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Studies on buccal microbiota of Black Bengal Goat (*Capra hircus bengalensis*) in some villages of Arambagh subdivision, West Bengal

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ABSTRACT
The goat is one of the promising animal of genetic resources and is commonly known as "poor man's cow. Black Bengal goat is dwarf breed, highly prolific and are resistance to diseases. Enzymes are biological catalyst that accelerate the rate of biochemical reaction. Bacterial enzymes are most significant to carryout biochemical reaction. It has been reported that different types of bacteria are prevalent in the upper respiratory tract. Despite their economical as well as cyto genetical and molecular characterization, no efforts have been under taken to study the biochemical characterization of buccal microbiota of Black Bengal goat. Therefore, a preliminary attempt has been undertaken to isolate and characterize the buccal micro biota of black Bengal goat in some villages of Arambagh subdivision, West Bengal.

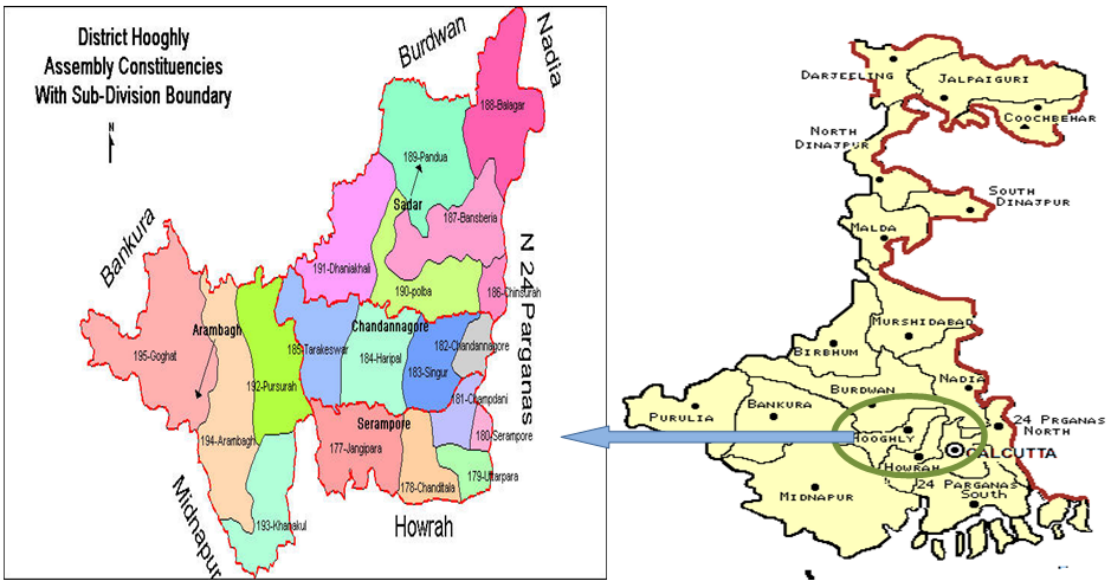
Keywords: Buccal microbiota, Growth curve, Antibiotic susceptibility, Biochemical enzymes.

1. Introduction
The goat is one of the promising animal genetic resources of India and serves as integral part of rural India's symbiotic system of crop and livestock production. Traditionally goat has served as source of livelihood and financial security to large section of society mainly comprising of resource poor people. In the present sceneries of changing agro climatic conditions, this small ruminant farm animal has tremendous potential to be projected as the 'Future Animal' for rural and urban prosperity. Various lines of data (10, 3) indicated that the black Bengal goats (*Capra hircus bengalensis*) are very valuable goat breeders in India and generates employment income and improves household nutrition. Goat is a hollow horned mammal belonging to the order Artiodactyla, family Bovidae and the genus *Capra.*
The black Bengal goat is a dwarf breed, distributed almost in all villages of West Bengal and adjacent part of West Bengal, Bihar, Orissa, Jharkhand and Assam (3). This breed is highly prolific and has natural resistant to common diseases and can produce excellent quality meat and skin. They are black, brown, grey and white in colour and both the sexes have small and medium horns directed upwards and sometimes backwards. In Bengal it is commonly known as "poor man's cow". It is precious germplasm of West Bengal (3). Poverty, hunger and healthcare represent some of the major challenges before rural India as well as West Bengal. The government of India launched "green revolution" for food and security ,'white revolution' for more milk production and the" pink revolution" in which meat from black Bengal goat play an important part (Go1-2004-05). In West Bengal small marginal and landless rural farmers traditionally rare goat.
Farmers and government are showing interest to utilize the species *Capra* to increase the supply of meat and to alleviate poverty through creation of employment. Various lines of data (8, 1and 10) highlighted the molecular and genetic diversity through the study of isozymes, genomic DNA as well as by the of study microsatellite markers. Enzymes are biological catalysts that accelerate the rate of biochemical reactions. Bacterial enzymes are most significant to fermentation, milk spoilage and cheese ripening but it is very important to distinguish between the enzyme and biochemical sources. It has been reported that *Staphylococcus* sp. (15%) and *Bacillus* sp. (18%) are prevalent in the upper respiratory tract of black Bengal goat of Bangladesh (9) Despite their economic importance as well as cytogenetical & molecular characterization and no efforts have been done up to study the In view of these reasons a preliminary investigation has been undertaken to isolate and characterize the buccal micro biota of black Bengal Goat.

