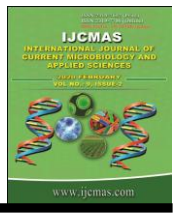
Int.J.Curr.Microbiol.App.Sci (2020) *9*(2): 584-591

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 2 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.902.073

Screening of Bacteria Present in Cow Dung

Ayush Tomar, Shalu Choudhary*, Lalit Kumar, Megha Singh, Nisha Dhillon and Sonam Arya

College of Applied Education and Health Sciences, Meerut, India

**Corresponding author*

Keywords

Bacteria, Cow dung, *Lactobacillus plantarum*

Article Info

Accepted:
08 January 2020
Available Online:
10 February 2020

A B S T R A C T

Cow dung has a wide range of micro-organisms, like- endospore forming *Bacillus, Enterococcus & protozoa*. 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 test. It was observed that spore forming bacillus were the predominant type of organism, which possibly helped in maturation of manure forming.

Introduction

Cow dung is excreted by bovine animal species, which are herbivores. It consists of undigested residues of consumed matter which has passed through the cow's gastrointestinal system (Teo & Teoh, 2011). Cow dung is widely used in formation of manure which is used as bio-fertilizers. Manure of cow dung enhances the minerals of soil and also develops resistance power of plants against pests and plant diseases. It contains about 80% of water and has some part of undigested plants material that have high amount of organic substance.

The microflora of cow dung contains a wide number of bacteria such as - *Bacillus*, like *lactobacillus* as central endospore forming *bacillus* some *cocci*, fungi and yeast such as *Saccharomyces* (Sharma & Singh, 2015). According to Ware *et al.*, (1988), lower part of the gut of cow has probiotic acitivity due to presence of different types of microorganisms like *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, Bacillus subtilis, Entrococcus diactylactis, Bifido bacteirum* and yeasts *(Saccharomyces cerevisiae).* There are several evidences to show that fresh cow dung and cow urine are antifungal and antiseptic in nature, which might be due to secretion of antimicrobial metabolites by cow dung micro-flora (Sharma & Singh, 2015).

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 test, so that we can identify which microorganism will possibly helped in maturation of manure formation.

Materials and Methods

In this study, cow dung was collected and left it for some days for the isolation of different types of bacteria. Using nutrient agar media (NAM), the bacteria from cow dung were characterized and identified on the basis of Gram's staining method and biochemical analysis.

For fulfilling the requirement of objective, we required following materials and chemicals:

Cow dung, Petri plates, test tubes, Nutrient agar media, Inoculating loop, sprit lamp, LAF, autoclave, Hot air oven, Sample container, Cotton, Foil paper, Slides, Distilled water, Cotton swab, Phosphate buffer saline solution (PBS solution), pH strip, Incubator, Microscope, Gram's staining kit and Normal saline solution.

Method

Cow dung was Collected and placed at a place from where samples were collected from upper surface and lower surface in sterile containers at a specific intervals- 2nd day, 7th day, 15th day, 21st day and 31st day. In this process streak plate method and pour plate method were used.

Isolation of bacteria

Nutrient agar media was used for the isolation of bacteria from cow dung.

Sample-1st (After 2 days)

Cow dung sample was streaked on nutrient agar media with the help of sterilized inoculating loop. Upper surface sample and lower surface sample were streaked on nutrient agar media separately. The plates were incubated at 37^0 C for 24 hours. After incubation, colony characteristics were studied. The colony was transformed to slants for further characterization.

Sample-2nd (After 7 days)

After 7 days, sample was collected from upper surface and lower surface of cow dung separately. Inoculum was made from the upper surface on nutrient agar medium, after that sterilized the loop again over the flame, till it became hot red. The plate was turned and streaked over agar surface. Incubation of all the plates was done at 37^0 C for 24 hours. Same procedure was repeated for lower surface sample.

Sample-3rd (after 15 days)

Sample of cow dung from upper surface and lower surface was collected after 15 days for isolation of bacteria. By applying the both pour plate method & streak plate method.

Streak plate method

The sample was taken with sterilized inoculating loop and streaked at the medium of the plates. Then the plates were incubated at 37^0 C for 24 hours.

Pour plate method

In pour plate method the sample was picked from upper surface and lower surface separately by the help of sterilized inoculating loop and transferred in Petri plates separately, after that the media (47^0 C) was poured in the

plates. The plates were rotated and left for solidification. All plates were incubated in incubator at 37^0 C for 24 hours.

