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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSPTABILITY OF BACTERIA FROM COW DUNG, BILASPUR DISTRICT, CHHATTISGARH STATE

Meesala Sudhakar
Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.), India, 495009

Preeti Choudary
Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.), India.-495009

DSVGK Kaladhar
Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.), India.-495009

Dharmendra Kashyap
Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.), India.-495009

Nikki Agrawal
Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.), India.-495009

Leena Preeti Lakra
Department of Food processing & Technology, Atal Bihari Vajpayee University,Koni, Bilaspur (C.G.), India, 495009

Aakriti Singh
Department of Food processing & Technology, Atal Bihari Vajpayee University,Koni, Bilaspur (C.G.), India, 495009

Varahalarao vadlapudi
Diabetomics Medical Private Limited, Muppieddypally Village, Medak dist, Telangana, India-502336

***Corresponding Author:** Meesala sudhakar
*Department of Microbiology &Bioinformatics, Atal Bihari Vajpayee University, Koni, Bilaspur (C.G.) ,India.-495009, Email-sudhakarmeesala55@gmail.com, Mobile-09000781791

ABSTRACT

The present study includes the collection, isolation and characterization of microorganisms from the cow dung in rural area of Bilaspur dist, Chhattisgarh state, India.The isolation and characterization of bacteria from the cow dung source provides a better understanding of their diversity, distribution, and potential impact on antibiotic use. Through this research, many types of bacteria were identified and their effects on antibiotics were determined. Isolation involves collection of cow dung sample and growing the bacteria in a suitable medium. In the present investigation attempts were made to isolate bacteria using nutrient agar media (NAM). The isolated bacteria were identified on the basis of their colony characteristics, morphology, Gram's staining and biochemical tests, antimicrobial activity test

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This study aims to find out type of organisms that are found in the rural area's cow dung. The Gram stain technique also showed that Gram-negative bacteria's were isolated from the cow dung. The isolate enzymatic activity indicates amylase production. Five antibiotics were used to test the antimicrobial activity of isolated microorganisms. The bacterial colony shows the zone of inhibition against several types of antibiotics including Amoxicillin, Ofloxacin, Streptomycin and Chiprofloxacin but Ofloxacin has no zone of inhibition.Plant extract has been used to test the antimicrobial activity by well diffusion method.

Keywords: Cow dung microorganisms isolation , antibiotics, biochemical tests, gram stain, enzymatic activity, antibiotic susceptibility.

1. INTRODUCTION

Cow dung or faece is a mixture of indigestible plant material, water, and other substances that is released from the animal's intestine.

Faeces are generally not a favourite topic of conversation, whether it comes from an animal or a human. Cow dung is worth discussing, though. Cow dung has a soft texture and tends to be deposited in a circular shape, which gives dung patches their alternate names of cow pies and cow pats. According to Ware et al., (1988), lower part of the gut of cow has probiotic activity due to presence of different types of microorganisms like Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, Bacillus subtilis, Entrococcusdiactylactis, Bifidobacteirum and yeasts (Saccharomyces cerevisiae) . Cow dung also contains various nutritional components, including minerals, vitamins, potassium, nitrogen, oxygen, carbon, cellulose, hemicellulose, mucus, lignin. ash,cellulose, magnesium, manganese, calcium, zinc, and trace elements . Cow dung manure is rich in carbon and nitrogen, which indicates that it could be a promising feedstock for the growth of microbes. There may two types of bacteria: gram's positive bacteria and gram's negative bacteria. archaea ,etc., as well as eukaryotic, such as protozoa, algae, fungi, etc. In the cow dung. The overall chemical formula for fresh cow manure is C10H20O6N2. Mass of one mole C10H20O6N2 is equal to 264.278 g.The aim and objective of this study was to isolate bacteria from cow dung, using nutrient agar media (NAM). The isolated bacteria were identified on the basis of their colony characteristics, morphology, Gram's staining and biochemical tests, so that we can identify which microorganisms their pathological, beneficial properties with novel characters.

