Screening and Identification of Bacteria and Fungus Present In Cow Dung

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ABSTRACT
Cow dung (CD) or cow manure is the waste product of bovine animal, cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. Composition of cow dung is about 80% of water and has some part of undigested plants material that have high amount of organic substance, due to secretion of antimicrobial metabolites of cow dung microflora. Manure of cow dung enhances the minerals of soil and also develops resistance power of plants against pests and plant diseases. The cow dung(CD) microflora used in agricultural domain such as biocontrol, growth promotion, organic fertilizer, phosphorus solubilisation. CD has been used for several other applications concerning environmental such as biodegradation, bioremediation, and biosorption of heavy metals etc. CD harbors a rich microbial diversity containing almost 60 species of bacteria (*Bacillus* sp., *Lactobacillus* spp., *Corynebacterium* spp.), fungi (*Aspergillus, Trichoderma*), 100 species of protozoa and yeasts (*Saccharomyces* and *Candida*).
In the present investigation we studied the microbial load of cow dung. Bacteria were isolated from cow dung by using nutrient agar, blood agar and MacConkey agar. Sabouraud Dextrose agar (SDA) used for fungal isolation. The isolated bacteria were identified on the basis of their colony characteristics, morphology, Gram's staining, microscopy and biochemical test. Microbial load of cow dung was calculating by cfu/gm of samples. The maximum number of bacterial population was exhibited in dilution 10^{-3} which ranged from 170×10^{-4}cfu/ml. A total of 20 isolate including Gram Negative bacilli, Gram Positive cocci, and Gram Positive bacilli *Escherichia coli*, *Microccocus* sp. and *Bacillus* sp respectively were isolated from cow dung. Sabouraud Dextrose Agar (SDA) used for fungal isolation. The maximum number of fungal population was exhibited in dilution 10^{-2} which ranged 35×10^{-3} cfu/ml different fungus colonies of *Aspergillus niger* and *Aspergillus fumigates* was observed. These beneficial microbes will be used for further research work.

Keywords: Cow dung, Microbial load, Bacteria, Fungi, Microbiome.

1. INTRODUCTION

In India, raising cattle has a long history and is primarily associated with agriculture. Numerous Ayurvedic formulations use various products made from cow's milk, ghee, curd, urine and dung (Sharma and Singh, 2015).
Cow dung (CD) is the undigested remnant of plant material that has passed through the digestive system. Its composition is made up of water (80%), undigested residues (14.4%), and microorganisms (5.6%), and its pH ranges from 7.1 to 7.4 (Nene et al. 2003; Teo and Teoh, 2011; Radha and Rao, 2014).
Due to the presence of various microorganisms including *Lactobacillus plantarum*, *Lactobacillus casei, Lactobacillus acidophilus, Bacillus subtilis, Entrococcus diactylactis, Bifidobacteirum*, and yeasts (*Saccharomyces cerevisiae*) with probiotic activity, the lower part of the cow's gut has probiotic activity Ware et al. (1988). It includes a large number of naturally occurring beneficial bacteria, *lactobacilli* and *cocci*, as well as some known and unknown actinomycetes, fungi and yeasts (Muhammad and Amusa, 2003; Radha and Rao, 2014; Sharma and Singh, 2015).
Cow dung harbors a rich microbial diversity containing almost 60 species of bacteria (*Bacillus* sp., *Lactobacillus* spp., *Corynebacterium* spp.), fungi (*Aspergillus* and *Trichoderma*), 100 species of protozoa and yeasts (*Saccharomyces* and *Candida*) (Gupta et al. 2016; Bhatt and Maheswari, 2019). According to Muhammad and Amusa (2003), soil contaminants like bacteria and fungi frequently invade old cow dung. Biotechnological applications (such as enzymes, biomethane and biohydrogen) and environmental applications, as well as biotechnological diversity of microbes, biodynamic preparation and uses of cow dung in agriculture. Since the turn of the century, biologists have been interested in the microbial diversity of CD (coprophilous organisms) (McGranaghan et al. 1999; Kim and Wells, 2016). CD microorganisms used in biotechnological, environmental and agricultural applications in the context of a sustainable circular economy. Numerous studies have demonstrated the antimicrobial and antifungal properties of fresh cow dung and urine, respectively, this may be because of microflora present in the dung secretes antimicrobial metabolites (Nene et al. 2003; Sharma and Singh 2015).
Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. Cow dung also harbors diverse groups of microorganisms that further enhance soil biogeochemical processes (Akinde and Obire 2008).