Sample-4th (after 21 days)

After 21 days sample was collected from both upper and lower surface of cow dung. Both pour plate method and streak plate method were applied for isolation of bacteria from cow dung.

Streak plate method

The sample was taken by sterilized inoculating loop and inoculum was made on nutrient agar media and line was drawn over agar surface, (streak plate method) for both upper and lower surface sample separately. All the plates were incubated for 24 hours at 37^0 C temperature, and then single colony was isolated and transferred this colony in nutrient agar slant.

Pour plate method

The sample was taken from upper surface by the help of sterilized inoculating loop and transferred on Petri plates separately and the media (nutrient agar media) was poured, sample was mixed gently and left the plates for solidification.

After solidification, all the plates were incubated at 37^0 C for 24hours and then single colony was transferred on nutrient agar slant, after that same process was repeated for lower surface sample. The bacterial culture was maintained on nutrient agar media.

Sample-5th (after 31 days)

After 31 days sample was collected from upper and lower surface. Both method (pour plate & streak plate method) applied for isolation of bacteria.

Streak plate method

Sample was taken by sterilized inoculating loop aseptically from upper surface and transferred over the sample by drawing straight line over agar surface. All the plates were incubated at 37^0 C for 24hours. The same process was repeated for isolation of bacteria from lower surface.

Pour plate method

The upper surface sample was transferred in the sterilized Petri plates by the help of sterilized inoculating loop, under aseptic condition. The nutrient agar media was poured in Petri-plates and mixed gently and the plate was left for solidification. After solidification all plates were incubated at 37^0 C for 24 hours. The same process was repeated for lower surface sample.

Characterization and Identification of micro-organisms

After isolation pure colonies of bacteria were identified and characterized on the basis of morphological analysis such as color, size and shape. These colonies of bacteria were identified by the Gram's staining method.

Effect of Different pH on the growth of bacteria

Isolated bacteria were grown on media with different pH values.

Preparation of Different pH media

Media of different pH was prepared by addition of different pH buffer solution named according to pH as pH-2, pH-4, pH-6, pH-7 & pH-8. Two plates of each pH buffer was prepared and the pure culture of isolated bacteria was transferred by the help of sterilize inoculating loop aseptically on each nutrient agar media done by streak plate

method. Among the streaked plates, one plate from each pH was incubated at 37^0 C for 24 hours, and second streaked plate of different pH plates was incubate at room temperature for 24 hours.

Results and Discussion

In this study, cow dung was collected and left it for 31st days. The sample was taken at different time interval of days- 2, 7, 15, 21, 31st days. Sample was taken from upper and lower surface in a sterilized container. Different bacteria were isolated from cow dung on nutrient agar media from upper and lower surface and identified by Gram's Staining.

Among the isolated bacteria the *Bacillus* was present in high amount as compared to other bacteria, such as *Enterococcus*, *Diplococcus*, *Pseudomonas* and thread like bacteria. *Enterococcus* was dominant on other bacterial growth. These bacteria were characterized on the basis of morphological and biochemical analysis as per Table 2. On the basis of gram's staining, bacteria were differentiated as gram positive and gram negative bacteria shown in Figure 10. *Bacillus*, *Diplococcus*, *Enterococcus* were gram positive bacteria and show purple colour after gram's staining. *Pseudomonas* show pink colour, rod shape after gram's staining. Isolated bacillus was central endospore forming bacteria.

Effect of different pH level *on Bacillus*

From the isolated bacteria by gram staining the bacillus was found in high amount than other bacteria so we check the effect of different pH level on growth of *bacillus*. There was no effect of pH on the growth of bacteria, optimal temperature for growth was 37^oC.

Table.1 Composition of NAM (Nutrient agar media)

S.No	Chemicals	Amount Required
1.	Peptone	5.0gm
2.	NaCl	5.0gm
3.	Beef extract	3.0gm
4.	Yeast extract	3.0gm
5.	Agar	15.0gm
6.	Distilled water	1000ml

Table.2 Growth of bacteria at different surface of cow dung

S.No.	Sample/Time duration	Gram's staining	Lower surface	Upper surface
1.	Cow dung sample (after 2nd days)	Purple color	*Bacillus*	*Bacillus*
2.	Cow dung sample (after 7days)	Purple Colour	*Bacillus*	*Bacillus*
3.	Cow dung sample (after 15days)	Purple & Pink colour	*Bacillus & pseudomonas*	*Diplococcus*
4.	Cow dung sample (after 21st days)	Purple colour	*Bacillus*	*Bacillus*
5.	Cow dung sample (after 31st days)	Purple colour	Thread like bacteria	*Enterococcus*