2. MATERIALS AND METHODS

All the chemicals used in the study were obtained from HiMedia, Mumbai, Maharashtra.
Cowdung used as a main source for microorganisms in research.

Instruments used:Autoclave, Hot air oven, Laminar air flow, Weighing Machine, Refrigerator, Microscope , Incubator shaker .

Glassware used: Conical Flask, Petri Plate, Beaker, Test tubes, Pipettes, Measuring cylinder, Glass rod, Cuvette.

Media used: For microbiological works Nutrient agar, Macconkey agar,EMB agar,Simmons citrate agar etc. are used.

Reagents: Gram's crystal violet, Gram's iodine, Safranin, Hydrogen peroxide, Mannitol 1%, Phenol red, Alcohol, Malachite green, Cresol red, Bromocresol purple, Basic fuchsin,

Bromocresol green ,antibiotic discs

Others: Different types of Antibiotics (Amoxiline, Ciprofloxacin, Cefixime & L.A., Ofloxacin, Streptomycin) Different types of plant extract (Azadirachtaindica (Neem), Mentha spicata (Mint), Murraya koenigii (Curry leaves), Tinosporacordifolia (Giloy), Calotropis gigantea (Crown flower).

METHODS PREPARATION OF COW DUNG SUSPENSION

Sample collection: The sample was collected from the rural area Koni , Ballarpur district in Chhattisgarh state ,India . The samples were collected in a sterile plastic bag and immediately taken to the laboratory for further analysis and stored for microbial analysis.

Media preparation: Weighed all the components of media then dissolved it in 100 ml distilled water, transfered it in sterile conical flask and cover it with cotton plug. After that, autoclaved the conical flask at 121°C temperature and 15 lbs pressure for 15 to 20 minutes. After 15 minutes taken out from autoclave, allowed it to cool a little bit for further use.

Isolation of microorganisms:

Transferred 1 mL of cow dung sample into a test tube containing 9 mL of sterile water. Prepared serial dilutions of the sample with an initial dilution of 10^{-1}. Inoculate 1 mL of sample from the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilution into nutrient agar medium (NAM) using the spread plate method. Inoculated plates were incubated at 37°C for 24 hours. After incubation, colonies were observed and were isolated in pure culture from using the streak plate method.

Isolation and screening of cows Dung microorganisms :10 ml serially diluted sample was spread over nutrient agar plates The inoculated plates were incubated at 37°C for 24 hour under aerobic condition in Incubation . The predominant and morphologically different colonies were then cultured using standard streak plate technique for the isolation of the microorganisms in the nutrient agar media. The isolated colonies were subsequently streaked on nutrient agar slant for pure culture preservation.

Preservation of pure culture: The isolated colonies were subsequently streaked on nutrient agar slant for pure culture preservation. Streaked slant original culture is directly diluted across an agar surface using and inoculating loop. This is a simple & rapid method. In this work, the collected sample had been preserved by using pure culture maintenance methods.

MORPHOLOGICAL TEST OF MICROORGANISMS

Gram's staining and motility test:The bacteria's were gram stained using gram staining chemicals-Gram's crystal violet, Gram's iodine, Safranin, the microorganisms,and observed under light microscope (40×).Later Motility was checked using hanging drop method by cavity slide .

SELECTIVE BIOCHEMICAL TEST OF MICROORGANISMS

a)Macconkey agar test : Suspended 1 gram of dehydrated macconkey agar medium in 20 ml of distilled water was added and heated to dissolve the components then autoclaved, plates were prepared to observe different types of bacterial colonies.

b)Mannitol test : Mannitol was added at a concentration of 0.5-1.0% to 100 mL of nutritional broth. An inoculum from a pure culture was transferred aseptically to a sterile tube containing diluted 0.0001 gm of phenol red and 0.5% mannitol. The test consisted of a colour change

from red to yellow,

c)EMB agar test :Eosin-methylene blue (EMB) agar favours gram-negative bacteria over gram-positive bacteria. To 0.7 grammas EMB agar in 20ml distilled water was mixed in a test tube and heated, autoclaved at 15 Da pressure (121°C) for 15 minutes, then added the diluted solution- 0.001% of methylene blue .On the same day, Petriplates were prepared and bacteria were streaked and incubated for 24 hours.

d)Simmons agar test :20 ml distilled water was introduced to 0.5 grammes of Simmons Citrate Agar, along with the dye, to examine the colour change.