Manure of cow dung enhances the minerals of soil and also develops resistance power of plants against pests and plant diseases. The cow dung (CD) microflora used in agricultural domain such as biocontrol, growth promotion, organic fertilizer, sulfuroxidation, phosphorus solubilization etc. The advances on genomics and proteomics of CD microflora for enhanced yield ofenzymes, organic acids, alternative fuels (biomethane and biohydrogen) and other biocommodities, and environmental applications such as biosorption of heavy metals, biodegradation of xenobiotics, etc. Cow dung is traditionally used as organic fertilizer in Asian and African agriculture for ages (Sharma and Singh 2015; Sawatdeenarunat 2016).

Traditional uses of cow dung in Asian households as burning for fuel purposes, biogas, biofertilizer and bioelectricity. CD has been used also in several other applications concerning environmental issues such as xenobiotics degradation, bioremediation and used as bioabsorbent for waste water treatment. CD is recognized as an eco-friendly and indigenous material for biosorption (removal) of heavy metal ions **(**Wang and Chen 2009; Barot and Bagla 2012; Geetha and Fulekar, 2013; Gupta et al. 2016).

In the present study, isolation and characterization of the microbes from cow dung of desi cow breed. Preliminary biological screening of microbes of cow dung on basis of morphological and biochemical test. The microbial load of bacteria inhabiting in cow dung will be used for improvement in nutritional properties of soil and water treatment.

2. MATERIALS AND METHODS

2.1 Collection of Cow dung sample

Cow dung was collected from Acharol village, aseptically in sterile poly bags and transport to microbiology laboratory of the department of Allied medical science and technology, Nims University Rajasthan, Jaipur

2.2 Preparation of Cow dung suspension

Cow dung suspensions were made in distilled water and the serial dilution method was used to determine the microbiological load. For adequate mixing of the sample, a 10gm sample of cow dung were mixed in 100 ml of sterilized distilled water and vigorously agitated in a shaker for an hour. Before plating, all the samples were incubated for 24h at 37°C in an incubator. Following incubation, each sample was diluted using the conventional dilution method using a sterilized pipette. In this method, each test tube containing 9 ml of sterilized distilled water. The labeled tubes were placed in a test tube stand, then 1 ml of activated standard solution was transferred aseptically to test tube number 1, and further 1 ml of sample was transferred to test tube number 2, and the same procedure was repeated for each dilution. In our studies, we used water to prepare cow dung suspension, and in previous work, two carrier materials, i.e., phosphate buffer and cow dung slurry (Sharma and Singh 2015;Dhiman et al. 2022).

2.3 Microbial load of cow dung

Nutrient agar, blood agar, macconkey agar, sabouraud dextrose agar (SDA) were used for the isolation of bacteria and fungi respectively from cow dung by using serial dilution method. Calculate the number of microorganism and fungi per gram of cow dung according to following formula.

$$\textit{Viable cells per gram cow dung} = \frac{Meanplatecount \times Dilutionfactor}{Dryweightofbuffalodung}$$

2.4 Isolation of bacterial and fungal colonies

To perform the streak-plate and spread-plate inoculation procedure to separate the microbes from mixed culture, so that discrete colonies were isolated.

2.4.1.Streak plate method

This method is a rapid and qualitative isolation for microbes. It is essentially dilution technique that involves spreading a loopful of culture over the surface of an agar plate. Although, many types of procedures are performed, the four-way, or quadrant, streak. The sample was taken with sterilized inoculating loop and streaked at the medium of the plates. Then the plates were incubated at 37°C for 24 hours for further isolation.