2. Materials and Methods

2.1 Collection of buccal swabs from goats

The Black Bengal goats are collected from the rural areas of three villages viz. Kanpur, Nakunda, and Kumursha (Figure 1 & 2) of Goghat Block under the subdivision of Arambagh, District Hooghly, West Bengal. Sterile non absorbant cotton swabs were prepared by autoclaving (121 ^{0}C, 15 lb/in^2 saturated steam pressure, 60 minutes) in a transfer reservoir and carried to the field site. Buccal swabs from goats were collected under quasi sterile conditions and the inoculated swabs were rapidly transferred into the transfer media. The media were transferred to the lab (within 20 minutes at 35+/- ^{0}C and 70% Relative Humidity) for further examinations.



A. Map of Hooghly showing Goghat block. **B.** Map of West Bengal

Fig 1: (A & B) collection site (Goghat block, Arambagh subdivision, Hooghly, West Bengal) of Black Bengal goat.

BL1 (Kanpur) BL2 (Nakunda) BL3 (Kumursha)

Fig 2: Black Bengal goat from three different villages of Goghat under Arambagh subdivision.

2.2 Isolation of Bacterial morphotype samples from goat buccal swabs

The bacterial population were aseptically streaked from the transfer media onto preformed sterilized solidified Nutrient agar universal media (peptic digest of animal tissue 5 gms/ltr.; NaCl 5 gms/ltr.; Beef extract 1.5 gms/ltr.; Yeast extract 1.5 gms/ltr.; Agar 15 gms/ ltr., distilled water 1000 mls; Final pH at 25 ^{0}C 7.4±0.2) cast in sterile disposable 90 mm petri plates (Himedia M173) via sterile inoculation loops according the standard 4 way streaking protocol of Loeffler and Gaffky (1881)(5,7). Each buccal swab sample collected were streaked in 10 plates to observe the variability of sample microbial populace according to standard probability theorems. The plates were incubated in a BOD incubator at35^{0}C±2 ^{0}C under uniform aeration for 24±24 hours until there appeared visible isolated growth colonies of the bacterial morphotypes on the plates. Each distinct growth colony were subsequently streaked several times on sterile nutrient agar plates (as mentioned earlier) to obtain an axenic population of the individual morphotypes. Upon obtaining, the morphotype pure cultures were streaked on separate sterile nutrient agar slants, grown in BOD under the aforementioned conditions until growth occurred. Upon occurrence of growth the slants were stored in a 4 ^{0}C refrigerator for further assays.

2.3 Identification colony morphological characters of isolated bacterial morphotypes

The specimen microbe morphotype slants were subcultured in sterilized nutrient agar Petri plates by standard 4 way streaking protocol of Loeffler and Gaffky (1881) [7]. The plates were incubated in a BOD incubator at 35±2 ^{0}C under uniform aeration for 24 hours and the colony characteristics were noted according to visual interpretation.

2.4 Identification of cellular morphological characters of isolated bacterial morphotypes

The specimen microbe slants were sub cultured in sterilized nutrient broth liquid media in sterile culture tubes and incubated overnight (18-20 hours) at 35±2 ^{0}C and 110 rpm shaker speed. Growth was observed visually. Under sterile conditions grease free pathological glass slides [that has been cleansed by standard ionic detergents as well as 70% alcohol (vol/vol)] was taken in an aseptic environment and 5-6 loopfuls of the broth culture of the specimen microbe was transferred on the slide by a sterile inoculation loop. The transferred culture was made into a uniform smear and left to air dry in the laminar air flow hood. Upon being dried the slide was heat fixed by passing the slide once or twice (not more than twice) over a standard methanol lamp flame. Having fixed the specimen to the slide ,the slide was mounted on a staining bridge and flooded with Gram's Crystal Violet staining solution as primary stain [Solution A : Crystal Violet powder (certified 90% dye content) 2 gms; Ethanol 95% (vol/vol) 20 ml; Solution B: Ammonium oxalate 0.8 gms; distilled sterile water 80 ml; mix Solutions A and B , store for 24 hrs and filter thorough Whatmann's filter paper prior to use], Gram's Iodine as mordant [Iodine 1 gm; Potassium iodide 2 gms; distilled water 300 mls. Grind the iodine and potassium iodide in a mortar and add water slowly with continuous grinding until the iodine is dissolved. Store in amber bottles], Ethanol as decolourizer (95% vol/vol) and Saffranine as secondary stain (Stocksolution: Saffranine O 2.5 gms; 95% Ethanol 100mls; Working solution: 10 mls of stocksolution and 90 mls of sterile distilled water) using standard protocol of gram staining of Hans Christian Gram (1884).

