prepared as shown in the Figure 2 for 250 ml for that 18 gram of nutrient agar was mixed with 250 ml distilled water. The media was prepared and glassware were sterilized by autoclaving.

Figure 2: Media preparation

C. Sample Preparation

The collected cow dung was taken 1 gram and it was diluted in beaker using distilled water.

D. Serial Dilution and plate count Technique

The prepared sample was serially diluted using distilled water and desired dilution factor was taken and plate count techniques was done as shown in the Figure 3. In plate count technique we have done spread plate technique for the isolation of the bacteria.

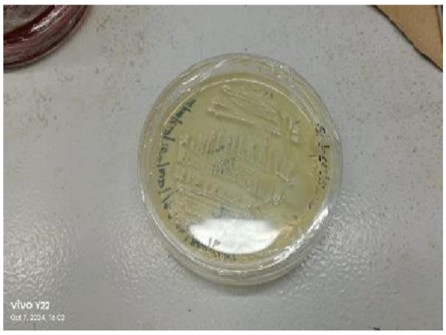


Figure 3: Spread plate technique

E. Colonies Observation

After 24 hours incubation the colony growth was observed as shown in the Figure 4. The streak plate technique is done for the separation of particular colony from the group of mixed population. After streaking, the colony were characterized.

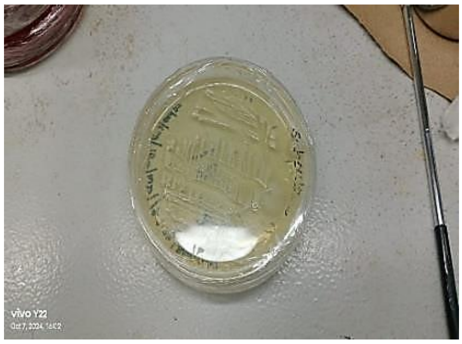


Figure 4: Spread plate technique

F. Growth Analysis

The growth of the colonies were observed more in nutrient agar as compared to the MacConkey agar as shown in the Figure 5.

Figure 5: Nutrient broth and MacConkey Agar

G. Testing the Bacteria fortThe Pigment Extraction

The bacteria was employed for the pigment extraction as shown in the Figure 6. 5 µl of bacterial culture is mixed with various organic solvents such as acetone, hexane, chloroform, isopropanol and methanol and centrifuged it for 10,000 rpm for 15 minutes.

Figure 6: Nutrient broth

H. Testing the Bacteria for the Pigment Extraction

The bacteria is employed for the pigment extraction. 5 µl of bacterial culture is mixed with various organic solvents as shown in the Figure 7, such as acetone, hexane, chloroform, isopropanol and methanol and centrifuged it for 10,000 rpm for 15 minutes.

Figure 7: Micro centrifuge tube containing organic solvent

Figure 9: Colony analysis

III. CHARACTERIZATION STUDIES

A. *Gram Staining*

The bacteria was identified as *Acinectobacter baumanii* using MALDI-TOF (Matrix assisted laser desorption ionization–time of flight) analysis. After the characterization the bacteria were sub cultured in the nutrient broth and kept in the rotary shaker for 24 hours for primary confirmation as shown in the given Figure 8 Gram staining was done.