2.4.2 Spread plate method

In spread plate method the sample was picked from cow dung by the help of sterilized inoculating loop and transferred to Petri plates separately. All plates were incubated in incubator at 37°C, for 24 hours and 27°C, for 72 hours for bacteria and fungi respectively.

2.5 Morphological and microscopic characterization of microbes

Unknown microorganisms were identified by morphology characters and the microscopic examination. Bacterial morphology characteristics such as colony diameter, growth, colour, form, elevation and margin were observed on culture medium. Morphological features such as shape and arrangement were observed after Gram staining according to standard protocol(Cappuccino and Sherman 2005).

2.6 Identification of an unknown bacterial and fungus

Streak the unknown bacterial and fungal culture on the surface of agar plates for isolation and identification. Incubated the inoculated petriplates at 37°C and 27°C for bacteria and fungi for 24 and 48 hours for isolation respectively. Perform a Gram staining from the 24hour old culture and Cotton blue lactophenol staining of fungi(Cappuccino and Sherman 2005).

2.7 Biochemical Test

For the identification of bacterial isolates routine standard biochemical tests such as Indole, Triple sugar, Citrate, Urease and Mannitol motility were performed. Isolates were inoculated in these medium and incubated at 37^0C for 18-24 hours. Next day various biochemical reactions such as indole production, urease hydrolysis, citrate utilization, fermentation of sugars and motility were observed.

2.7.1 Indole

The indole test evaluates an organism's capacity to break down the amino acid tryptophan and generate indole. It is a component of the IMViC procedures, a set of diagnostic tests used to identify between members of the Enterobacteriaceae family. Indole is extracted from the medium into the reagent layer by the acidified butyl alcohol component and forms a complex with the *p*-dimethylaminobenzaldehyde, yielding the cheery red colour.

2.7.2 Triple sugar iron

Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate. The acid base indicator phenol red is also incorporated to detect carbohydrate fermentation that is indicated by a chance in colour of medium from orange red to yellow in the presence of acids with gas production.

2.7.3 Citrate test:

Recognizes the organism's ability to use citrate as its sole carbon source and for energy. In middle of the growth, even if the color is not changed, is considered positive. We would see a color change in the medium if the test organism forms acid or alkali during its growth. The usual color change that is observed is from green (neutral) to blue (alkaline).

2.7.3 Urease test:

This test serves to rapidly distinguish members of this *Proteus* genus from lactose-non fermenting enteric microorganisms. Urease is a hydrolytic enzyme that attacks the nitrogen and carbon bond in amide compounds such as urea and forms alkaline end product ammonia. The presence of urease is detectable when organisms are grown in a urea broth medium containing the pH indicator phenol red to turn to deep pink. This is a positive reaction for the presence of urease.

2.7.4 Mannitol motility

Mannitol motility test media is designed to differentiated bacteria on the basis of their motility and the ability to ferment mannitol. Semisolid media contains 0.3 percent agar help to detect motility. Motile bacteria produce diffused growth throughout the media while non- motile bacteria grow only along the line of inoculation. Fermentation of mannitol produces acidity in the media. Phenol red used as a pH indicator, which detect acidity by exhibiting a visible colour change from yellow to red.

3. RESULTS AND DISCUSSION

The cow varied in terms of their breeds (three main breeds: Krishna valley, Red sindhi, and Sahiwal), developmental stages (calf, breeding and adult) and sexes (oxen and cows). In addition to their roles in food digestion and nutrient absorption, the rumen and gut microbiota have been linked to more pronounced phenotypes, such as the milk production and quality of cattle. Different processed products obtained from cow such as milk, curd, ghee, urine and by-product (dung) are widely used in medicinal formulations. Cow dung is an excellent fertilizer. Besides this, it also contains beneficial minerals, such as phosphorus, potassium and nitrogen that support the growth of soil microorganism. Some cultures are using cow dung for making paper and insect repellent. Microbial load of cow dung includes *Fibrobacter, Ruminococcus, Bacillus, Proteus mirabilus, Pseudomonas aeruginosa, Enterobacter xiangfangensis,* and *Butyrivibrio* bacteria; similarly, *Prevotella,* a group of bacteria capable of degrading non-cellulose plant fibres, is abundantly present in the rumen in buffalo (Dhiman et al.2020).