2.5 Antibiotyping of bacterial morphotypes

Sterile Mueller Hinton agar (Beef infusion 300 gms/ltr; Casein acid hydrolysate 17.5 gms/ltr; Starch 1.5 gms/ltr; Agar 17 gms/ltr; distilled water 1000 mls; Final pH at 25 ^{0}C 7.3±0.1) was prepared and poured in sterile disposable petri plates. A logarithmic phase microbe culture was prepared overnight (18-20 h.) in Mueller Hinton broth liquid cultures at 35±2 ^{0}C at 110 rpm shaker speed. Growth was observed. About 0.2 ml (200 µl) of the culture suspension was taken and uniformly spread over the solidified Mueller Hinton Agar by the standard protocol of Spread plate technique. The suspension was allowed to soak for 20 minutes at 25 ^{0}C under sterile conditions. Subsequently standard antibiotic discs (Himedia) of commercially available concentrations were used to check the antibiotic sensitivity of the isolates using the standardized single disc diffusion method of Bauer and Kirby et al. 1966 (2) method of determining antibiotic sensitivity. Nine different antibiotics were used for each isolate [(Ticarcilin (10 µg); Colistin (10 µg); Polymyxin B (30 units); Tetracyclin (30 µg); Imipenem (10 µg); Ciprofloxacin (5 µg); Netilin (30 µg); Gentamycin (10 µg) and Amikacin (30µg)]. All experiments were performed in triplicate.

2.6 Growth Curve Assay of the bacterial morphotypes

Logarithmic phase overnight grown (18-20 h) cell suspension was inoculated to 250 ml conical flask containing 75 ml of Nutrient broth and incubated at 35±2 ^{0}C on incubator shaker (at 110 rpm). The growth responses were measured as a change in optical density at 600 nm at every 2 h of interval for a duration of 36 h using Jasco UV-Visible double beam spectrophotometer (Model V600) using a quartz cuvette (Kozima) at 600 nm.

2.7 Specific Biotyping

a) Amylase Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Starch agar medium (Starch soluble, 20 gm/ltr; Peptone, 5 gms/ltr; Beef extract, 3 gms/ltr; Agar, 15 gms/ltr; Final pH at 25 ^{0}C 7.0±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the Starch agar plates using standard 4 way discontinuous streaking protocol and incubated at 35±2 ^{0}C for 18 to 48 hours. After incubation the growth colonies on the plates were scraped off using a sterile inoculation loop and the plates were flooded with a dilute iodine solution for 60 seconds. Excess iodine drained off. Results were observed.

b) Protease Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Standard count Caseinate agar medium (Casein enzymichydrolysate 5 gms/ltr; Yeast extract 2.5 gms/ltr; Dextrose 1 gm/ltr; Sodium caseinate 10 gms/ltr.; Trisodium citrate 10 gms/ltr; Calcium chloride 2.2 gms/ltr.; Agar 15 gms/ltr; Final pH at 25 ^{0}C 7.2±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way streaking protocol of Loeffler and Gaffky (1881) and incubated at 35± 2 ^{0}C for 18 to 48 hrs. Results were observed.

c) DNase Production Detection Assay

Overnight (18-20 h.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile DNase Test Agar w/ Toluidine blue medium (Tryptose 20 gms/ltr.; DNA powder 2 gms/ltr.; NaCl 5 gms/ltr.;Toluidine blue 0.1 gm/ltr.; Agar 15 gms/ltr.; Final pH at 25 ^{0}C 7.2±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspension was streaked onto the medium plates using standard 4 way streaking protocol of Loeffler and Gaffky (1881) and incubated at 35±2 ^{0}C for 18 to 48 h. Results were observed.

d) Phosphate Solubilization Activity Detection Assay

Overnight (18-20 h) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Pikovskaya agar medium (Yeast extract 0.5 gm/ltr; Dextrose 10 gms/ltr; Calcium phosphate 5 gms/ltr; Ammonium sulphate 0.5 gms/ltr; Potassium chloride 0.2 gm/ltr; Magnesium sulphate 0.1 gm/ltr; Magnesium sulphite 0.001 gm/ltr; Ferrous sulphate 0.001 gm/ltr; Agar 15 gms/ltr; Final pH at 25 ^{o}C 7.0±0.2) were prepared and cast on sterile disposable petri plates. The isolate suspensions were stabbed into the agar plates and incubated at 35±2 ^{0}C for 24 to 120 h. Results were observed.