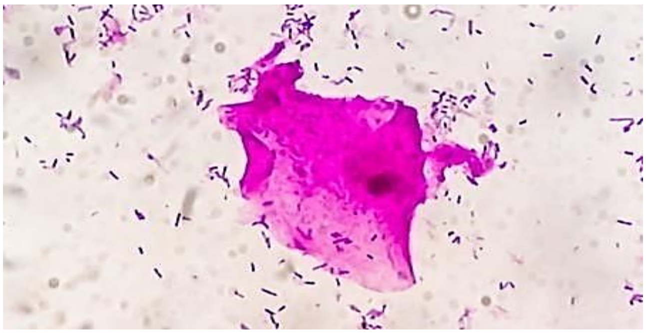
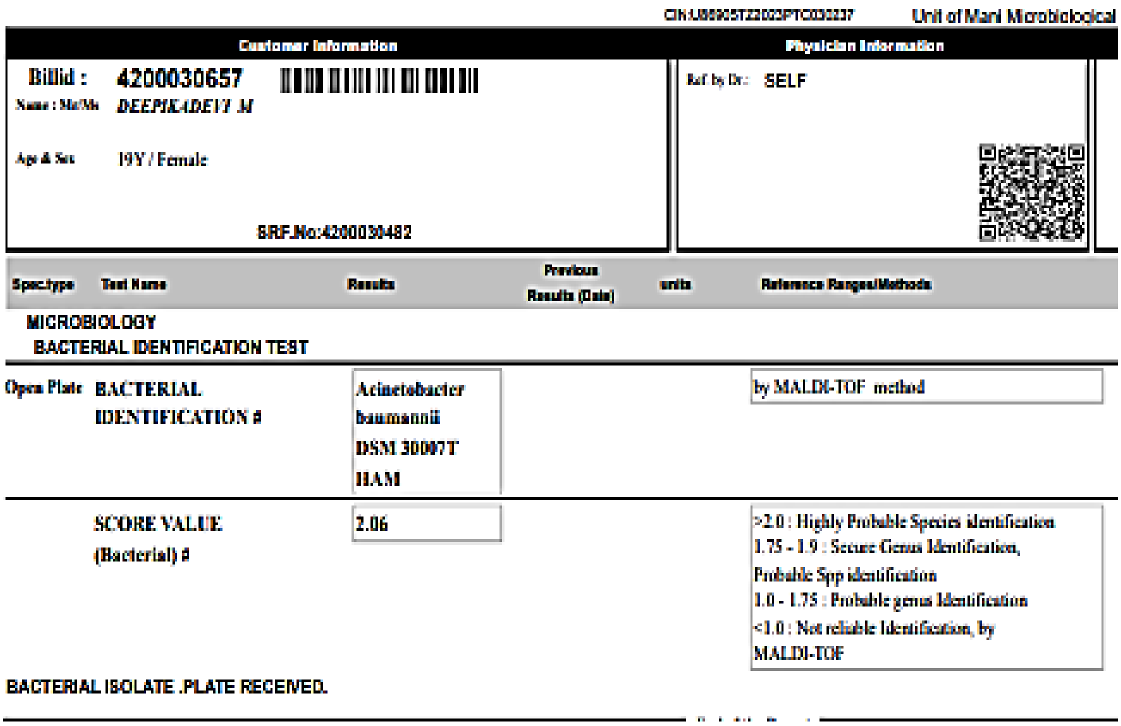


Figure 8: Gram staining

B. *Malti-tof Analysis*

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) as shown in the Figure 10. Mass spectrometry is a technique that uses a laser and mass spectrometer to analyse biological samples. By MALDI – TOF analysis the bacteria was identified as *Acinectobacter baumanii*. It is a round shaped gram negative bacteria.



Unit of Mani Microbiological

Customer Information | Physician Information

Billid : 4200030657

Name : Mr/Ms DEEPIKADEVI M

Age & Sex 19Y / Female

SRF.No:4200030482

Ref by Dr.: SELF

Spec.type	Test Name	Results	Previous Results (Date)	units	Reference Ranges/Methods
MICROBIOLOGY					
BACTERIAL IDENTIFICATION TEST					
Open Plate	BACTERIAL IDENTIFICATION #	Acinetobacter baumannii DSM 30007T HAM			by MALDI-TOF method
	SCORE VALUE (Bacterial) #	2.06			>2.0 : Highly Probable Species identification 1.75 - 1.9 : Secure Genus Identification, Probable Spp identification 1.0 - 1.75 : Probable genus Identification <1.0 : Not reliable Identification, by MALDI-TOF

BACTERIAL ISOLATE .PLATE RECEIVED.

Figure 10: MALTI – TOF analysis

IV. CONCLUSION

Cow dung has many microorganism in that we specifically isolate our desired microorganism (*Acinetobacter baumannii*). it is a rod shaped gram negative bacteria. By MALDI – TOF analysis the bacteria was identified as *Acinectobacter baumanii* .It is a round shaped gram negative bacteria. The bacteria was employed for pigment extraction. By centrifuging the bacterial culture with organic solvents as a result, a white color pigment was obtained. This procedure illustrates the possibility of using microbes to produce valuable substances, such pigments, which can be used in a variety of industries.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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