3.1 Cow dung suspension and its characterization

Cow dung suspension carrying large numbers of microbe's serial dilution technique was used for microbial load analysis. That involves spreading a suspension over the surface of Nutrient agar for the isolation of bacteria and sabouraud dextrose agar media (SDA) for fungus isolation from cow dung. Further characterization of isolated bacterial and fungal colonies was performed according to standard protocols. Previous studies shows cow dung is one of the best sinks of microorganisms on the other hand, several bacterial genera present in dung.

3.2 Microbial load and isolation of bacteria by Serial dilution of cow dung

The collected samples of cow dung were enumerated for their total bacteria in microbial load of cow dung were calculating cfu/gm of samples. Serially diluted cow dung suspension was plated of nutrient agar plates. The maximum number (TNTC) of bacterial population was exhibited in initial dilution, then 10^{-3} to 10^{-4}showed which ranged from $170\times10^{-4}$to 100×10^{-5}cfu/ml and minimum concentration (TNFC) was exhibited in dilution10^{-6}.Table 1 and Figure 1 shows the microbial count of cow dung in initial dilution large numbers of microbes are present as further dilution shows isolated pure colonies.

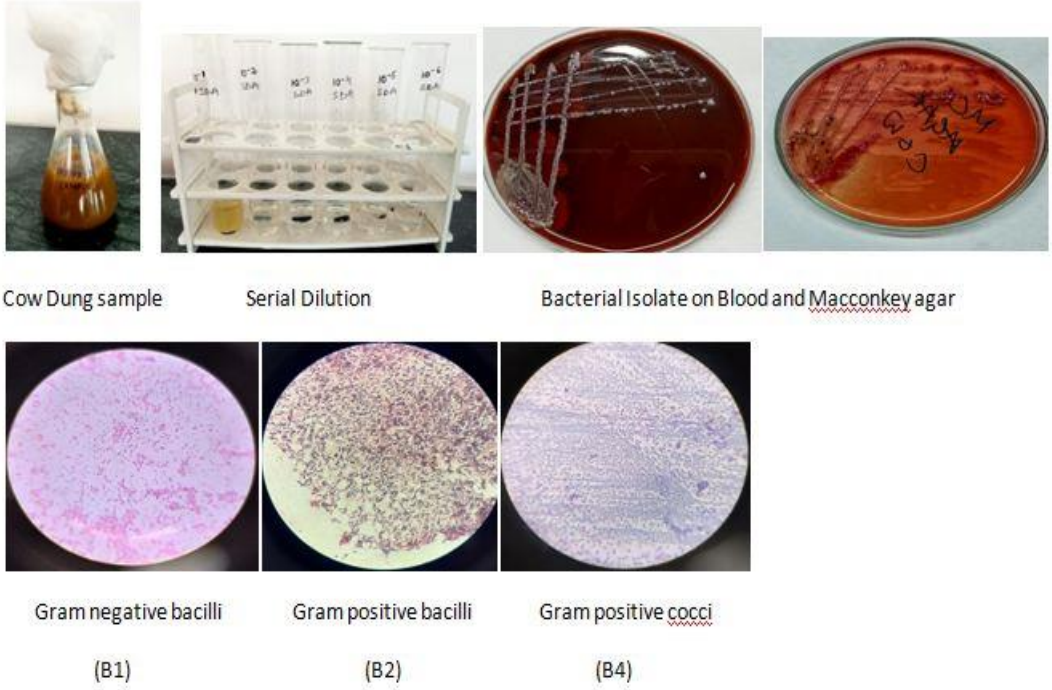


Figure 1: Isolation and identification of bacterial isolates from cow dung by serial dilution method.

