Probiotic Lacticaseibacillus of human origin to tackle colistin resistant *Klebsiella pneumoniae*

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INTRODUCTION

- Infectious diseases caused by multi-drug resistant bacterial pathogens are a major threat to public health globally and are responsible for ~ 4.95 million deaths yearly (Murray, 2022).
- Probiotics are an alternate strategy to combat antibiotic resistance by protecting from infections as well as a complementary approach alongside antibiotics.
- Probiotics are considered to be "health-friendly bacteria" that help to restore the gut microbial composition.
- They exert antimicrobial effects through competitive exclusion, production of antimicrobial substances, modulating immune response and inhibiting pathogen adhesion.

RELEVANCE OF THE WORK

- Escalating colistin resistance among *K. pneumoniae* made current treatment regimes more complicated.
- Present study aims to evaluate the prophylactic potential of probiotics against colistin resistant Klebsiella
- Human-origin probiotics were selected as it has prolonged survivability in gut and genome based screening for antibiotic resistance will erase the possibility of resistance transfer

OBJECTIVES

❖ Isolation and characterization of probiotic bacteria of human origin

❖ Assessment of antibacterial property of potential probiotic strain against colistin resistant *K.pneumoniae*

❖ Genome analysis, safety evaluation and anti-infectious property of selected probiotic against pathogen in *in vivo* models

METHODOLOGY

Sample collection, screening and selection of probiotics

Identification, Gastric tolerance, Virulence testing and antibiotic susceptibility

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Antibacterial activity and adhesion assay

Broth microdilution, SEM imaging and cell line adhesion ability

↓

***In vivo* studies**

Microbiological analysis and Histopathological imaging

↓

Genome analysis

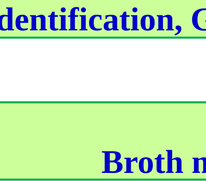
Genome sequencing, screening of AMR genes, prediction of bacteriocin

RESULTS

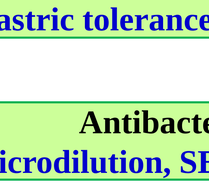
1.Sample collection and probiotic characterisation

Subjects data	
Total no: of subjects	**40**
Infant age	**0-6 months**
Subjects with normal delivery	**25**
Subjects with cesarean section	**15**
Infants with breast feeding only	**30**
Infants with bottle as well as breast fed	**10**
Antibiotics after delivery	**Cephalosporins**

Probiotic characterisation



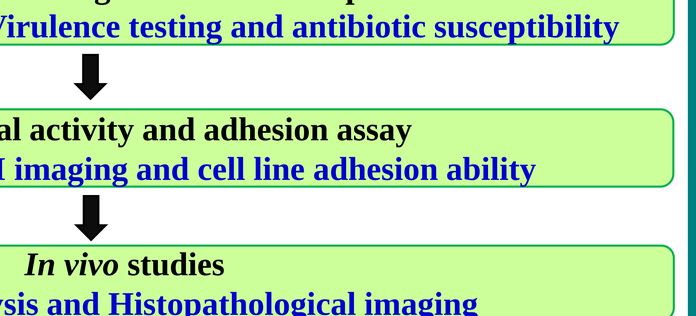
Lacticaseibacilli colonies



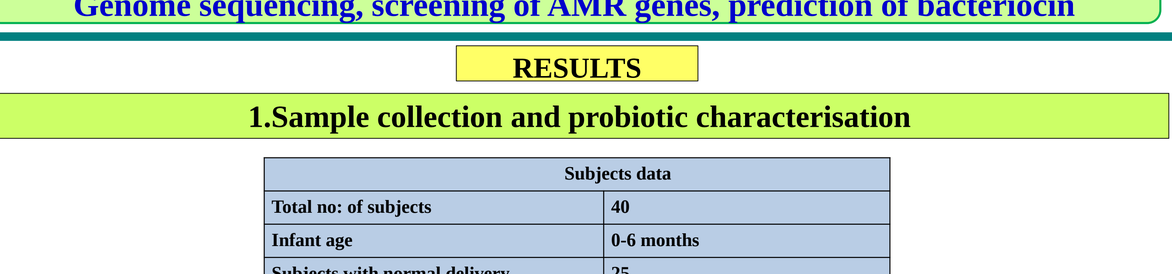
Gram positive bacilli

16 S rRNA Gene amplification: Lane M - 100bp ladder, N-negative control, Lane 1 –13-Lacticaseibacilli isolates

Antibiotic susceptibility of Lacticaseibacilli



INTER-Intermediate, RES-Resistant, SEN- Sensitive. AMP-ampicillin, Van-Vancomycin, CIP-ciprofloxacin, IMP-imipenem, MT-metronidazole, CTX-cefotaxime, GEN-gentamicin, TET-tetracycline, COL-colistin, MET-methicillin, C-chloramphenicol, E-erythromycin, AZM-azithromycin, LZ-linezolid