BIOCHEMICAL TEST OF MICROORGANISM

a) **Production of the enzyme catalase** : Catalase is a protein that aids in the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. Mix a little quantity of isolated bacteria into a 3% hydrogen peroxide solution determines the bacterial enzyme catalase production. In the present study it was found that oxygen bubbles developed quickly after adding H_2O_2 into the bacterial culture.

b) Production of acid carbohydrate fermentation test: A little amount of Phenol red was introduced (0.05 ml of 0.25% phenol red diluted solution) in a 30 ml nutritional broth in a test tube to made Phenol red carbohydrate broth, and a bacterial culture was also added utilising an incubation loop to observe the colour change.

c) Production of the enzyme gelatinase :A heavy inoculum of an 18- to 24-hour-old test bacteria was stab-inoculated onto culture plates prefilled with nutrient gelatine (100ml nutrient agar Peptone and 0.8 gm gelatine). Inoculated nutrient gelatine plates were incubated at 37°C for 24 hours then cooled it under the refrigerator, After 30 minutes in an ice bath,A nutrient gelatin tube inoculated with bacteria observed for the gelatin hydrolysis by medium liquefaction.Hydrolysis of gelatin in the medium indicates the secretion of gelatinase by the test organism..

d) Production of enzyme amylase: Starch hydrolysis test is used to determine if the organism is capable of breaking down starch into maltose through the activity of the extra-cellular α-amylase enzyme.Added 0.06 gm beef extract , 0.2 gm starch , 0.24 gm agar in 20 ml distilled water and boiled it at 121°c for 15 , and cooled and poured on suspended petri plates , after solidification a small amount of organisms was streaking on the plates and Incubated and observed for the result.

BACTERIAL DEGRADATION Of DYE :

Bacterial oxidoreductases are important for the degradation of synthetic dyes. This dynamic metabolism of bacteria causes them to use complex xenobiotic dye compounds as substrates. In the process, they break down into less complex metabolites.

Following are the five types of synthetic dyes is used for the degradation:

a)Malachite Green
b)Cresol Red,
c)Bromocresol purple
d) Basic fuchsin
e) Bromocresol Green

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Added 5 ml of distilled water to 1 ml of bacterial suspension and diluted form of 5 different types of dyes (0.01 of the above dyes dissolved in 100 ml of distilled water) in 5 different test tubes, after complete addition, checked colour change after 7 days, which indicates that media culture degradation by microorganisms.

ANTIMICROBIAL SENSITIVITY

Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. They all have different modes of action by which they act to suppress the infection. two methods have been employed for this purpose :

1. Disk diffusion method 2. Well diffusion method

SENSITIVITY TEST BY PLANT EXTRACTS: Sohilet apparatus was used to make the five plant extracts listed below. with 1mg/1ml was prepared with aqueous solvent. The antibacterial activity of these plant extracts was tested using nutrient agar with diffusion techniques &well diffusion methods. In this procedure, cow dung microbial cultures are employed.

a)Neem (Azadirachtaindica (A. Indica)
b)Mont (Mentha piperita)
c)Curry leaves(Murraya koenigii)
d)Crownflower(Calotropisgigantea)
F)Giloy (Tinosporacordifolia)