e) Lipase Production Detection Assay

Overnight (18-20 hrs.) Nutrient broth culture suspensions of the isolates were prepared (as mentioned earlier). Sterile Tributyrin agar medium (Peptic digest of animal tissue 5 gms/ltr; Yeast extract 3 gms/ltr; Agar 15 gms/ltr; Final pH at 25 ^{0}C 7.5±0.2) supplemented with 10% (vol/vol) sterile Tributyrin oil were prepared and cast on sterile disposable petri plates. The isolate suspensions were streaked onto the medium plates using standard 4 way discontinuous streaking protocol and incubated at 35±2 ^{o}C for up to 48 hours. Any zone formation was observed.

3. Observation /Result

3.1 Colony morphology of each morphotype after isolation.

Two morphotypes of bacterial colony were selected each from two sample plates (BL1 and BL2) of buccal swab of goats while the fifth was from another sample plate (BL3).

Table 1: Colony characteristics of the morphotypes

Sample zoonose code	Sample plate code	Bacterial Morphotype	Colony shape	Colony morphostructural	Colony size (mean)	Colony opacity	Colony texture	Colony colour	Colony surface topology
GA	BL1	M1	circular	diffuse	1-1.5 mm	opaque	Matte	Creamish white	elevated
GA	BL1	M2	irregular	nucleated	2-3 mm	translucent	Matte	Yellowish white	elevated
GA	BL2	M1	Circular	nucleated	0.5-1 mm	translucent	Glossy	Cloudy white	depressed
GA	BL2	M2	reticulate	nucleated	3-4 mm	Opaque	Matte	Cloudy white with greenish pigmentation	elevated
GA	BL3	M1	circular	nucleated	2-3 mm	opaque	matte	Cloudy white	elevated

3.2 Gram Characteristics of each morphotype post fixing after Gram's Staining

Of the five morphotypes tested, the isolates M1 and M2 were from plate BL1, while the two isolates M1 and M2 from plate BL2.The final isolateM1 from plate BL3.The Gram character, morphology and arrangement of cell in each morphotype are shown in Table 2.

Table 2: Gram character, morphology and arrangement of cells in each morphotype [l =cell length; b= cell width; dia= cell diameter]

Bacterial morphotypes	Gram character	Cellular morphology	Cell size (as per micrometry) (in µm) (mean)	Cell association
GA: BL1: M1	Gram positive	bacillio-coccoidal, tapered ends	1.31 x 1.13 (l x b)	Mostly clustered, some in pairs
GA: BL1: M2	Gram positive	Coccoidal	0.98 (dia.)	Mostly clustered, some single
GA: BL2: M1	Gram negative	Bacillary, rectangular ends	1.76 x 0.57 (I x b)	Mostly in pairs and triads, some single
GA: BL2: M2	Gram negative	Bacillary, rectangular ends	2.15 x 0.43 (l x b)	Mostly clustered, some in chains
GA: BL3: M1	Gram positive	Bacillary, tapered ends	1.45 x 0.57 (l x b)	Mostly in pairs, seldom clustered

3.3 Growth curve assay of bacterial isolates

Growth curve assay of each of the five bacterial morphotypes are shown in Table 3 and the graphical representation in Figures1 to 5.

Table 3: Tabular depiction of comparative growth curve assay of the 5 bacterial morphotypes

Time (hrs.)	O.D. of Morphotype GA: BL1: M1	O.D. of Morphotype GA: BL1: M2	O.D. of Morphotype GA: BL2: M1	O.D. of Morphotype GA: BL2: M2	O.D. of Morphotype GA: BL3: M1
0	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.0923	0.0784	0.1986	0.5703	0.5011
4	0.3143	0.3697	0.5574	0.7788	0.7886
6	0.3390	0.5963	0.9250	1.0698	1.1339
8	0.7652	0.9984	1.1244	1.3378	1.8541
10	1.2256	1.6522	1.7523	1.9961	2.0013
12	1.7425	1.9742	1.9953	2.2451	2.4121
14	1.8499	2.1112	2.3265	2.4623	2.5579
16	2.1324	2.3356	2.5741	2.5124	2.5495
18	2.4719	2.6012	2.5542	2.5222	2.6846
20	2.5214	2.5845	2.5929	2.6821	2.6002
22	2.5844	2.5662	2.2784	2.6856	2.6012

