Potential probiotic lactic acid bacteria from naturally fermented vegetable products

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INTRODUCTION

Consumption of fermented vegetable products represent a gastronomic trait of human diet known for improving the nutritional profile. Such traditionally prepared fermented vegetable products of Sikkim include *sinki* and *gundruk*, which serve as an excellent source of probiotics as they are dominated mainly by lactic acid bacteria (LAB).

OBJECTIVES

The present study was to screen the best probable candidate with probiotic properties from fermented vegetable products, namely *sinki* and *gundruk* and to study their characteristics with a purpose to utilize them in functional food formulations.

METHODOLOGY

Sinki

Gundruk

A) Preliminary Screening

B) Molecular Characterization

C) Genetic Screening

D) *In vitro* Screening of Probiotic attributes

RESULTS AND DISCUSSION

A) Preliminary Screening

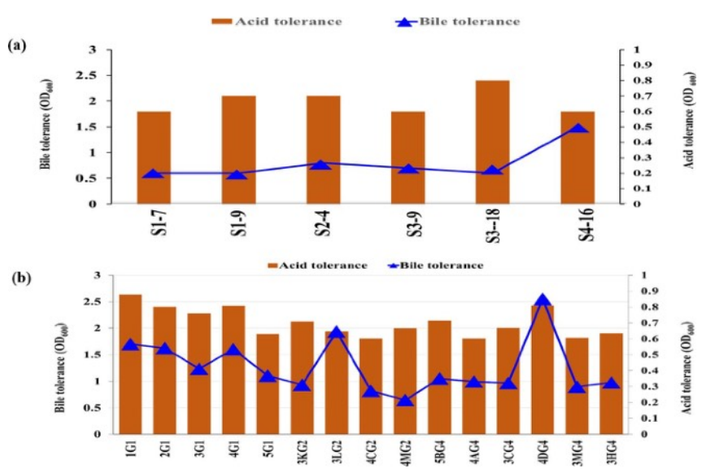


Fig 1a and 1b : Acid and bile tolerance

B) Molecular Characterisation

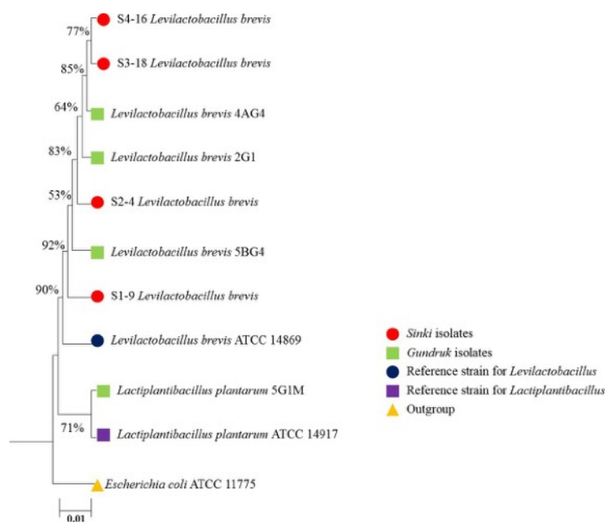


Fig 2 - Evolutionary relationships of lactic acid bacteria

C) Genetic Screening

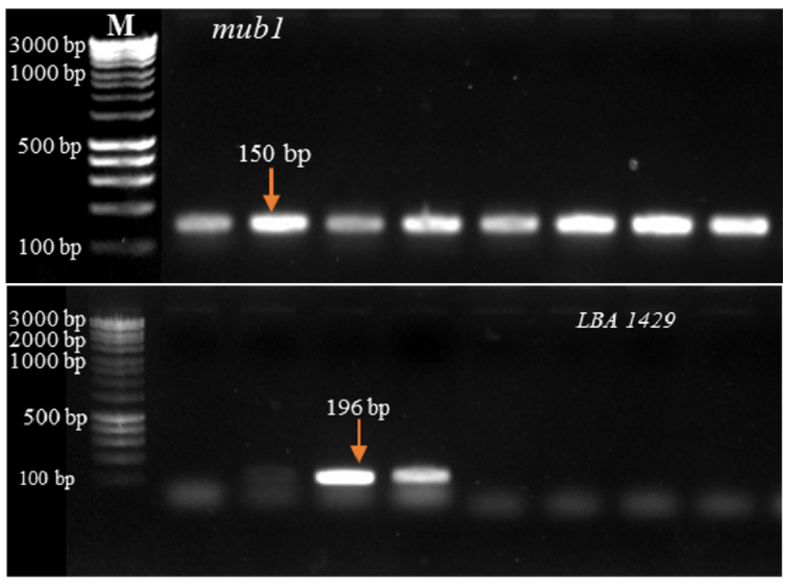
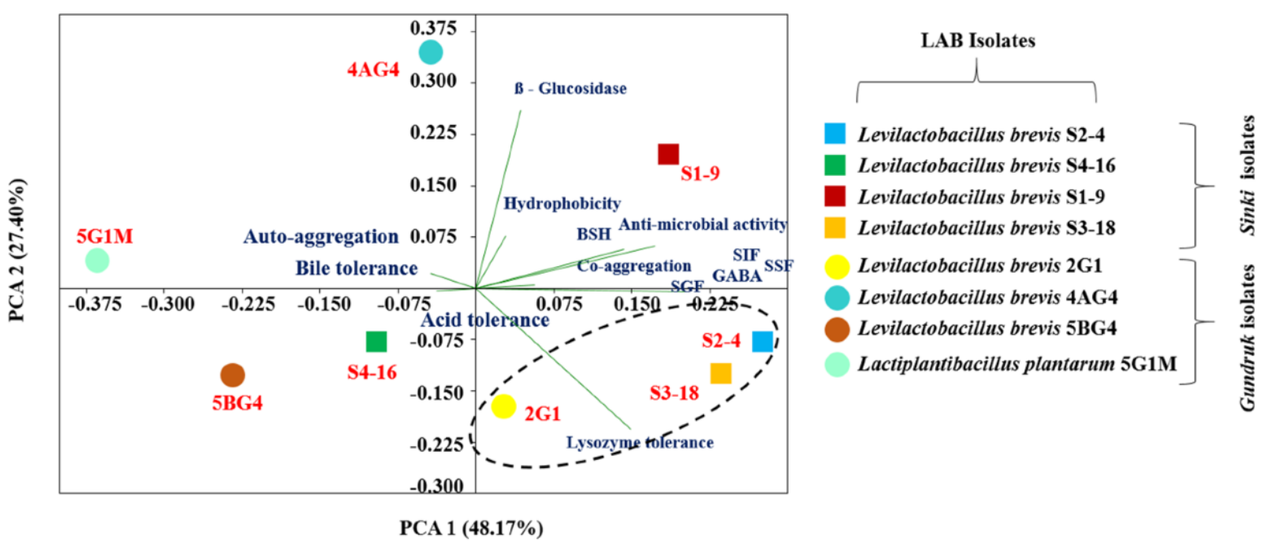


Fig 3a and 3b: Detection of probiotic marker gene

D) *In vitro* Screening of Probiotic attributes



SIF: Simulated intestinal fluid; SGF: Simulated gastric fluid; SSF: Simulated salivary fluid

Fig 4 : PCA biplot

- **Initially, screening was based on tolerance to acidic conditions, adjusted to a pH-3 and bile concentration of 0.3 %, considered to be the prerequisites for distinguishing the isolates having probiotic characteristics (Fig. 1a and 1b).**
- **Selected 8 isolates were identified by 16S rRNA gene sequencing as *Levilactobacillus brevis* (7) and *Lactiplantibacillus plantarum* (1).**
- **The evolutionary relationships of classified LAB strains were constructed and aligned along with reference type strains acquired from NCBI database (Fig. 2).**