Although bacteria and fungi are both important contributors to the composting process of cattle dung, bacteria are more abundant. The general microflora inhabitant of the cattle gut involves *Bacillus, Bifidobacterium,* and *Lactobacillus.*

Table 1 Microbial load of the cow dung sample by serial dilution method

S.No	Dilutions	Methods used	Total bacteria count
			Sample
1.	10^{-1}	Serial dilution method	TNTC
2.	10^{-2}	Serial dilution method	TNTC
3.	10^{-3}	Serial dilution method	170×10^{-4}
4.	10^{-4}	Serial dilution method	100×10^{-5}
5.	10^{-5}	Serial dilution method	TFTC
6.	10^{-6}	Serial dilution method	TFTC

Similarly in previous studies of cow dung showed microbial population and mixture of strains growth (9.4 × 108 cfu/ml) for 120 DAI followed by molasses (9.1 × 108 cfu/ml) and rice gruel (7.9 × 108 cfu/ml). These useful strains were further applied for crop productivity and slurry-based formulation with mixture of strains exhibited incredible plant growth. This research disseminates a successful technology to develop an eco-friendly bioformulation of buffalo dung slurry augmenting the crop growth in an eco-friendly manner leading to sustainable agriculture (Dhiman et al. 2020)

3.2.1. Streak plate method

The sample was taken with sterilized inoculating loop and streaked on blood agar medium plates. Then the plates were incubated at 37° C for 24 hours. Haemolytic activity of cow dung bacteria was observed on blood agar plates. The haemolytic activity was done on prepared blood agar plates. Mostly bacteria showed Haemolysis and similarly in previous studies. The selected bacterial colonies were streaked from nutrient agar (NA) agar plates to blood agar plates by using a sterile inoculating loop. Haemolysis was observed by the development of clear halo around the colonies after 24 h of incubation at 30°C in Figure 1.

3.3 Morphological and microscopically characterization of bacteria

Microorganisms produce colonies with characteristics which could be seen by naked eyes that are called as cultural characteristics. The cultural characteristics were observed on Nutrient agar, blood agar and Macconkey agar after incubation. These morphological characteristics were observed in different forms such as colony form, colony elevation, surface of the colony and colony colour (Figure 1). The morphological examinations of the isolates were determined by standard procedure of basic stain, gram stain and endospore stain (Cappuccino and Sherman 2005).

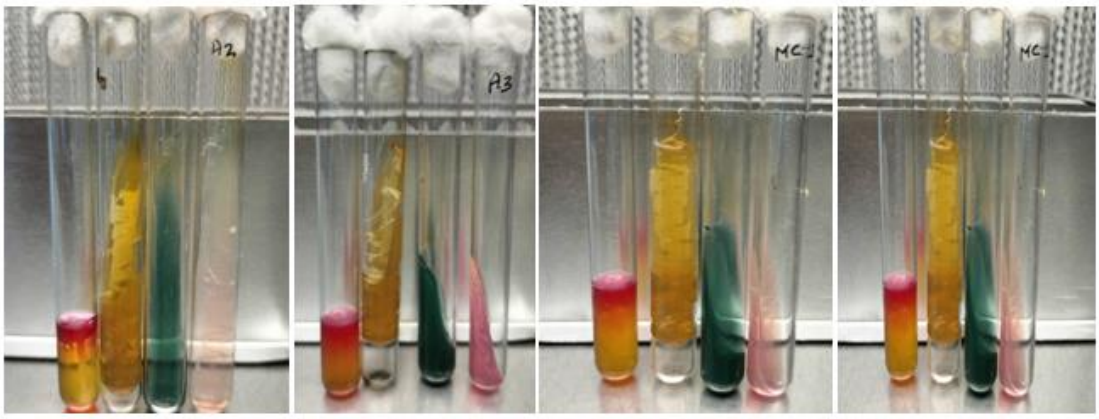
These isolated bacteria were characterized on the basis of morphological and microscopically as showed in Table 1; Figure 1. On the basis of Gram's staining, bacteria were differentiated as Gram positive and Gram-negative bacteria shown in Figure 1. Gram-positive bacilli C2 is *Bacillus* sp., Gram-positive cocci C4 *Micrococcus* sp, respectively. Gram positive bacteria and show purple colour after Gram's staining. C1, C3, C5, C6, and C7 all are Gram-negative bacilli is *Escherichia coli* show pink colour, rod shape after Gram's staining.C2 isolate was showing central endospore forming bacteria (Table 2 and 3). Three beneficial bacteria *Proteusmirabilis*, *Pseudomonas aeruginosa* and *Enterobacter xiangfangensis* were isolated from cow dung to evaluate for their effects individually as well as in consortium (Zhang et al.2017; Dhiman et al. 2020; Tomar et al. 2020).

