Pathogenic (Human) Bacterial Strains Identified from preserved Goat Milk

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INTRODUCTION

Goat milk and their products are nutritionally versatile (Bartlett, J. M. S.; Stirling, D. 2003), due to their potential nutraceutical properties. Goat milk can easily get contaminated by spoilage microorganisms (mainly bacteria) (kim *et al* 2008) during various stages of milk processing and storage from farm. Previously,(Login GR *et al,*) some of the pathogenic (especially *Mycobacterium)* and spoilage bacteria such have *Listeria* and *Micrococcus* have been isolated from fresh raw goat milk and also preserved goat milk in various parts of the world. However, the count might increase upto 100 fold or more once stored at ambient temperature for an extended period of time

Hence, the main objective to undertake the present study was to screen for the microbiological quality of fresh goat milk collected from two popular, small-scale dairy farms in karakudi, Tamil Nadu, India. Results of in this study are expected to be useful for health conscious consumers, as well as the local economy.

Goat milk samples were analyzed for the prevalence of selected bacterial pathogens. Enumeration of total plate count , Enumeration of *Coliforms and K. pneumoniae* in goat milk was performed by employing three-tube most probable number (MPN) technique. The typical colony found was confirmed based on their IMViC pattern based on BAM method. For determination of *pneumoniae* in samples, International Standard Organization protocol (ISO, 1990) was employed and the modified method described by the Food and Drug Administration (FDA) was employed (Westoo and Peterz, 1992; FDA, 2001). Presumptive *Listeria species* isolates were confirmed based on Gram reactions and catalase tests (Yala D, 2009).

MATERIAL AND METHODS

Sample Collection

Sample collection was carried out on a goat's farm in the karaikudi, Tamil Nadu, India. At regular intervals, total of 58 samples of raw goat's milk and 50 samples of pasteurized goat's milk were obtained.

On the farm, there were 90 goats of the white short-haired breed in the 1st to 8th lactation. The average daily milk yield is 2–3 liter and the average annual milk yield is 400–600 liters.

Processing

Pre-milking, semi-dry udder cleaning is carried out. After milking, the milk is cooled down promptly to 4–7 °C and then stored for 6–12 hours until further processing, which is stationary pasteurization in a tank at 79 °C for 14 seconds.

Sampling

Milk samples were collected after cooling at 4–7 °C and pasteurized milk samples were collected after the heat treatment and adequate cooling at 4–7 °C. The samples were changed to the laboratory at a maximum temperature of 10 °C and processed.

MORPHOLOGICAL IDENTIFICATION

The morphological identification is usually carried by the identity the characters of the bacteria which all are present in the samples. The morphological characters are also used to separate the bacteria which based on the various staining method, and biological characters.

Each staining techniques were used to identify the specific characters likewise the Gram staining is used to enumerate the gram negative and gram positive bacteria. The negative staining method is used to diagnostic the specimen in black color. The biochemical test is widely used to identify the *Staphylococcus aureus.*

DNA Isolation

The cells were grown overnight in nutrient rich broth (Nutrient broth). Incubate at 55˚c for 2 hours. After incubation it was chilled on ice for 10 minutes. 250µl of 6M Nacl was added. Again it was kept on freezer for 5 minutes. The supernatant was removed and rinse with 500µl of 70% ethanol. 5µl of DNA sample was added to the 0.8% agarose gel. Visualized under the UV Transilluminator.

The amplified sequences of EUBAC were confirmed by similarity index built in the NCBI's BLAST program. Based on the percentage similarity and query coverage against the reference species, the species were confirmed.

RESULTS

Staining Of Bacteria

TABLE: 1 Illustrated That Staining Of Bacteria

	Staining Techniques	Figure A *Mycobacterium tuberculosis*	Figure B *Pseudomonas aeroginosa*
1.	Gram's staining	Positive	Positive
2.	Capsule staining	Negative	Negative
3.	Negative staining	Negative	Negative
4.	Simple staining	Negative	Negative
5.	Motility test	Negative	Negative
6.	Acid fast staining	Positive	Negative

Biochemical Tests

Table: 2 Illustrated That Biochemical Tests

	Biochemical Tests	Figure A *Mycobacterium tuberculosis*	Figure B *Pseudomonas aeroginosa*
1.	Indole test	Negative	Negative
2.	Methyl red test	Negative	Negative

3.	Vogesproskauer test	Negative	Negative
4.	Simmon citrate agar test	Negative	Positive

Antimicrobial Activity

Table: 3 Illustrated That Antimicrobial Activity

	Antimicrobial Activity	*Mycobacterium tuberculosis*	*Pesudomonas aeroginosa*
1.	*Klebsillela pneumoniae*	Positive	Positive

SUMMARY AND CONCLUSION

Two bacterial species, *Mycobacterium tuberculosis, Pseudomonas aeruginosa were* collected by karaikudi goat farm. The morphological characters of these two species were compared and found they are distinct. DNA was isolated from fresh broth culture by standardized method and the purity was checked. The PCR conditions for the amplification of EUBAC and 16S rRNA gene were standardized. All the sequences were checked for species confirmation by BLAST in NCBI. Based on the similarity search the two species were identified as *Mycobacterium tuberculosis, Pseudomonas aeruginosa*. This study serves as a basis for future studies possibly involving the conservation and management of the species.

REFERENCES

Bartlett, J. M. S.; Stirling, D. (2003). "A Short History of the Polymerase Chain Reaction". PCR Protocols. Methods in Molecular Biology. 226 (2nd ed.).

Kim JT, Park SY, Choi WB, Lee YH, Kim HT. Characterization of Colletotrichum isolates causing anthracnose of pepper in Korea. Plant Pathol J. 2008.

Kim S, Park M, Yeom SI, Kim YM, Lee JM, et al. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species.

Kim ST, Yun SC. Biocontrol Activity of Myxococcus sp. KYC 1126 against Phytophthora blight on hot pepper. Res Plant Dis.

Kim TS, Lee GH, Kim GJ, Lee SW, Park KS, Park JW. Antifungal activity of bacterial strains isolated from tidal mudflat and salted seafood (traditional jeotgal) against six major plant pathogens. Korean J Pestic Sci.

Kim YK, Hong SJ, Shim CK, Kim MJ, Choi EJ, Lee MH, Park JH, Han EJ, An NH, Jee HJ. Functional analysis of Bacillus subtilis isolates and biological control of red pepper powdery mildew using Bacillus subtilis.

Login GR, Schnitt SJ, Dvorak AM (1987) Methods in laboratory investigation – rapid microwave fixation of human tissues for light microscopic immunoperoxidase identification of diagnostically useful antigens. Lab Investig 57:585–591.

NOAA 2005. Fisheries of the United States, 2005. *National Marine Fisheries Service, Silver Spring, MD.*

WHO/FAO. Risk assessment of Vibrio parahaemolyticus in seafood. Rome: FAO; 2011. Microbiological Risk Assessment Series No. 16.

HO/FAO. Risk assessment of Vibrio vulnificus in raw oysters: interpretative summary and technical report. Rome: FAO; 2005. Microbiological Risk Assessment Series No. 8.

Zall R R 1990 Control and destruction of microorganisms. In Dairy microbiology Vol. 1. Elsevier Applied Science Publishers, London: 115–161.

Zeng S S, Escobar E N 1996 Effect of breed and milking method on somatic cell count standard plate count and composition of goat milk. Small Ruminant Research 19:169–175.