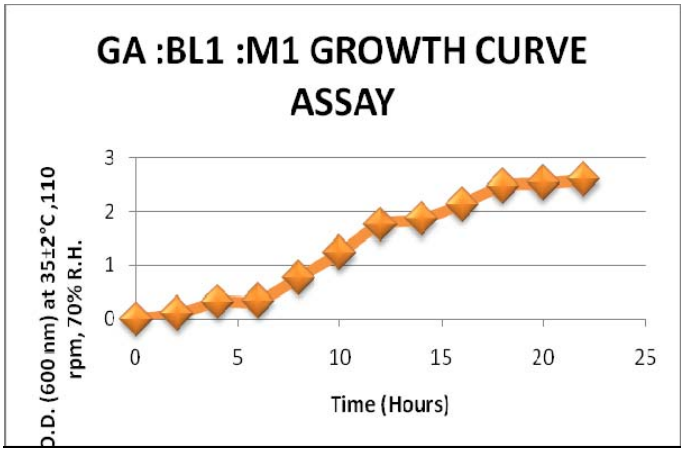


Fig 1: Morphotype GA: BL1: M1 growth curve

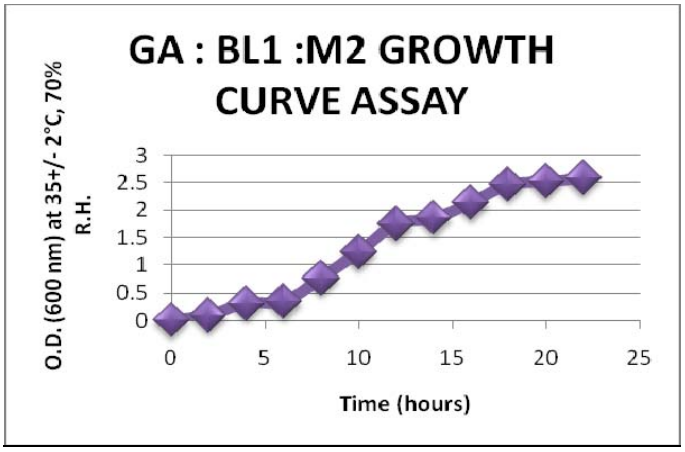


Fig 2: Morphotype GA: BL1: M2 growth curve

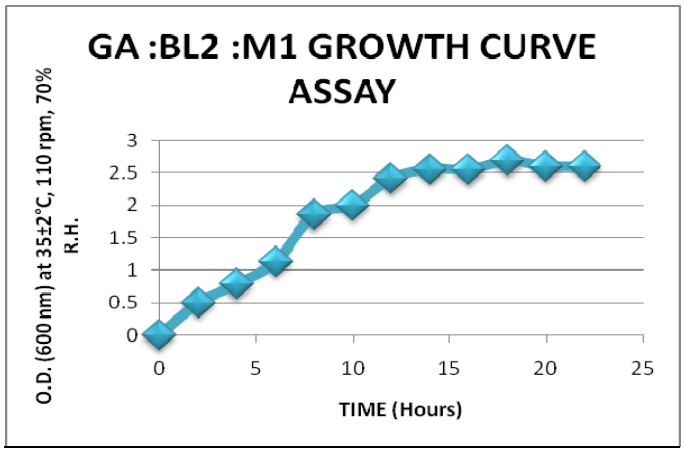


Fig 3: Morphotype GA: BL2: M1 growth curve

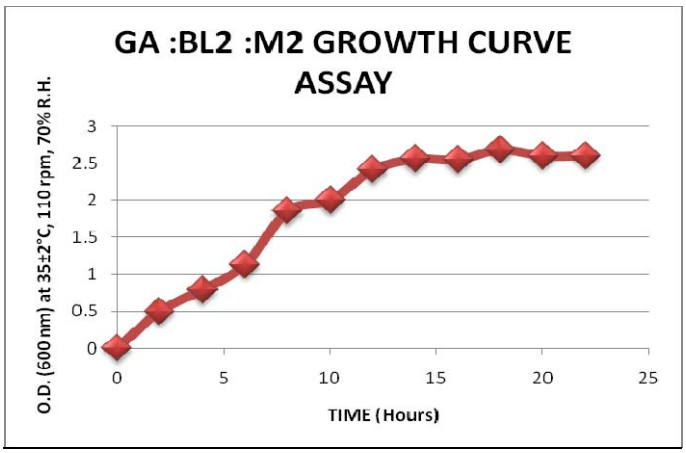


Fig 4: Morphotype GA: BL2: M2 growth curve

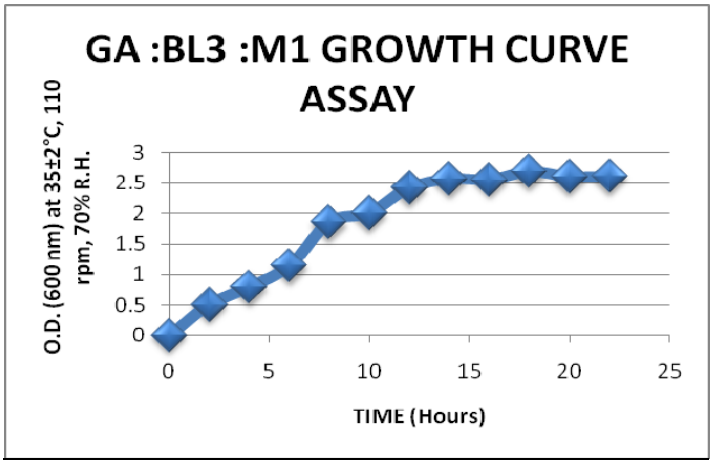


Fig 5: Morphotype GA: BL3: M1 growth curve

3.4 Antibiotyping: Antibiotic susceptibility test

Mean zone inhibition of the 5 bacterial morphotypes against the aforementioned nine antibiotics are presented in Table 4 and Figures6 to 10.

Antibiotic	Morphotype GA :BL1: M1			Morphotype GA :BL1: M2			Morphotype GA :BL2: M1			Morphotype GA :BL2: M2			Morphotype GA:BL3: M1		
	Recorded zone of inhibition (mm)	Mean zone of inhibition (mm)	Sensitivity type	Recorded zone of inhibition (mm)	Mean zone of inhibition (mm)	Sensitivity type	Recorded zone of inhibition (mm)	Mean zone of inhibition (mm)	Sensitivity type	Recorded zone of inhibition (mm)	Mean zone of inhibition (mm)	Sensitivity type	Recorded zone of inhibition (mm)	Mean zone of inhibition (mm)	Sensitivity type
