



## Evaluation of the Fecal Microbes in Pre-Adult, Adult and Castrated Male Goats (*Capra Hircus*)

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### ABSTRACT

The importance of animal gut microbiota (GM) is widely acknowledged because of its pivotal role in metabolism, immunity, and health maintenance. The skin, vagina, and oral cavity also provide important niches for distinct bacterial communities, contributing to the immune system's defense against potential pathogens. These microbial communities significantly affect host health by affecting host metabolism, immunity, and hormones. Goats are often used in biological research, surgical training, and education. However, little is known about what constitutes a healthy microbiota to manipulate reproductive efficiency successfully. We plan our study to evaluate the preliminary fecal microbes in pre-adult, adult and castrated male goats (*Capra hircus*). In this study, we have identified eight microbes in three reproductive stages of a male goat. *Proteus mirabilis* is the only species present in all three reproductive stages of a male goat. On the other hand, six microbes, namely *Citrobacter*, *Freundin*, *Proteus vulgaris*, *E. coli*, *Salmonella SPS*, *Morganella morgani*, *Shigella sonnei*, and *Shigella* (A, B, and C), are present in the castrated male goat. The study of gut microorganisms is of interest because of its close relationship to the well-being of the host. Here we conclude that these bacteria population from the feces of male goat may be in symbiotic relationship with their host as gut micro flora, which may involve in biological activities of the host. Hence, further studies are warranted to provide strong evidence to confirm the same.

**Keywords:** Gut Microbiota (GM), Goat (*Capra hircus*), *Proteus mirabilis*. Fecal, Host metabolism.

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### INTRODUCTION

Goats are native to West Asia and Eastern Europe's hilly regions and graze on slopes and plains. Domesticated goats are modern-day common goats that are closely related to sheep. According to the United Nations' Food and Agriculture Organization, there were over 924 million goats in the country in 2011. For thousands of years, goats have been used for meat, hair, milk, and skin. Goats are also used to help lift heavy loads across several areas. The goat is a domestic ruminant animal that is a member of the bovid family, which includes sheep and cattle. Goats are more often used in biological research, surgical training, and education. Medical, orthopedic, psychological, chemotherapeutic, and physiologic research also use them. Prions, the protein that causes Mad Cow Disease and Creutzfeldt-Jacob Disease, were initially discovered in sheep and goats. In 1997, this research was awarded a Nobel Prize.

Microbes can be multi-cellular or single-celled organisms and include bacteria, protozoa, and some fungi and algae. The human body is home to a unique microbial population, including bacteria, archaea, fungi, and viruses. The differences in microbiota composition at each body site are shaped by the varying environmental conditions such as pH, levels of oxygen, availability of nutrients, humidity, and temperature, enabling various populations to thrive and perform different functions while interacting with the human host [1].

These microbial communities have a significant impact on host health by affecting host metabolism [2], immunity [3], and hormones [4]. Most microbes in the human body live in the gut, which is home to hundreds of bacterial species [5]. Firmicutes and Bacteroidetes are the most common gut bacteria types. The skin, vagina, and oral cavity also provide important niches for distinct bacterial communities, contributing to the immune system's defense against potential pathogens [6-9]. A wide range of factors can cause shifts in the microbiota composition (termed dysbiosis). Dysbiosis is usually associated with harmful effects and may have long-term consequences, leading to disease. Examples of dysbiosis-related disease states include obesity, inflammatory bowel disease (IBD), diabetes, and metabolic syndrome [10].

The importance of animal gut microbiota (GM) is widely acknowledged because of its pivotal role in metabolism, immunity, and health maintenance. The dynamic distribution of gut microbiota can reflect the level of health. As the largest and most complex mammalian micro-ecosystem, the gut microbiota (GM) regulates body health and plays an important bridging role between diet and host [11, 12]. Studies indicate that the GM is a reflection of evolutionary selection pressures acting at the levels of the host and microbial cell.

The GM is intimately involved in numerous aspects of normal host physiology, from nutritional status to behavior and stress response. GM is a primary cause of many diseases, affecting both near and far-flung organ systems [13-15]. This is especially true for ruminants, which have demonstrated their unique digestive properties and microbial groups that help them adapt to high-fiber foods but also make them susceptible to various diseases and conditions [16]. Accordingly, GM in ruminants plays a more prominent role in various physiological states than in most other mammals [17, 18]; some potential links may also exist between age factors and intestinal microbiota. However, whether the participation of age-related factors will affect the micro intestinal ecology of ruminants has rarely been reported. Based on the above report we plan our study to characterization the preliminary fecal microbes in pre adult, adult and castrated male goat (*Capra hircus*).