Table 2 Morphological characteristics of bacteria isolates cow from dung

	Bacterial Isolates			
Characteristics	**C1**	**C2**	**C3**	**C4**
Form of colony	Circular	Circular	Circular	Circular
Translucency and opacity	Opaque	Opaque	Opaque	Opaque
Elevation of colony	Convex	Convex	Convex	Convex
Surface of colony	Smooth	Smooth	Smooth	Smooth
Pigmentation	Creamy white	Creamy white	Creamy white	Creamy white
Margin	Entire	Undulate	Entire	Entire

3.4 Biochemical tests

Biochemical tests such as Indole acetic acid production test, Triple sugar iron, citrate utilization test, urease test was performed for identification of bacterial isolates as shown in the Figure 2. Bacterial isolates C1, to C8 showing red colour ring formed in test tube indicating positive result for indole test. Bacterial isolate C1, C3, C5, C6 and C7 all showing mannitol motility test positive, this test for *Escherichia coli*. Triple sugar iron test showing orange colour converted in to yellow colour with gas formation. All bacterial isolates showing negative result for citrate utilization and urease test. (Cappuccino and Sherman 2005).



A, B, C, D A, B, C, D A, B, C, D A, B, C, D

Figure 2: Biochemical test (A) Indole, (B)Triple sugar iron, (C)Citrate, (D) Urease test of bacterial isolates C1 to C4 respectively

Table 3 Biochemical test of bacterial isolates from cow dung

	Bacterial Isolates				
Biochemical Tests	**C1**	**C2**	**C3**	**C4**	**C5**
Indole	+	+	+	+	+
Mannitol Motility	+	+	+	+	+
Triple Sugar Iron	A/A with gas	A/A with gas	A/A with gas	A/A with gas	A/A with gas
Citrate	-	-	-	-	-
Urease	-	-	-	-	-
Bacterial Identification	*Escherichia coli*	*Bacillus* spp	*Escherichia coli*	*Micrococcus*	*Escherichia coli*

Table 4 Name of bacterial isolates from cow dung on the basis of microscopy and biochemical test

Bacterial isolates	Name of Bacteria
C1	*Escherichia coli*
C2	*Bacillus* spp
C3	*Escherichia coli*
C4	*Micrococcus*
C5	*Escherichia coli*
C6	*Escherichia coli*
C7	*Escherichia coli*
C8	*Bacillus* spp.

3.5 Microbial load and Isolation of fungi by Serial dilution of Cow dung

The collected samples of cow dung were enumerated for their microbial load of total fungi. The serial dilution plating method was used to make suspension of cow dung in distilled ware purpose to minimizing the fungi in the dung in each dilution. The dung sample was diluted six times and labelled as 10^{-1} to 10^{-6} dilution. Serially diluted cow dung suspension was plated of sabouraud dextrose agar media (SDA). Microbial load of cow dung was calculating cfu/gm of sample. The maximum number (TNTC) of fungal population was exhibited in initial dilution, then 10^{-2} to 10^{-4} showed which ranged from 30×10^{-3} to3×10^{-5}cfu/ml and minimum concentration (TNFC) was exhibited in dilution 10^{-6}.Table 5 shows the microbial count of cow dung in initial dilution fungal colonies were present.

