



Isolation and identification of microorganisms from goat intestine

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ABSTRACT

The present study has been carried out to evaluate the microorganisms from goat intestine. The microorganisms were isolated by streak plate method, and the microorganisms were identified by the serial dilution method. The components present in the goat intestine were detected by biochemical test such as indole, methyl red, vogesproskauer's test, mannitol test, urease test, triple sugar ion test, sucrose test and glucose test. The microorganisms were screened under microscope for its structural elucidation.

Keywords: *Clostridium perfringens*, *Escherichia coli*, *Escherichia fergusonii*, *Enterobacterium*, *Yersinia enterocolitica*

INTRODUCTION

Microbial communities make the essential elements of oxygen, carbon, nitrogen, and sulfur available for other life on our planet. Without microbial decomposer communities, life would be smothered in dead organisms. Microorganisms also carry out almost half of the photosynthesis on our planet, increasing oxygen levels and lowering carbon dioxide [1]. Both animals and plants are closely associated with microbial communities that make nutrients more available, provide protection from diseases, make essential vitamins, or a combination. Recent discoveries are highlighting the roles that microorganisms play in the human intestine. Some microbes are free-living organisms and others are parasites. Intestines are a profound source of gelatin and glutamine which are a key for stabilizing and improving leaky gut function.

Goats are prone to attack by a number of pathogens, toxic substances, and nutritional causes. The most common causes of diarrhea in adult goats are parasite, including *Coccidia*, *Clostridium perfringens* type D, and *Salmonella spp.* infection, nutritional factors; toxic agents; liver disease; and copper deficiency. An uncommon cause is Johne's disease, but diarrhea may occur in the terminal stages of the disease [2]. This case in a goat with diarrhea and wasting yielded *Escherichia fergusonii* in faeces and internal organs.

The number and type of bacteria in the gastrointestinal tract vary dramatically by region. In healthy individuals the stomach and proximal small intestine contain few microorganisms, largely as a result of the bactericidal activity of gastric acid; those that are present are aerobes and facultative anaerobes. One interesting testimony to the ability of gastric acid is to suppress bacterial populations as seen in patients with achlorhydria, a genetic condition which prevents secretion of gastric acid. Such patients, which are otherwise healthy, may have as many as 10,000 to 100,000,000 microorganisms per ml of stomach contents.



Goat intestine - A View

The gastrointestinal tract is sterile at birth, but colonization typically begins within a few hours of birth, starting in the small intestine and progressing casually over a period of several days. It is also clear that microbial populations exert a profound effect on structure and function of the digestive tract.

Intestinal bacteria also have an important role in sex steroid metabolism. Bacterial populations in the large intestine digest carbohydrates, proteins and lipids that escape digestion and absorption in small intestine. This fermentation, particularly of cellulose, is of critical importance to herbivores like cattle and horses which make a living by consuming plants. In the present study, we came to isolate and identify the microorganisms from goat intestines which includes *Escherichia coli*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Escherichia fergusonii*, *Enterobacterium*.

EXPERIMENTAL SECTION

SAMPLE COLLECTION

In the present study the goat intestine was collected from Thanjavur District in Tamil Nadu. The collected samples were brought to the laboratory for isolation and identification of bacteria by using following techniques.

SERIAL DILUTIONS OF THE SAMPLE

The nutrient agar medium were prepared and sterilized. The medium was poured in sterile petri plates and allowed to solidify. 10gm of the sample was added to 90ml of the distilled water in a flask.



Serial dilution plates

It was shaken vigorously and 1ml was transferred from 10^{-1} dilution to the next dilutions up to 10^{-9} dilution. After solidifying, the nutrient agar plates with dilution 10^{-4} and 10^{-5} were taken. 0.1ml sample was poured in petri plates using spread plate technique. The plates were incubated for bacterial growth at 37°C for 24 hrs. After incubation, the plates were observed.

REASON FOR THE SAMPLE UNDERGOING SERIAL DILUTION

A Pure culture may be obtained by serially diluting the sample with sterile water to the point of extinction in number of cells. This method is used to isolate the organisms, if it is present in large number in the mixture.

ISOLATION AND IDENTIFICATION OF BACTERIA

The media is of complex type that is rich in vitamins and nutrients. The following components were used to prepare nutrient agar medium.