Table.3 Growth of different pH level and temperature

Different Temperature	Different pH level				
	pH-2	pH-4	pH-6	pH-7	pH-8
Room Temperature	+	+	++	++	++
Incubator temperature (37°C)	++	++	++	++	++

* + Sign represents moderant growth; ++ Represents good growth

Figure.1 Sample 1st after 2 days

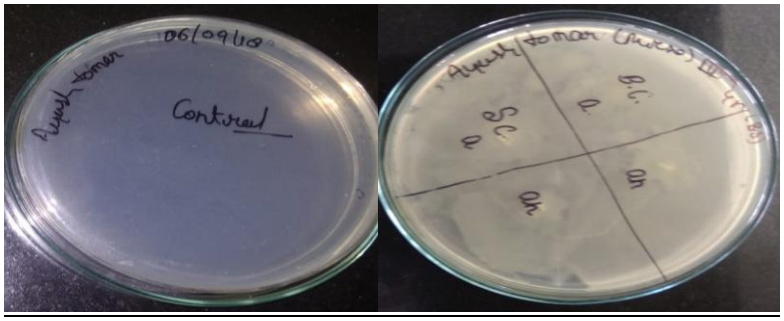


Figure.2 Sample 2nd after 7 days

Figure.3 Sample 3 rd after 15 days

Figure.4 Sample 3rd after 15 days (Pour Plate Method)

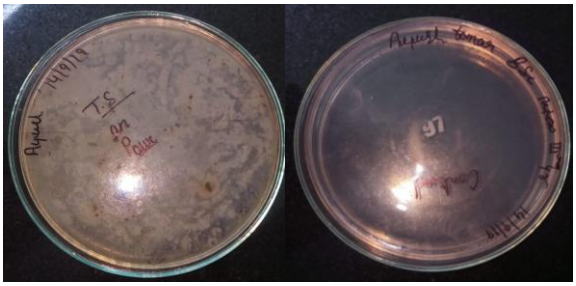


Figure.5 Sample 4th after 21 days (Streak plate method)

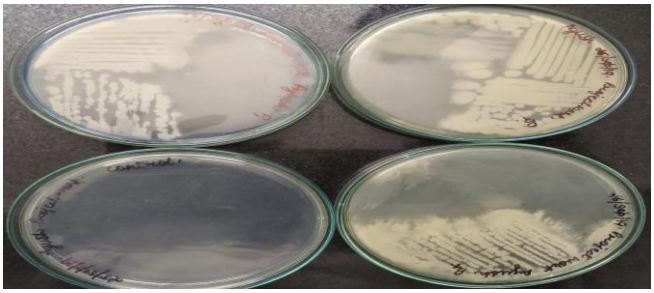


Figure.6 Sample 4th after 21 days (Spread Plate Method)

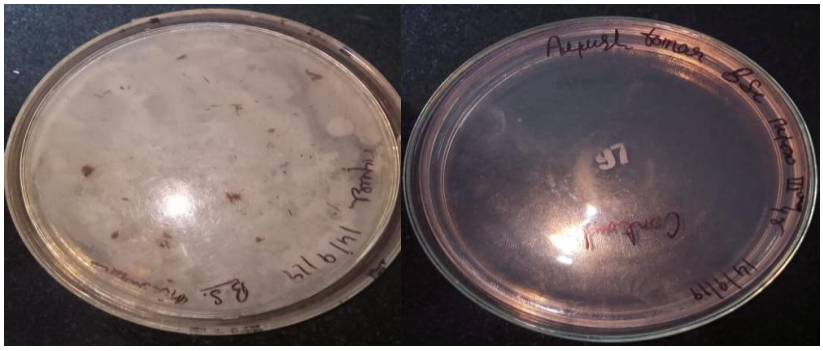


Figure.7 Sample 5th after 31 days (Streak plate method)