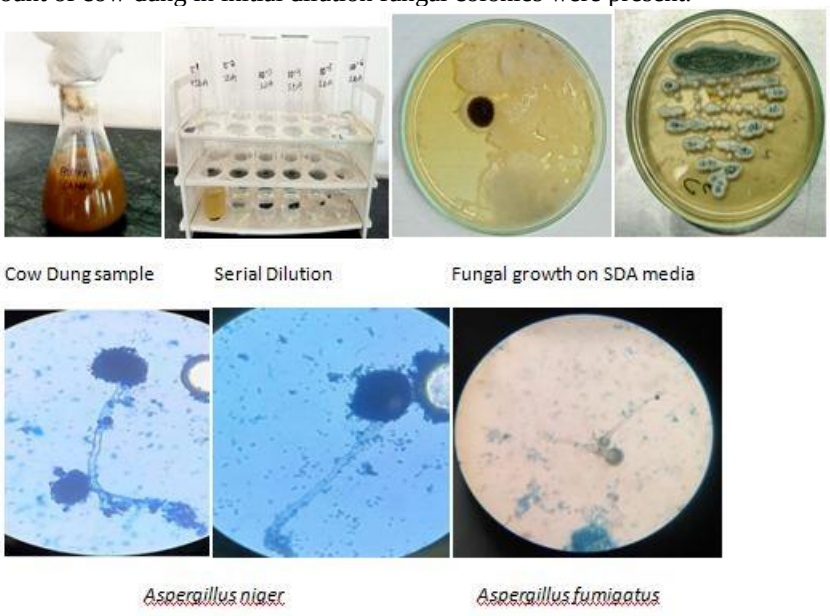


Figure 3: Isolation and identification of fungal isolates from cow dung by serial dilution method

Table 5 Microbial count of the fungal isolates

S.NO	Dilutions	Method Used	Total Fungal Count
			Sample
1.	10^{-1}	Serial dilution method	TNTC
2.	10^{-2}	Serial dilution method	35×10^{-3}
3.	10^{-3}	Serial dilution method	8×10^{-4}
4.	10^{-4}	Serial dilution method	4×10^{-5}
5.	10^{-5}	Serial dilution method	TFFC
6.	10^{-6}	Serial dilution method	TFFC

3.5.1 Streak plate method

The cow dung sample was taken with sterilized inoculating needle and transfer on Sabouraud dextrose agar media (SDA) agar medium plates. Then the plates were incubated at 27°C for 72 hours. Figure 3 shows the fungal colony appeared on the plate after incubation was then transfer to new plate. After incubation fungal colonial characteristic such as growth rate, texture, pigmentationon the surface and reverse side and the folds or ridges on the surface. The fungal isolates were identified up to genus level by standard protocol (Cappuccino and Sherman 2005).

3.6 Morphological and microscopically characterization of fungi

The isolation of fungi from dung was carried out in order to identify each fungal species. The cultural characteristics of fungal colonies were observed on Sabouraud dextrose agar media (SDA) agar medium plates after incubation. The morphological examinations of the isolates were determined by standard procedure (Cappuccino and Sherman 2005). These morphological characteristics were observed in different forms such as colony form, colony elevation, surface of the colony and colony colour.It was observed that isolated different pure colony of same fungus (Figure 3).