ANTIMICROBIAL SENSITIVITY TEST BY DRUGS: The antimicrobial sensitivity test for commercially available drugs tested by well diffusion method . The method was be performed by applying bacterial inoculum of approximately 1–2×108 CFU/mL to the surface of two nutrient agar plates, five commercially , fixed concentration , paper antibiotic disks were placed on the inoculated agar surface to check inhibition zone

In this test five drugs were used :

1)Amoxicillin
2)Chiprofloxacin
3)Cifixime and L. A,
4)Ofloxacin,
5)Streptomycin

BIOFUEL PRODUCTION: Alcohol can be produced by fermentation, however for many years it has been manufactured utilising the catalytic hydration chemical method. Microbial fermentation is employed in this study to manufacture ethanol for medical and fuel purposes. In the ethanol business, both yeast and bacteria are employed. Following steps in employed in the biofuel production :-

1. Prepared of nutrient broth
2. Added cow dung microorganisms and yeast to nutrient broth and went through fermentation process and employed fermentation conditions . (pH 5, 35 °C) and production started in 12 hours. After fermentation was completed, the cells are separated to obtain yeast cell biomass, which can be used as single cell protein (SCP) for animal feed. The medium or supernatant was processed to recover the ethanol .

Results

Screening results from cow dung samples

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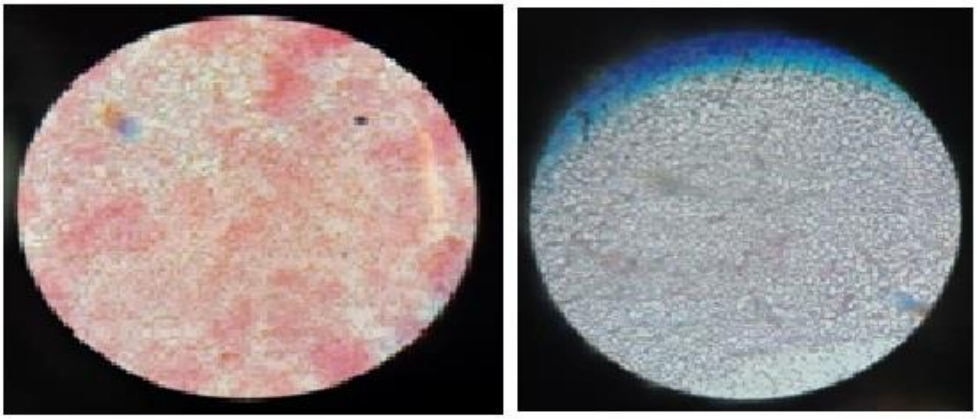
Cow dung microorganisms are cultured in Petri plates, colonies were seen on nutrient agar and preserved in an incubator for additional dilutions to get a pure cow dung microbial culture. This screening is required due to the presence of a large number of impure bacteria.

a)Pure culture isolation b)Pure culture maintainance

Fig 1: Cow dung microorganisms

extraction

Colonies were collected from cow dung sample Petri plates and sample repeated dilutions of Petri pates were prepared, later Slants were prepared by streaking method in a test tube to get pure culture.



a)Gram stianing b)Motility of Bacterial culture

Fig 2: Gram staining and motility of bacterial culture

a) In the gram staining technique saffron colour was observed which indicates presence of gram negative bacteria in the microbial culture and rod shapes microbial cultures were observed. Along with that some uncoloured microbial cultures were also seen.

b) Hanging drop test was performed for motility of the bacterial cultures. Observed motility of the microbial cultures which indicates presence of flagella's on its surface and helps in the movement of its body from one place to another place.

Table 1: Selective media for Bacterial culture

selective media test	MacConkey Agar test	Mannitol hydrolysis test	Eosin methylene blue test	Simmon's citric agar test
Test results	+	+	+	+

a)In the Maconkey agar test certain bacterial colonies were observed which indiacates presence of gram negative bacteria.Along with bacteria growth certain other mass cultures, some spores were observed

b)As the mannitol sugar was used and an acid by-product was formed, the pH of the medium surrounding the colonies falls, causing the indicator to become yellow. Both salt resistance and mannitol usage are recorded.

c) Bacterial colonies were discovered in this EMB (Eiosin methalene blue) agar associated with the breakdown of methelene, indicating the presence of gram negative bacteria.

d)) Simmons Citrate agar was performed and observed blue colour indicates the conversion of ammonium ions to ammonia.