15 g of agar was dissolved in 250 ml of distilled water and boiled till the agar was melted. In a 1 liter beaker, 3.0 g of beef extract, 5.0 g of peptone, 5.0 g of NaCl and the melted agar were poured and made to 1000 ml with distilled water. The medium turns to turbid. It is heated, until the agar peptone was dissolved. Adjust the pH to 6.5 - 7.0 using Bromothymol blue as an indicator. Disperse 250 ml, to each of four conical flasks which were sterilized by autoclaving at 121°C for 20 minutes. After sterilization, the liquefied agar was poured into the two sterilized Petri plates which were marked as control, with 10^{-4} and 10^{-5} dilution. The agar was poured of about 15-20ml in each of the two petri plates. The Plates were then allowed to remain undisturbed until the agar was cooled and hardened.

INOCULATION OF THE SAMPLE

The two petri plates with the solidified agar were marked as control 10^{-4} and 10^{-5} was taken. Inoculation was done with the help of 0.1 ml of micropipette inside the inoculation chamber. Using sterile micropipette, 0.1 ml of the diluted sample was taken from the 10^{-5} , 10^{-6} and 10^{-7} dilution and was transferred to the Petri plates containing culture medium which was already marked as dilution plate. The plate was rotated gently to get uniform distribution of inoculums. After inoculation the Petri plates were incubated at 37°C or 24 - 48 hours. After incubation some of the dispersed cells of the colonies were developed.

SUBCULTURE OF THE BACTERIAL COLONIES

STREAK PLATE METHOD

The streak plate method offers a most practical method of obtaining discrete colonies and pure culture. The streak plating technique was done by usual method.

Culture of Microbial Floras

Clostridium perfringens *Escherichia fergusonii*



Yersinia enterocolitica *Escherichia coli*



Enterobacterium

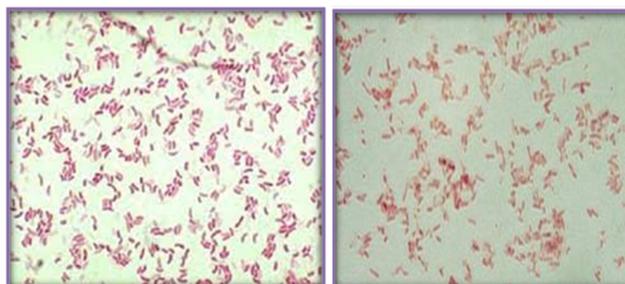


ISOLATION OF BACTERIA**GRAM STAINING**

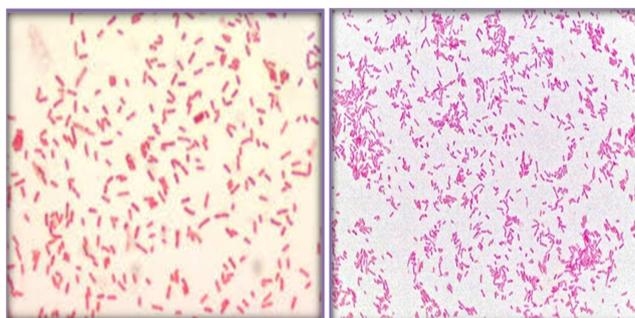
This method was developed by Hans Christian's Gram a Danish bacteriologist. It is used to differentiate the Gram positive and Gram negative bacteria. The test was also done by usual method.

MICROSCOPIC OBSERVATIONS OF MICROBIAL SPECIES

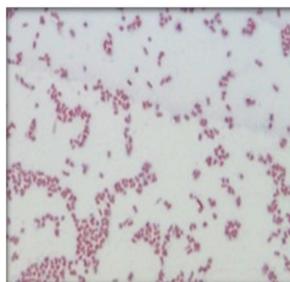
Clostridium perfringens *Escherichia fergusonii*



Yersinia enterocolitica *Escherichia coli*



Enterobacterium

**BIOCHEMICAL TESTS**

The following biochemical tests were performed to characterize the isolates:

INDOLE TEST

Tryptophan broth was prepared and sterilized by autoclaving at 121°C for 15 minutes. The broth was cooled and culture was inoculated. After 24 hours of incubation, 0.3 ml of Kovac's reagent was added, after adding Kovac's reagent there is an appearance of red color ring formation which indicates the result as positive.