Representative image showing tolerance to acid, bile and sodium chloride

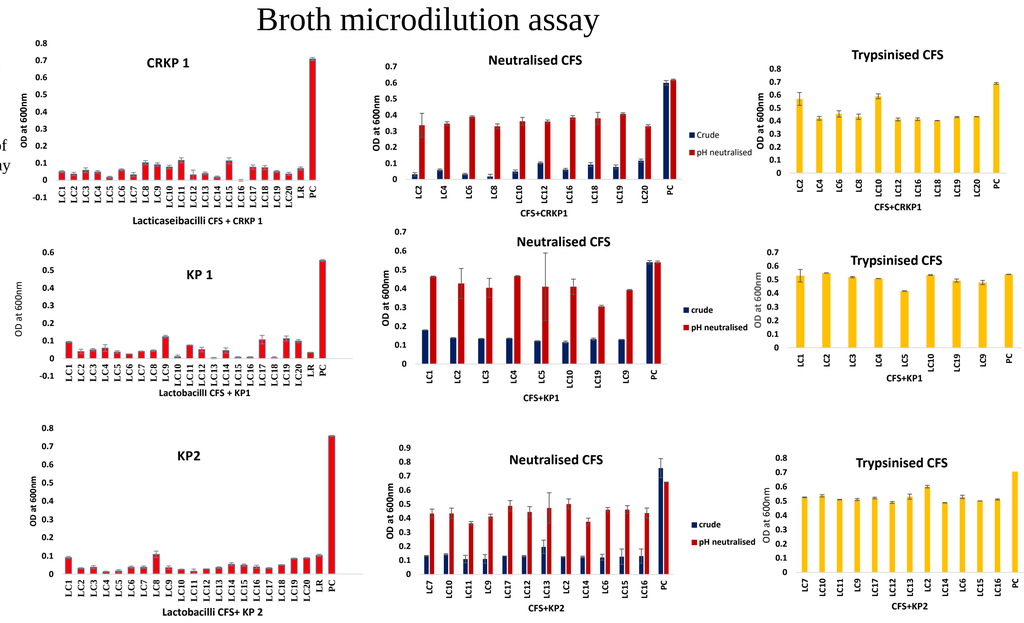
***L.rhamnosus* ATCC 53103 was used as control strain**

2. Antibacterial activity of cell free supernatant

Pathogens used in the study are colistin resistant *K.pneumoniae* CRKP 1, KP1 and KP2 (colistin MIC ≥64 µg/ml)

Broth microdilution assay

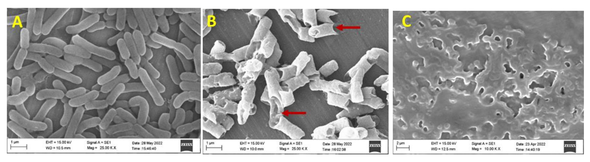
Representative image of agar well diffusion assay



- Twenty Lacticaseibacilli were selected based on probiotic characterization
- *K.pneumoniae* CRKP1 was selected for downstream studies as it is hypervirulent and whole genome sequenced
- Antibacterial activity was evaluated and two isolates (LC 10 and LC 2) showed suspected proteinaceous substances, among which LC 10 showed more adhesion

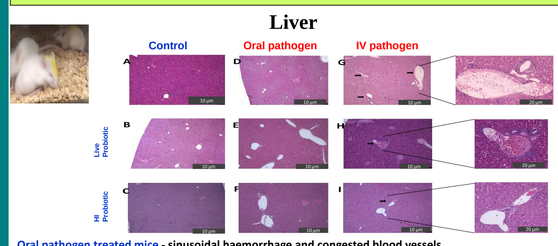
Lacticaseibacilli isolates	% Adhesion
LC10 (infant feces)	31 ± 2
LC2 (infant feces)	12 ± 2
L. rhamnosus GG	26 ± 3