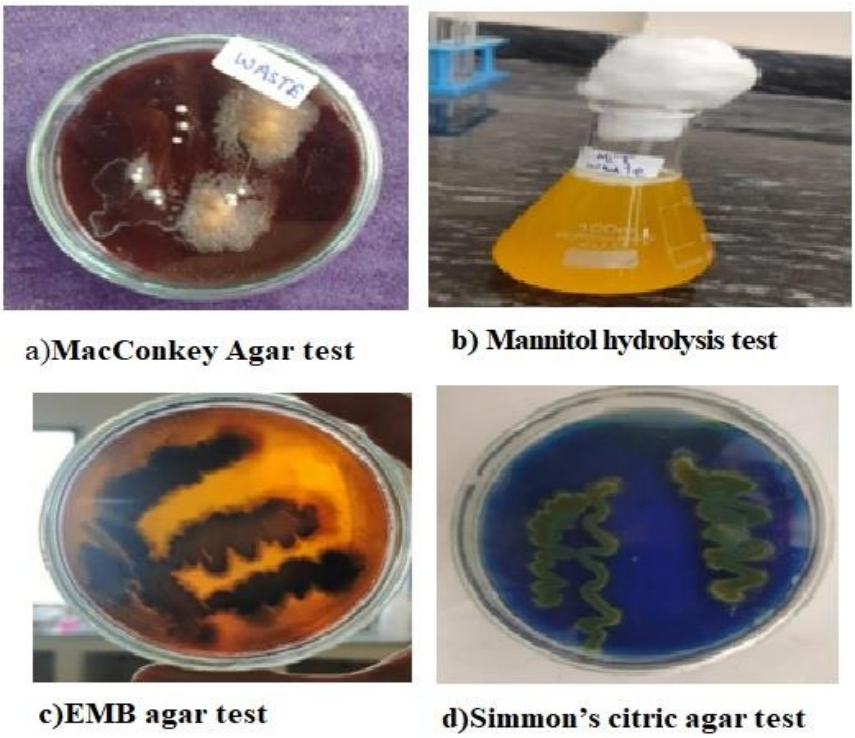


Fig 3: Selective media for gram negative bacteria

Table 2 Bacterial Biochemical tests

Biochemical test	1.Catalase test	2.Carbohydrate fermentation test	3.Gelatine hydrolysis test	4.Amylase test
Test results	+ Bubble formation	_ Negative results of Acid production	+ Gelatine hydrolysed	+ Starch Degradation

a) Catalase is an enzyme that aids in the breakdown of hydrogen peroxide into oxygen and water. The existence of the enzyme in a bacterial strain was demonstrated when a modest inoculum was added to hydrogen peroxide, resulting in the fast formation of oxygen bubbles indicates positive result.
b) Due to the lack of acid production, no colour change was detected, which may suggest the presence of proteobacteria.
c) The test tube displays a gelatin medium that has become more liquid after cooling, showing thatgelatin hydrolysis was occured (gelatin hydrolysis positive).control was set to cross check the test.
d) Except for brocerolpurple, all of the test tubes lowered dyes.In this approach, bacterial enzymes transformed dyes, causing a colour shift to be detected.