METHYL RED AND VOGES PROSKAUER'S TEST

MR – VP broth was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs. The culture was inoculated into the tubes containing broth. The tubes were incubated at 37°C for 24 hrs. Then 0.5ml of MR reagent, 0.2ml of VP reagent A and B were added into the tubes.

CITRATE UTILIZATION TEST

The media was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs. The media was transferred into the tubes and then the slants were prepared by keeping in slanting position for solidification. The culture was inoculated into the tubes and incubated at 37°C for 24 hrs.

UREASE TEST

The media was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs/Inch². The media was added and added 20% urea solution to it. The media was transferred into the tubes and then slants were prepared by keeping them in slanting position for solidification. The culture was inoculated into the tubes and incubated at 37°C for 24 hrs.

TRIPLE SUGAR ION TEST

The media was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs / Inch². The media was transferred into the tubes and then the slants were prepared. The culture was inoculated into the tubes and incubated at 37°C for 24 hrs. After incubation were observed the results. By convention the tubes are read in two parts; colour change on the slant and colour change in the butt. Alkaline slant & alkaline butt (K/K) indicates the absence of carbohydrate fermentation and Alkaline slant & acid butt (K/A) presence of Glucose fermented, Lactose and sucrose not fermented. Alkaline slant & acid/black butt(K/A/H₂S) denotes Glucose fermented, Lactose and sucrose not fermented and H₂S produced in the medium. Acid slant & acid butt (A/A) indicates the glucose fermented, Lactose and /or sucrose fermented on the medium.

CATALASE TEST

A clean microscope slide was taken. A drop of culture broth was placed on the slide Two or three drops of hydrogen peroxide solution was added to culture broth on the slide.

OXIDASE TEST

A clean microscope slide was taken. Oxidase disc coated with 1%N-N, tetra methyl paraphenylenediamine dihydrochloride was placed at the centre of the slide. A drop of culture broth was placed over the surface of the oxidase disc.

NITRATE REDUCTION TEST

Dissolve 9 g of Nitrate Broth in 1 litre of distilled water. Dispense 10 ml aliquots of the broth into tubes fitted with Durham tubes. Sterilize by autoclaving at 121°C for 15 minutes. Inoculate the tubes heavily with a fresh culture of the suspected organism. Inoculate at least 1 ml of the sample in a tube or take a big part of a colony with an inoculating loop.

CARBOHYDRATE FERMENTATION**PHENOL RED BROTH BASE SUGAR FERMENTATION**

Dissolve all the components in boiling water and adjust pH at 7.5 and autoclaving at 121 °C for 15 minutes.

To prepare the Glucose broth, Sucrose broth and Mannitol broth and autoclaving at 121 °C for 15 minutes. Transfer a portion of the colony to be tested to the media containing the appropriate carbohydrate for 18-24 hours. Incubation of up to 3 days may be required for some organisms. Check results daily. Acid production is indicated by a change in colour of the indicator used. Bromocresol purple is most commonly used which is purple in alkali and yellow in acid. Gas production is indicated by displacement of liquid from the inverted Durham tube.

CONFIRMATION TEST

The identified organisms were allowed to conformation test on the specific medium of each individual organism namely, *Escherichia fergusonii* was identified on Macconkey agar, Chocolate agar was used to identify *Clostridium perferigenes*.

The medium was prepared and allow to sterilization to inoculate the media in to the Petri plate. The culture was streaking on the medium after solidification process. The plate was incubated for 24 to 48°C at room temperature. After incubation process the specific colonies confirmed based on the morphological characters of growing microbes.

CULTURE MAINTENANCE

The isolated cultures of bacteria are maintained at 4°C in nutrient agar.

RESULTS AND DICUSSSION**MICROSCOPIC EXAMINATION**

Based on the gram staining, the morphological study of the test organism was tabulated. Motility test was demonstrated in order to conclude whether the organism is motile or non-motile as mentioned in the table-1.