Ticarcillin	5 9 7	7	Resistant	12 8 10	15	Intermediate to sensitive	20 18 21	19.67	sensitive	15 20 18	17.67	sensitive	15 17 16	16	sensitive
Colistin	8 8 5	7	Resistant	15 16 17	16	sensitive	12 11 13	11	Intermediate to sensitive	13.5 17 18	16.17	sensitive	17 13 12	14	sensitive
Polymyxin B	12 12 13	12.33	sensitive	15 16 14	15	sensitive	15 14 12	13.67	sensitive	7 5 4	5.33	resistant	21 14 20	18.33	sensitive
Tetracyclin	15 12 17	14.67	Intermediate to sensitive	13 16 17	15.33	Intermediate to sensitive	25 24 23	24	sensitive	23 24 22	23	sensitive	27 22 15	21.33	sensitive
Imipenem	38 40 43	40.33	sensitive	40 35 42	39	sensitive	38 35 40	37.67	sensitive	31 29 29	29.67	sensitive	37 39 38	38	sensitive
Ciprofloxacin	30 35 38	34.33	sensitive	25 27 24	25.33	sensitive	25 26 24	25	sensitive	30 33 31	31.33	sensitive	26 22 24	24	sensitive
Netilin	30 28 29	29	sensitive	20 22 23	21.67	sensitive	27 26 25	26	sensitive	30 28 32	30	sensitive	3 7 4	4.67	resistant
Amikacin	26 23 27	25.33	sensitive	7 7 9	7.67	resistant	20 19 21	20	sensitive	20 22 21	21	sensitive	23 26 24	24.33	sensitive
Gentamycin	25 26 29	26.67	sensitive	16 18 17	17	sensitive	22 23 25	23.33	sensitive	11 14 13	12.67	resistant	5 8 7	6.67	resistant