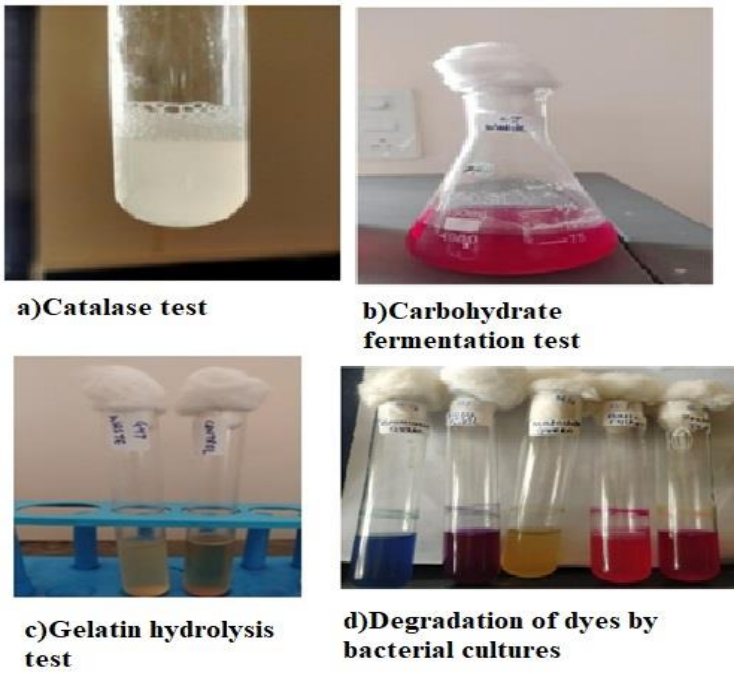


Fig 4: Biochemical tests for bacterial cultures

TABLE 4 a): Antimicrobial activity against plant extracts

S. No.	Plants leaf extract	Test results	Zone of inhibition (mm)
1	Azadirachtaindica (Neem)	+	2.8 mm
2	Mentha spicata (Mint)	_	No zone
3	Murraya koenigii (Curry leaves)	_	No zone
4	Tinosporacordifolia (Giloy)	_	No zone
5	Calotropis gigantean (Crown flower)	_	No zone

All the tested plant extracts shown no zone of inhibition under given concentration(1mg/1ml) except Neem which shown 2.8 mm zone of inhibition.

4b)Antimicrobial activity of cow dung microbes against drugs (30µg/paper disc)B

S.No.	Antibiotic	Test result	Zone of inhibition (mm)
1.	Amoxiline	+	2..9 mm
2.	Ciprofloxacin	+	4. 8 mm
3.	Cefixime & L.A.	-	No Zone
4.	Ofloxacin	+	4 .3 mm
5.	Streptomycin	+	4.0 mm

Bacterial culture shows are more sensitive towards Ciprofloxacin antibiotic whereas Where as Cefixime & L.A has no impact.

Fig 4: Antimicrobial activity of bacterial cultures against plant extracts,drugs

Biofuel production:

There was no gas production indicated shows no capability to produce ethanol by fermentation method.

DISCUSSION:

Bacterial isolation and characterisation from cow dung samples provide important insights into the microbial diversity and functional potential of this rich waste material. The purpose of this debate is to go into the main results and consequences of cow dung microorganisms. Cowdung bacteria's play an important part in the adverse effects on the human body, as evidenced by antimicrobial sensitivity, degradation, nutrient absorption, and enzyme and acid generation, all of which are critical processes in waste identification and the sustainability of various forms of production. Furthermore, the found microorganisms may have potential uses in immunology, manufacturing, agriculture, and other fields.

Finally, the isolation and characterization of bacteria from cow dung samples provides valuable insights into the microbial, sensitivity, enzyme production, acid production, motility, dye degradation, biofuel production, and biochemical analysis within this abundant waste material. The findings add to our understanding of the microbial communities connected with cow dung, as well as the relevance of the pathogenic nature of their microorganisms, which have a negative influence on a human body's helpful nature for the creation of useful goods. The discoveries include practical detection of microorganisms in animal manure and a research study aimed at identifying pathogenic qualities that are damaging to our health using microbiological methods. Further study in this area includes DNA sequencing, phylogenetic analysis, and insilico investigations.

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