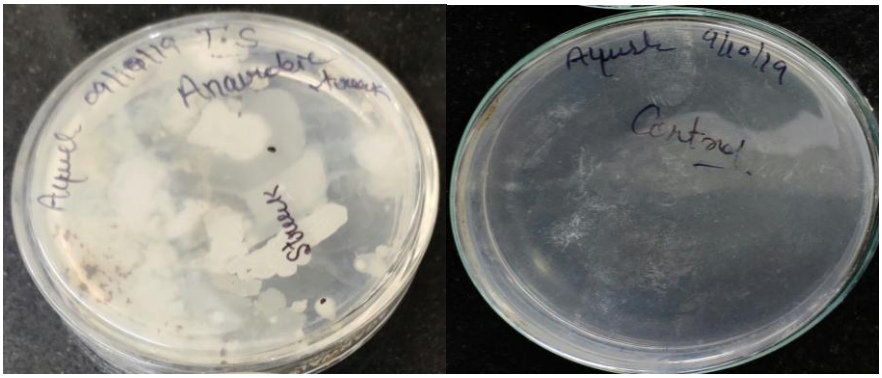


Figure.8 Sample 5th after 31 days (Pour Plate Method)

Figure.9 Isolated Pure Culture Plates of Different pH by Streak Plate Method (A- at Room temperature; B- at Incubator Temperature)

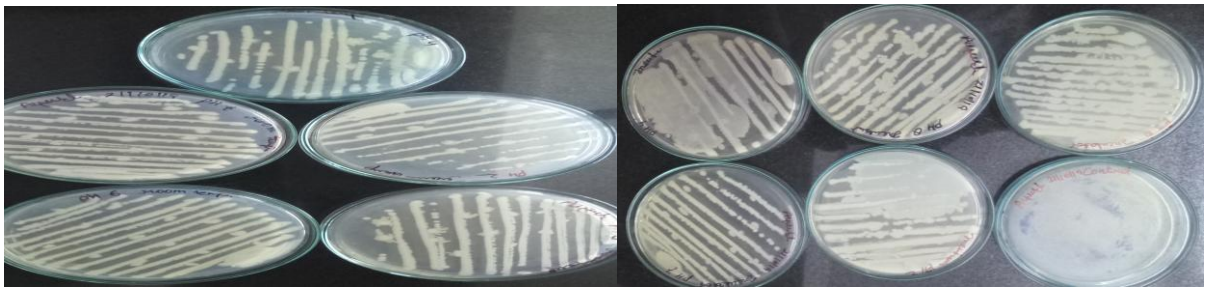
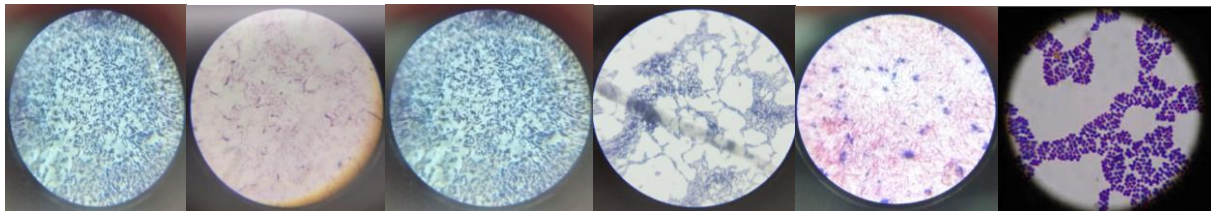


Figure.10 Different types of bacteria were identified such as A: *Bacillus*; B: *Pseudomonas*; C: Endospore forming *Bacillus*; D: *Enterococcus*; E: Thread like Structure; F: *Diplococcus*



In conclusion, **i**t was found that various species of gram positive bacteria were present in cow dung such as - *Bacillus spp.*, *Enterococcus*, *Diploccocus* and gram negative bacteria such as - *pseudomonas*. *Enterococcus* was present in dormant condition which affects the growth of other bacteria. According to time, number of *Bacillus* species was increasing in cow dung and present in motile condition. It is concluded that the micro-flora of cow dung shows the isolated bacteria is endospore forming bacillus. The *Enterococcus* show dormant nature on nutrient agar media and inhibit the growth of other bacteria. Hence, the *Enterococcus* cultures work as a bacteriostatic for the growth of other bacteria.

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How to cite this article:

Ayush Tomar, Shalu Choudhary, Lalit Kumar, Megha Singh, Nisha Dhillon and Sonam Arya. 2020. Screening of Bacteria Present in Cow Dung. *Int.J.Curr.Microbiol.App.Sci.* 9(02): 584-591. doi: https://doi.org/10.20546/ijcmas.2020.902.073