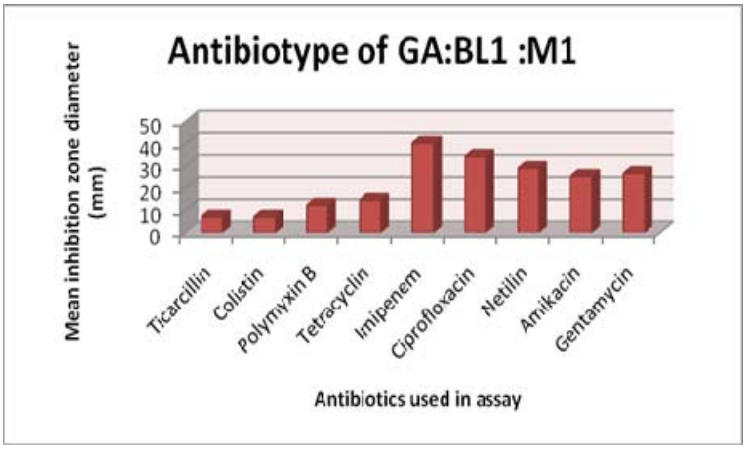


Fig 6: Antibiotic susceptibility profile of GA: BL1: M1

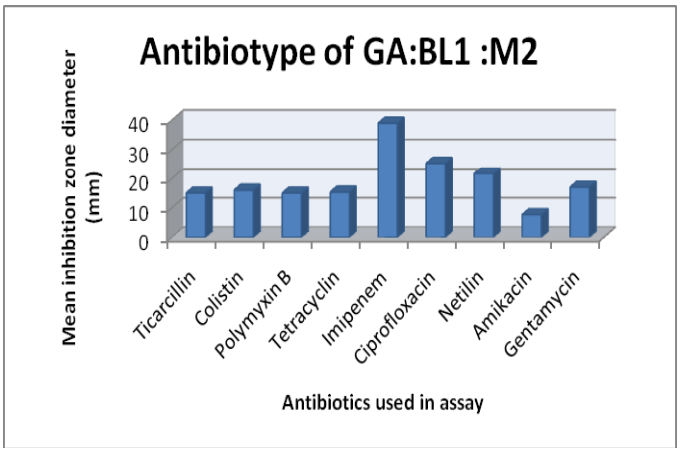


Fig 7: Antibiotic susceptibility profile of GA: BL1: M2

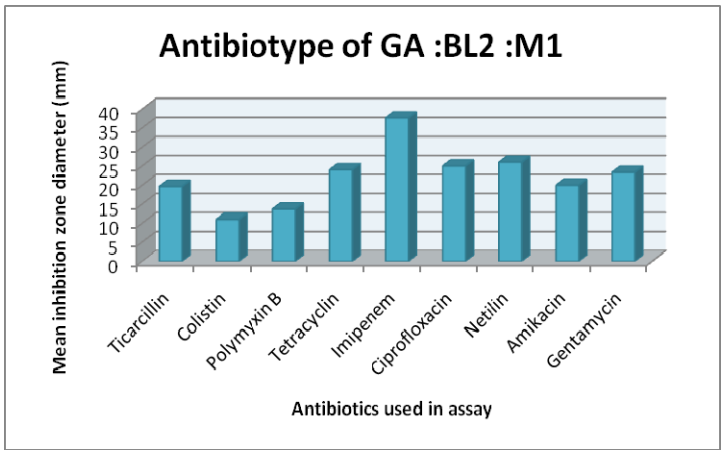


Fig 8: Antibiotic susceptibility profile of GA: BL2: M1

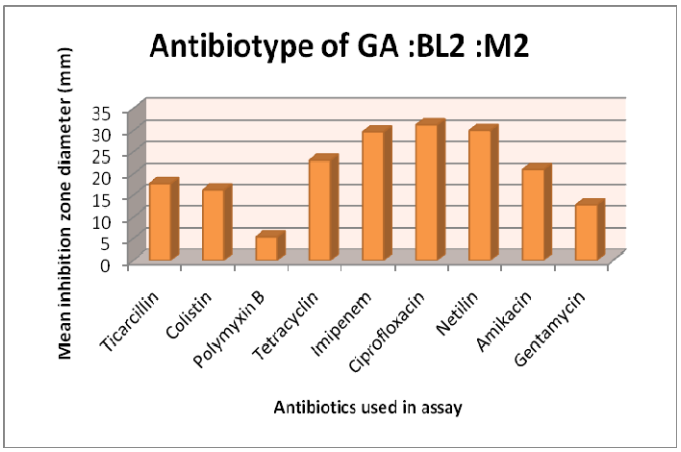


Fig 9: Antibiotic susceptibility profile of GA: BL2: M2

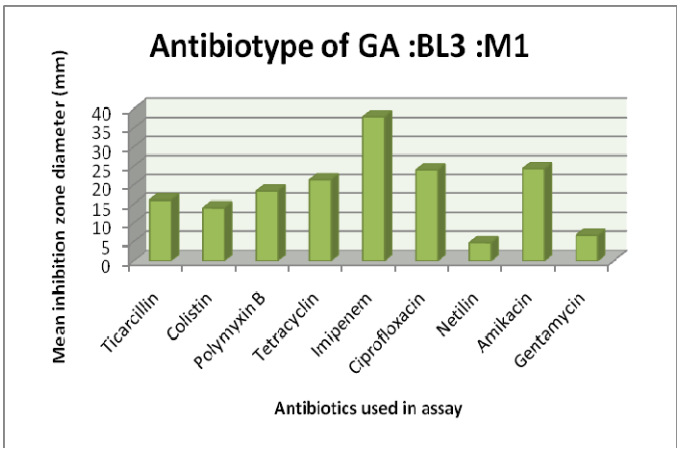


Fig 10: Antibiotic susceptibility profile of GA: BL3: M1

3.5 Specific Biotyping Assays

These assays were performed to assess the economic and commercial viabilities of the isolates. Observation shows that the ability of the isolates to secrete different types enzymes which can be commercially harnessed and marketed. (Table -5)

Table 5: Comparative assay results of all the five morphotypes (The multiple "+" signs indicate the intensity of growth and/or the colour formed with respect to the respective control setups.)