## MATERIAL AND METHODS

**Experimental animals:** Pre-pubertal (two months old), adult (seven-twelve months old), and castrated male (twelve months old) goats were chosen from the Sustainable Integrated Farming System (SIFS), Bharathidasan University, Tiruchirapalli-620 024. They were maintained on the farm as free roaming from the day they hatched out. These goats were caged only at night (6 PM to 6 AM). They were allowed to roam freely on the farm during the daytime (6 AM to 6 PM). The animals were fed their natural foods like grass, etc.

**Isolation of microorganisms from the gut region of goat:** Pre-pubertal (two months old), adult (seven-twelve months old), and castrated male (twelve months old) goats were chosen for the isolation of bacterial strains. Fecal samples will be collected in large zip lock covers, labeled and kept in ice immediately after collection, and transported to the laboratory. The samples were kept in a deep freezer (20 °C) until further use. Fecal samples will be collected immediately after observation of fecal samples are transferred to permanent freezing facilities and stored at -20 °C for further analysis. At the time of feces collection, sterilized bags will be fitted to the animals for a few minutes to ensure the collection of a sufficient amount of feces.

**Biochemical characterization of microbe:** Nine colonies from adult goats, Thirteen colonies from pre-puberty, and six colonies from castrated were tested for biochemical analysis viz., Indole, Methyl red, Voges Proskauer, Citrate, Urease, TSI (Triple Sugar Iron), Catalase, and Gram staining for the preliminary confirmation of species type.

**Indole production test:** Tryptophan is an essential amino acid that can undergo oxidation through the enzymatic activities of some bacteria. The enzyme tryptophan then mediates the conversion of tryptophan into metabolic products. Tryptophan with indole production is not a characteristic feature of all microorganisms and therefore serves as a biochemical marker. The presence of indole is detectable by adding Kovac's reagent. This test is done to determine the ability of microorganisms to degrade the amino acid tryptophan.

**Methyl red test:** Hexose monosaccharide glucose is the primary substrate of all enteric organisms for energy production. This process's products will vary depending on the specific enzymatic pathway process and the specific enzymatic pathway present in the bacteria. This test is done to determine the ability of microorganisms to oxidize glucose with the production of acid products.

**Voges – Proskauer's test:** Voges - Proskauer's test determines the capability of organisms to produce non-active or neutral and produces acetyl methyl carbinol, organic acids that result from glucose metabolism. This test is performed to differentiate further among enteric organisms such as E-coli and Klebsiella.

**Citrate utilization test:** In the absence of fermentable glucose or lactose, some microorganisms are capable of using citrate as their carbon source for their energy. The ability depends on the presence of citrate in the cell. Citrate is the first significant intermediate in the Krebs cycle, produced by the condensation of active acetyl with oxaloacetic acid. This test is performed to differentiate among enteric organisms based on their ability to ferment citrate as a sole carbon source.

**Triple sugar iron test:** The triple sugar iron (TSI) agar test is designed to differentiate among the different groups or genera of Enterobacteriaceae. Which are gram-negative Bacilli capable of fermenting glucose with the production of acid, and distinguish *Entero bacteriaceae* from other gram-negative intestinal bacilli. This differentiation is made based on differences in carbohydrate fermentation patterns and hydrogen

sulfide production by various groups of intestinal organisms. This test is performed to learn a rapid screening produced that will differentiate among the members of *Enterobacteriaceae*.

**Urease test:** Urease, which is produced by some organisms, is an enzyme that especially helps in the identification of *Proteus Vulgaris*. Although other microorganisms may produce urease, their action is found to be slower than that seen in *Proteus* species. This test serves to distinguish the members of these fermenting enteric organisms rapidly. This test is performed to determine the ability of microorganisms to degrade urea utilizing the enzyme urease.

**Catalase test:** The enzyme catalase splits hydrogen peroxide into water and oxygen. When a small portion of the colony is introduced into H<sub>2</sub>O<sub>2</sub>, the rapid evolution of bubbles indicates a positive 48-hour result. To the 5ml of 48-hour-old culture broth, 1ml of 3% H<sub>2</sub>O<sub>2</sub> was added. The release of bubbles was observed and compared with the control.