Cell adhesion assay: **LC10** showed good adhesion to CaCo-2 cells *in vitro.*



Scanning electron microscopy: A- untreated cells, B- 10^8 CFU /ml pathogen treated with lactobacilli cell free supernatant (CFS, LC10) for 6h, C- $3x10^8$ CFU/ml treated with CFS for 24h

3. *In vivo* mice model study for probiotic prophylaxis

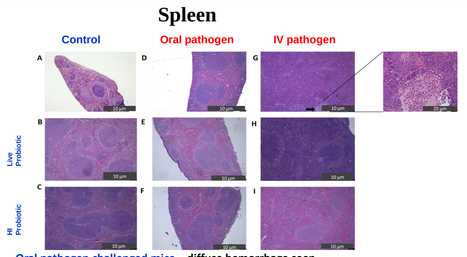


Oral pathogen treated mice - sinusoidal haemorrhage and congested blood vessels

IV challenged mice - dialated and congested blood vessels ,severe neutrophilic and macrophage infiltration in many blood vessels,partly thrombosed portal vessels having mixed population of inflammatory cells.

Probiotic prophylactic + IV pathogen challenge - one lesion of periportal focal haemorrhagic necrosis and neutrophilic infiltration

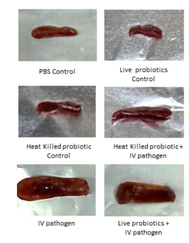
Inactivated probiotic+IV pathogen challenge - neutrophilic infiltration in one blood vessel and also dialated and partly thrombosed portal vessels



Oral pathogen challenged mice – diffuse hemorrhage seen

IV challenged mice-lymphocytic proliferation and megakaryocytes detected

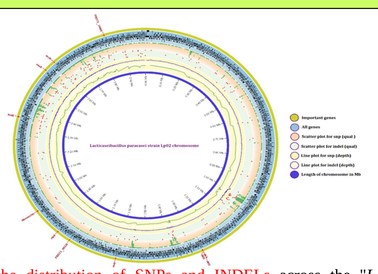
Both live and heat inactivated probiotic prophylactic groups has least pathological changes as compared to pathogen treated groups



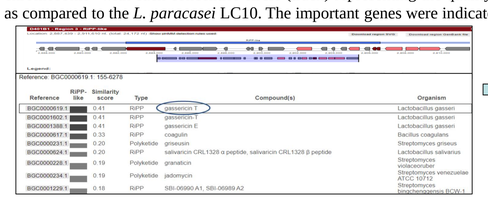
- Safety evaluation of *L. paracasei* LC10 showed promising results with no translocation to other organs
- Microbiological analysis proved that live probiotic administration was more effective as compared to heat inactivated Lactobacilli in terms of pathogen reduction.

4. Whole genome sequencing of *Lacticaseibacillus casei* LC10

Tools	Genes identified
ResFinder (Antibiotic resistant genes)	No antibiotic resistance genes detected
CARD analysis	No antibiotic resistance genes detected
VirulenceFinder (Virulence/genes)	No virulence genes detected
PlasmidFinder	No plasmids detected
Tox Finder	Toxin genes not detected
ANTISMASH	Ripp like proteins detected in region 2 and 3 with similarity percentage less than 50% with existing bacteriocins



Circos plot illustrating the distribution of SNPs and INDELs across the "*L. paracasei* strain Lp02 chromosome" with each concentric circle (track) representing the quality and depth of each class of variant as compared to the *L. paracasei* LC10. The important genes were indicated in the outer track



AntiSMASH analysis

CONCLUSION

❑ All the isolates exhibited desirable probiotic characteristics like tolerance to gastric conditions such as pH, bile and 0.5% phenol.

❑ *L. paracasei* LC 10 and LC 2 showed promising antimicrobial activity against Colistin resistant *K. pneumoniae in vitro.*

❑ SEM imaging indicated morphological changes like pore formation on the cell surface of CFS treated *K. pneumoniae* which is similar to bacteriocin activity.

❑ *In vivo* studies proved the administration of *L. paracasei* LC10 was effective in terms of pathogen reduction and improved histopathological changes in colistin resistant *K. pneumoniae* challenged mice.

❑ Whole genome analysis of *L. paracasei* LC10 revealed that the strain is devoid of antibiotic resistance genes, plasmids and virulence genes and also predicted the presence of thermostable cyclic class II bacteriocin.

Key message:

Development of a probiotic formulation having prophylactic activity against colistin resistant *K.pneumoniae* is a promising strategy to tackle emerging AMR.

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