Biotyping assays	Biotyping parameters *("+" means positive result, "-" means negative result)*				
	Morphotype GA :BL1 :M1	**Morphotype GA :BL1 :M2**	**Morphotype GA:BL2 :M1**	**Morphotype GA :BL2 :M2**	**Morphotype GA:BL3 :M1**
Amylase production detection assay	Growth +++++ Enzyme production +++	Growth +++++ Enzyme production ++++	Growth ++ Enzyme production ++	Growth +++++ Enzyme production +	Growth ++++ Enzyme production ++++
Protease production detection assay	Growth +++++ Enzyme production +	Growth +++++ Enzyme production -----	Growth +++++ Enzyme production -----	Growth +++++ Enzyme production +++++	Growth +++++ Enzyme production ++
DNase production detection assay	Growth +++++ Enzyme production ----	Growth +++ Enzyme production +	Growth ++++ Enzyme production ----	Growth +++++ Enzyme production ----	Growth +++++ Enzyme production +
Phosphate solubilization activity detection assay	Growth +++++ Enzyme production +	Growth +++++ Enzyme production -----	Growth + Enzyme production -----	Growth ----- Enzyme production -----	Growth ++ Enzyme production +
Lipase production detection assay	Growth +++++ Enzyme production +	Growth ++ Enzyme production ++	Growth +++ Enzyme production ---	Growth + Enzyme production -----	Growth +++++ Enzyme production +++

4. Discussion

Present investigations provide comprehensive information about colony characters gram staining properties and biochemical characteristics of buccal micro biota of black Bengal goat (*Capra hircus bengalensis*) of some rural villages of Goghat block under Arambagh sub division, Hooghly, West Bengal. Our data (Table-1) reveal that the goat buccal microbiota colony are more less circular or irregular with elevated or depressed surface. Most of the buccal microbiota are found to display gram positive staining property but some showed gram negative staining property (Table -2). Different bio chemical test regarding antibiotic assay indicated that some morphotype of buccal microbiota of black Bengal goat are found to be sensitive (Table-4). However, a few are resistant to gentamicin colistin and ticarcillin. It has been reported that the buccal micrbiota which are resistant to ticarcillin may express beta lactamase which cleave beta lactam ring of ticarcilin. Another antibiotics like colistin which is poly cationic anti biotic having both hydrophilic and lypophilic moieties. These poly cationic region interact with the bacterial outer membrane by displacing bacterial counter ions in the lipopolysaccharides. The resistance may be due to the modification or loss of polysaccharide portion of LPS through which the drug may not displace the ions, favouring survival of the bacteria in the presence of drugs.

The data in table -5 reveal that the buccal micro biota Black Bengal goat demonstrate their unique property of secreting important enzymes like amylase and lipase. On the contary they are found to secrete less quantity of enzymes viz protease and DNase.

5. Acknowledgement

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6. References

1. Anita Yadav, Yadav BR. DNA Fingerprint: Genetic Relationship in Six Indian Goat Breeds. Ind Jour of Biotech 2008; 7(10):487-490.
2. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4):493-496.
3. Debraj Nandi, Roy S, Bera S, Kesh S, A Kumar A, Samanta, The Rearing System of Black Bengal Goat and Their Farmers in West Bengal India. Vet World 2011, 4(6):254-257.

4. GO1 (Government of India) 2004-2005, Annual Report. Department of Animal Husbandry and Dairying, Ministry of Agriculture New Delhi.
5. Harrigan WF, MCcance M. Laboratory Methods in Food and Dairy Microbiology (Revised Edition) 452 S 24 Abb London-New York-San Francisco 1978; 18(3):226-227.
6. Kasai T, Nishino T, Kazuno Y, Tanino T. The antibacterial activity of ticarcillin/clavulanic acid (BRL28500) against ticarcillin-resistant bacteria. J Antibiot (Tokyo) 1986; 39(10):1450-1460.
7. Koch Robert, "Methodes for the study of pathogenic organisms".s Zur Untersuchung Von pathogen Organismen (1881), 1-48.
8. Rahaman MA, Rahaman SMM, Jalil MA, Uddin NS, Rahaman MM. Molecular Characterization of Black Bengal and Jamunapari Goat P G Breeds by RAPD Markers. Amer Jour of Animal and Vet Science 2006; 1(2):17-22.
9. Assaduzzaman MD, Khan R, Hussain H, Najil MD, Rahaman M, Ahmed KS. Isolation and Identiofication of Bacteria from Upper Respiratory Tract of Black Bengal Goat in Bangladesh and Investigation of Some Epidemiological Parameters in Realated To Pneumonia Scientific Jour of Microbiology 2013; 2:11.
10. Kumari N, Singh LB, Kumar S. Molecular Charaterization of Goats using Random Amplified Polymorphic DNA. Amer Jour Animal Vet Science 2013; 8(1):45-49.