**Oxidase test:** The oxidase test is a necessary differential procedure that should be performed on all gram-negative bacteria that are to be identified. In this test, oxidase discs were placed over the individual cultures on an agar medium. An immediate color change of oxidase discs from white to blue indicates the positive results, and no color change indicates the negative result.

## RESULTS AND DISCUSSION

In this study, we have identified eight microbes in three reproductive stages of a male goat. *Proteus mirabilis* is the only species present in all three reproductive stages of a male goat. *Edwardsiella tarda* species were present only in pre adult male goat whereas absent in all other groups. On the other hand, six microbes, namely *Citrobacter*, *freundii*, *Proteus vulgaris*, *E. coli*, *Salmonella SPS*, *Morganaellamorganii*, *Shigellasonnei*, and *Shigella* (A, B, and C), are present in castrated male goat whereas absent in other experimental groups. Intestinal microbes play a crucial role in the maintenance of host health. They act as a defense barrier against transient pathogens, support the host in digestion and energy harvesting from the diet, stimulate the immune system, and provide nutritional support for enterocytes. Among these and other animal species' regions of the GI tract, the distal part of the intestinal tract (i.e., fecal microbiota) has been the most widely studied. The goat complex microbiota. The study of GI microorganisms is of interest because of its close relationship to the well-being of the host. In addition, an increasing number of investigations suggest that GI microorganisms may play a role in the etiology of various GI disorders. However, little is known about what constitutes a healthy microbiota, its normal biological variations within and among individuals, and how to manipulate reproductive efficiency successfully. Gastrointestinal microbial populations vary significantly among host species and regions of the gastrointestinal tract. Therefore, we decided to study the fecal microbes in adult, pre-puberty and castrated male goats.

In many ways, the results of the current study were similar to previous studies [16-18], but there were some interesting observations in our study, namely that the bacterial isolates from fecal samples mainly were gram-negative strains, and some were gram-positive strains. The bacterial strains in male goat fecal samples are (*Proteus mirabilis*, *Proteus Edwardsiella tarda*, *Proteus vulgaris*, *Salmonella SPS* *Citrobacter*, *Salmonella choleraesuis*, *Shigellasonnei*, and *salmonella paratyphs*) are present in male goat fecal samples. Previous studies using molecular techniques have shown the presence of at least four different bacterial phyla in the intestinal tract of dormant males, namely Firmicutes (47.7%), Proteobacteria (23.3%), Fusobacteria (16.6%), and Bacteroidetes (16.6%). Uropygial gland secretions usually include chemicals that are active against the growth of some gram-positive bacterial strain birds [11, 18].

Researchers isolated *P. mirabilis* from chicken bumble-foot. Based on their theory, these organisms were reported to be involved in septicemia in quails. In addition, to nosocomial and urinary tract infections. The three genera (*Proteus*, *mirabilis*, and *Serratia*) present male fecal samples in goats. All are motile, gram-negative rods and peritrichous flagella and are assigned to the *Enterobacteriaceae* family based on shared biochemical characteristics. Most significantly, they are characterized by their ability to oxidatively deaminate phenylalanine and, in most cases (except for some *Providencia* spp.), to hydrolyze urea [6]. Both *P. mirabilis* and *P. vulgaris* are widely distributed in the environment and have been isolated from the intestinal tracts of mammals, birds, and reptiles.

Despite well-documented evidence that the intestinal microbiota plays, a role in the actual mechanisms of the host-microbe interactions remains elusive but is believed, to be mediated in part by microbial products (metabolites) derived from the GI microbiota and locally and systemically absorbed by the host. Therefore, studies that assess functional aspects of the GI microbiota are needed. Metabolomics, the comprehensive study of small molecules present in biological samples, is an emerging method for better understanding of disease pathophysiology and host-microbe interactions, with the potential to develop novel diagnostic or treatment approaches. Metabolomics studies have been reported in human patients with IBD as well as animal models of IBD and provided new insights into disease pathogenesis. They are using metabolomics analysis to understand inflammatory bowel diseases metabolomics as a diagnostic tool in gastroenterology.

Using mass spectrometry platforms, an untargeted metabolomics approach can simultaneously identify hundreds of metabolites in biological samples and therefore provides a comprehensive functional overview of biochemical pathways that are up or down regulated during different physiologic or pathophysiologic states.

