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## **Diversity of** *Enterococcus* **Strains in Raw Donkey milk: Evaluating their** technological attributes and safety profiles

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### **INTRODUCTION & AIM**

Raw donkey milk, a lesser-known but nutritionally valuable resource, has gained recognition due to its unique biochemical composition and potential health-promoting properties. In this study, the diversity, technological attributes, safety profiles, and probiotic potential of *Enterococcus* spp. isolated from raw donkey milk were thoroughly examined. The primary objective was to assess whether these isolates could serve as viable probiotic candidates for incorporation into donkey milk-based fermented products.

From 20 raw donkey milk samples collected in Southwestern Haryana, 25 Grampositive, catalase-negative bacterial isolates were obtained using selective media. Identified through phenotypic and molecular methods as strains of *Enterococcus* and evaluated for their probiotic and technological properties. They demonstrated strong tolerance to acidic conditions (pH 2) and 0.3% bile salts, with increased bacterial viability over time. Antibiotic susceptibility tests showed that most strains were sensitive to clinically important antibiotics like Ampicillin, Chloramphenicol, Gentamycin, and Vancomycin, with no detected virulence factors or hemolytic activity, indicating their safety for food applications. The isolates exhibited significant cell surface hydrophobicity, enhancing their potential adherence to the gut lining. Notably, these strains displayed strong antimicrobial activity against common foodborne pathogens such as Staphylococcus aureus, Pseudomonas spp., and Escherichia coli, which was further enhanced using specific induction techniques. Phylogenetic analysis revealed close similarities to known probiotics like Bifidobacterium breve DSM 20213 and Lacticaseibacillus rhamnosus GG. These findings suggest that donkey milk-derived bacterial strains have promising potential as safe, natural probiotics for use in functional fermented foods, contributing to gut health and food safety.

#### **RESULTS & DISCUSSION**

#### Phenotypic and molecular characterization



## **METHOD** Purification Screening of Probiotics Isolation of the Microscopic Probiotics examination of Probiotics **Biochemical characterization** Biosafety assessme Milk quality analysis

Donkey

Milk

#### In vitro Safety assessment





Keys: (+) = Positive, (-) = Negative and ND = Not determined: Te = Trehalose, Ce= Cellobiose, Ga= Galactose, Mb= Melibiose Su= Sucrose, Xy= Xylose, Ma= Maltose, Mo= Mannose, Rf= Raffinose, Sb= Sorbitol, La=Lactose



Fermentation of Te, Ce, Ga, Mb, Sb, Su, Xv, Ma, Mo and Rf sugar by DM bacterial isolate A20

Fermentation of Ga, La, Te, Ce, Mb, Xy, Ma, Mo and Rf sugars by DM bacterial isolate A72.

Antibiotic sensitivity assay of A2 strain against,

the antibiotics: LZ, E, COT, AZM, CD, CX, TEI





A5, A2, A9 and A20 strains anta istic effect of A35, A58, A90 and A2



#### CONCLUSION

In conclusion, the bacterial isolates recovered from raw donkey milk, predominantly from the Enterococcus genera, exhibited promising probiotic and technological properties. Their demonstrated tolerance to low pH and bile salts, antibiotic susceptibility and absence of virulence factors or hemolytic activity underscore their potential safety for use in food applications. The notable antimicrobial activity against key foodborne pathogens, including Staphylococcus aureus, Pseudomonas spp., and Escherichia coli, further supports their role as functional probiotics.

## FUTURE WORK / REFERENCES

The probiotic efficacy and antimicrobial mechanisms of these strains will be validated through clinical trials.

✤The development of new functional fermented foods using these probiotics, coupled with the optimization of their industrial applications, represents a promising direction.

◆Genomic and metabolomic analyses of these strains will reveal further health benefits, while advanced encapsulation technologies will improve their viability in commercial products.

(Martini, M., Salari, F., Licitra, R., La Motta, C., and Altomonte, I. (2019). Lysozyme activity in donkey milk. International Dairy Journal, 96, 98-101

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