- **Results of genetic screening denotes the presence of most of the genes selected for studying probiotic attributes (Fig. 3a and b).**
- **All isolates were found to show aggregation properties, antimicrobial activity, bile salt hydrolase activity, tolerance to lysozyme and others.**
- **All isolates displayed good survivability (>70%) after being exposed to simulated salivary fluid (SSF), salivary gastric fluid (SGF) and salivary intestinal fluid (SIF).**

CONCLUSION

- **The acidic nature of *sinki* and *gundruk* facilitates the growth of LAB while inhibiting the growth of spoilage microorganisms.**
- **Determination of cell surface properties indicates the ability of LAB isolates to adhere to the host mucosal epithelia followed by colonisation to exert their probiotic potential.**
- **LAB strains exhibited antagonistic activity against tested pathogens (*Escherichia coli* KL96 MTCC, *Salmonella enterica* MTCC 3223, *Staphylococcus aureus* MTCC 740 and *Bacillus cereus* MTCC 127) which can be ascribed to their ability to produce bactericidal compounds known to play role in eliminating growth of spoilage microorganisms of food.**
- **Genetic screening confirmed the presence of biomarker genes responsible for various probiotic traits, such as acid tolerance (*groEL, clpL*), bile tolerance (*LBA1446*), BSH activity (*apf*, *ir0085*), adhesion (*msa*, *mub1*) and antimicrobial (*pedA*, *PlnEF*).**
- **Hence, *Levilactobacillus brevis* S2-4 from *sinki* and *Levilactobacillus brevis* 4AG4 from *gundruk* fulfil the criteria to be claimed as probable probiotic bacteria.**

KEY MESSAGE

With growing demand for probiotics worldwide, traditionally prepared fermented vegetables with probiotic properties proves to be a suitable alternative available to many consumers intolerant to lactose.

ACKNOWLEDGEMENT

I would like to acknowledge my supervisor Prof. Tamang Sir for support and encouragement. Further, I would like to thank Gut Microbiota and Probiotic Science Foundation (India) for giving me this present oppurtunity.