**Table 1: Biochemical Test result of isolated microbes from the Pre pubertal male goat (*Capra hircus*).**

Culture	Indole	Methyl Red	Voges Proskauer	Citrate	Urease	TSI	catalase	Gram staining	Identification of Bacteria
Control	-	-	-	-	-	-	-	-	-
Pre pubertal 1	+	+	+	+	-	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 2	+	+	+	+	+	-	+	-	<i>Proteus mirabilis</i>
Pre pubertal 3	-	+	+	+	-	-	+	-	<i>Proteus mirabilis</i>
Pre pubertal 4	+	+	+	+	+	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 5	+	+	+	-	+	-	+	-	<i>Proteus mirabilis</i>
Pre pubertal 6	+	-	+	+	+	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 7	+	+	+	+	+	-	+	-	<i>Proteus mirabilis</i>
Pre pubertal 8	+	-	+	+	-	-	+	-	<i>Proteus mirabilis</i>
Pre pubertal 9	+	+	+	+	-	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 10	+	+	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 11	+	+	+	+	-	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 12	+	+	+	+	-	+	+	-	<i>Proteus mirabilis</i>
Pre pubertal 13	+	+	-	+	-	+	+	-	<i>Edwardsiella tarda</i>

+ - Present - = Absent

**Table 2: Biochemical Test results of isolated microbes from the Adult male goat (*Capra hircus*).**

Culture	Indole	Methyl Red	Voges Proskauer	Citrate	Urease	TSI	Catalase	Gram staining	Identification of Bacteria
Control	-	-	-	-	-	-	-	-	-
Adult 1	+	+	+	-	+	-	+	-	<i>Proteus mirabilis</i>
Adult 2	-	-	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Adult 3	+	+	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Adult 4	+	+	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Adult 5	+	+	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Adult 6	+	+	+	-	-	+	+	-	<i>Proteus mirabilis</i>
Adult 7	+	+	+	+	+	-	+	-	<i>Proteus mirabilis</i>
Adult 8	+	+	+	-	+	+	+	-	<i>Proteus mirabilis</i>
Adult 9	+	+	+	-	+	-	+	-	<i>Proteus mirabilis</i>

+ - Present - = Absent

**Table 3: Biochemical Test results of isolated microbes from the Castrated male goat (*Capra hircus*).**

Culture	Indole	Methyl red	Voges Proskauer	Citrate	Urease	TSI	Catalase	Gram staining	Identification of bacteria
Control	-	-	-	-	-	-	-	-	-
Castrated Male 1	-	+	-	-	+	+	+	-	<i>Proteus mirabilis</i>
Castrated Male 2	-	+	-	-	+	+	+	-	<i>Proteus mirabilis</i>
Castrated Male 3	+	+	-	-	-	-	+	-	<i>Edwardsiella tarda</i>
Castrated Male 4	-	+	-	+	-	+	+	+	<i>Salmonella sps</i>
Castrated Male 5	+	+	-	-	-	-	+	-	<i>Proteus vulgaris</i>
Castrated Male 6	-	+	-	-	-	+	+	-	<i>Shigellasonneni</i>

+ - Present - = Absent

**Table 4: Comparative microbes profile as identified in the faeces of male in pre pubertal, adult and castrated male goats (*Capra hircus*)**

S.NO	Microbes	Pre pubertal	Adult	Castrated male
1	<i>Proteus mirabilis</i>	✓	✓	✓
2	<i>Edwardsiella tarda</i>	✓	x	X
3	<i>Citrobacter, freundii</i>	x	x	✓
4	<i>Proteus vulgaris</i>	x	x	✓
5	<i>E.Coli</i>	x	x	✓
6	<i>Salmonella sps</i>	x	x	✓
7	<i>Morganaellamorganii</i>	x	x	✓
8	<i>Shigellasonneni, shigella(A,B,C)</i>	x	x	✓

✓ - Present X = Absent

## CONCLUSION

Bacterial strains present in fecal sample of male goat are *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhi*, *Enterobacteraerogenes*, *Salmonella mercerscens*, *Serratiamarcescens*, *Shigellasonnei*. *Proteus mirabilis* and *Proteus vulgaris*. The identified bacterial populations shows pathogenic effect on other animals, in some cases it may lead to death. However, in our studies these bacterial population did not infect their host and the animal physiology was normal with these bacterial population in gut. Here we conclude that these bacteria population from the feces of male goat may be in symbiotic relationship with their host as gut micro flora, which may involve in biological activities of the host. Hence, further studies are warranted to provide strong evidence to confirm the same.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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