This isolated fungus was characterized on the basis of morphological cultural characteristics such as form of colony, colour of colony, texture of colony, aerial hyphae, pigmentation, margin, sporangiospores circular, white cottony colony, with the black dots and covers the entire plate, and microscopically like mycelial non-septate, creamy white, entire and columella is present on the top of sporangiospore, root like rhizoids are present shown in Figure 3; Table 5. Only *Aspargillus niger* and *Aspergillus fumigates* was isolated from cow dung and evaluate for their effects for further work. This isolated fungus was characterized on the basis of morphological cultural characteristics such as form of colony, colour of colony, texture of colony, aerial hyphae, pigmentation, margin, sporangiospores circular, white cottony colony, with the black dots and covers the entire plate, and microscopically like mycelial non-septate, creamy white, entire and columella is present on the top of sporangiospore, root like white colonies become greenish-blue, black, or brown as culture matures. Single-celled spores(conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle; long conidiophores arise from a septate mycelium are present shown in Figure 3; Table 5. Only *Aspargillus niger* and *Aspergillus fumigates* and was isolated from cow dung and evaluate for their effects for further work. Various authors reported different fungi from CD. For example, *Aspergillus niger, Aspergillus flavus, Aspergillus rapens, Aspergillus fumigatus, Rhizopus stolonifer, Mucor mucedo, Fusarium* spp. and *Vericosporium* spp. were reported in CD (Adegunloye et al. 2007); saprophytic fungi (yeast and molds) such *as Alternaria* sp., *Aspergillus sp., Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidum* sp., *Candida* sp.,.*Rhodotorula* sp., *Saccharomyces, Sporobolomyces, Trichosporon, and Torulopsis* sp. were reported by others (Obire et al.2008). Some fungi such as *Blastomyces* sp., *Botryodiplodiatheobromae, Fusarium* sp., *Nigrospora* sp., *Penicillum chrysogenum, Penicillum glabrum, Pleurofragmium* sp. and *Trichoderma harzianum* isolated from CD were reported as petroleum oil-degraders in aquatic environments in Nigeria (Orji et al. 2012).

Table 5 Morphological characteristics of fungal isolate isolated from cow dung

	Isolates
Characteristics	***Aspargillus niger***
Form of colony	*Aspergillus niger*, grow as colonies consisting of a network of branching and fusing hyphae that are often considered to be relatively uniform entities
Colour of colony	*Aspergillus niger* reveals that their growth is initially white but they change to black after a few days producing conidial spore.
Texture of colony	Cottony, dark brown to black, velvety, granular, and pale to yellow. Fruiting bodies were absent
Aerial Hyphae	Septate
Pigmentation	Black
Margin	The edges of the colonies appear pale yellow producing radial fissures
Sporangiospores	Single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle, long conidiophore arise from a septate.
Conidial head	Conidial head biseriate, radiate, conidia in chains or detached and dispersed. Single or paired conidia may resemble yeast cells. Conidial head uniseriate, columnar, conidia in chains or detached and dispersed.
	Aspargillus fumigates
Form of colony	Colonies are suede-like surface consisting of a dense felt of conidiophores.
Colour of colony	Colonies are typically blue-green
Texture of colony	Cottony, dark green,
Aerial Hyphae	Septate
Pigmentation	Green
Margin	Dark green in colour with a white border.
Sporangiospores	The asexual propagules that form inside a sporangium, which can be mostly spherical or cylindrical, through a process involving cleavage of the cytoplasm are named sporangiospores
Conidial head	Conidial head uniseriate, columnar, conidia in chains or detached and dispersed. Single or paired conidia may resemble yeast cells

4. CONCLUSION

In the present study, we analyzed the microbial load and isolate bacteria and fungi from cow dung. Isolated microbes were identified on the basis of their colony characteristics, morphology, Gram's staining, microscopically. The maximum number of bacterial population was exhibited in dilution 10^{-3} which ranged from 155×10^{-4}cfu/ml. Gram Positive cocci, Gram Positive bacilli, Gram Negative bacilli. 20 strains were isolated from cow dung. Among them, 4 isolates C1, C2, C3 and C4 were identify as *Micrococcus* sp. *Bacillus* sp. and *Escherichia coli* respectively on the basis of morphology and microscopically. The maximum number of fungal population was exhibited in dilution 10^{-2} which ranged 30×10^{-3} cfu/ml different fungus colonies of *Aspergillus niger* and *Aspergillus fumigatus* were observed. It was observed that spore forming bacillus were the predominant type of organism, which possibly present in cow digestive tract. These beneficial microbes will be used for further research work.

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CONFLICT OF INTEREST The authors declare that there is no conflict of interest.

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