Table 1: Gram staining and Motility test results of Isolated Pathogens

S. No	Name of the organism	Gram staining	Motility
1.	<i>Escherichia coli</i>	Gram negative, rod	Non-Motile
2.	<i>Clostridium perferingens</i>	Gram negative, rod	Non-Motile
3.	<i>Yersinia enterocolitica</i>	Gram negative, rod	Non-Motile
4.	<i>Escherichia fergusonii</i>	Gram negative, rod	Non - Motile
5.	<i>Enterobacterium</i>	Gram negative, rod	Non-Motile

BIOCHEMICAL TESTS

The biochemical test results for various tests like Indole, Voges Proskauer's, Citrate - utilization, Catalase, Carbohydrates fermentation, Nitrate reduction, Triple sugar iron and Oxidase tests were observed and tabulated in table 2.

Table 2: Biochemical Characterization of Isolated Pathogens

S. No	Biochemical Tests	<i>E.coli</i>	<i>Clostridium perferingens</i>	<i>Yersinia enterocolitica</i>	<i>Escherichia fergusonii</i>	<i>Enterobacterium</i>
1.	Indole	+	-	-	+	-
2.	Methyl Red	+	-	+	+	-
3.	VP	-	-	+	-	+
4.	Mannitol	-	-	-	-	-
5.	Urease	-	+	-	-	-
6.	Nitrate	+	+	+	+	+
7.	Citrate utilization	-	-	+	-	+
8.	Triple sugar iron	A/G	A/K	+	A/G	+
9.	Sucrose	-	+	-	-	-
10.	Glucose	-	+	-	-	-

(-) – Negative, (+) – Positive, (A) – Acid, (K) – Alkaline, (G) – Gas.

ISOLATION AND IDENTIFICATION OF TEST ORGANISMS

The present study shows the isolated bacterial species were the micro organisms which were found and isolated from the various fast food samples. The biochemical test results, like Indole, Methyl red test, Voges Proskauer's test, catalase, Urease, Oxidase, citrate utilization, TSI, Nitrate reduction test and carbohydrate fermentation test, for bacterial species were observed and tabulated in table 2. All the isolates were characterized by their cultural characteristics and they were confirmed as shown in table (3).

Table 3: Shows the Colony Morphology of Isolated Pathogens on the Selective Medium

S. No	Test organisms	Medium used	Colony morphology
1.	<i>Escherichia e.coli</i>	Chocolate agar	Zone formation
2.	<i>Clostridium perfringens</i>	Chocolate agar	Zone formation
3.	<i>Yersinia enterocolitica</i>	Macconkey agar	Translucent with dark pink centers
4.	<i>Escherichia fergusonii</i>	Macconkey agar	Yellow smooth, rose pink colour colonies.
5.	<i>Enterobacterium</i>	Macconkey agar	Yellow smooth, rose pink colour colonies.

SELECTIVE PLATING

There are three bacterial species were isolated in the goat intestine sample. *Escherichia fergusonii* (Gram-ve) and *Yersinia enterocolitica* produce Translucent with dark pink centers (Gram-ve) was observed in Macconkey agar medium. *Clostridium perfringens* (Gram +ve) produce Zone formation in Chocolate agar.

E. coli was found to be pathogenic for mice. Antibio gram studies revealed that Ciprofloxacin was highly sensitive against all the isolated bacteria. Diversified bacterial species are prevalent in broiler. However, *E. coli* and *Salmonella spp.* infection might make the bird vulnerable for easy access of infection. Proper vaccination and use of selective antibiotics are crucial in protecting broilers from these pathogens [3].

SUMMARY AND CONCLUSION

The isolated organisms were obtained by selective media and confirmed using standard biochemical tests. A pure bacterial culture is the first step in the process of bacterial identification. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics, and susceptibility to antimicrobial agents of a particular bacterial strain. Pure cultures are suitably obtained by using solid media, by streak plate or pour plate methods.

Yersinia species produce enterotoxins but are also invasive and produce inflammatory change within the intestine. In calves, infection commonly progresses to a bacteremia. These organisms are normally "laying low" in the small

and large intestine that is, they are present. When unusually high levels of these nutrients reach the intestine, *Clostridium perfringens* undergoes explosive growth, increasing its numbers rapidly within the intestine. As the organism grows in number, it releases very potent toxins (bacterial poisons) that harm the animal. These toxins can cause damage to the intestine as well as numerous other organs. This can result in fatalities, particularly in the non-vaccinated animal.

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