Comprehensive genomic analysis of hypocholesterolemic probiotic *Enterococcus faecium* LR13 reveals unique genes involved in cholesterol assimilation

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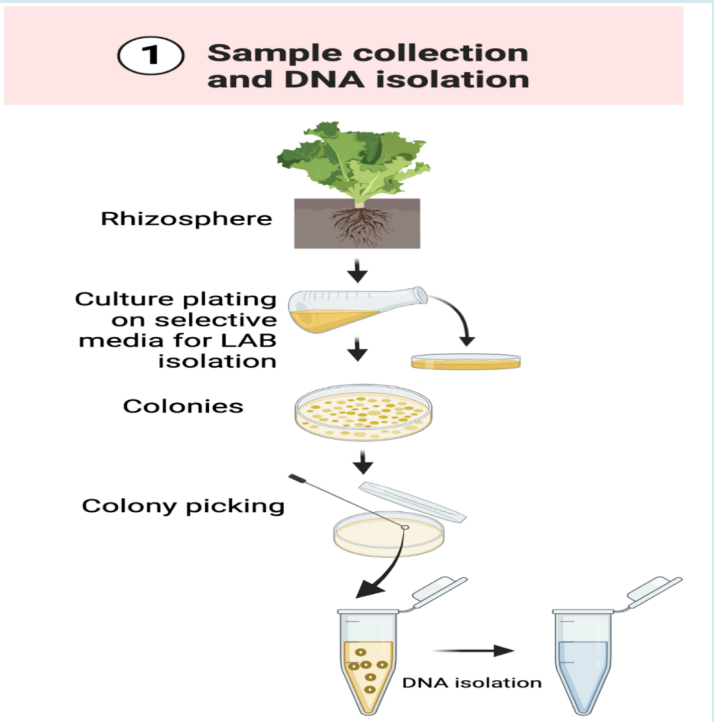
INTRODUCTION

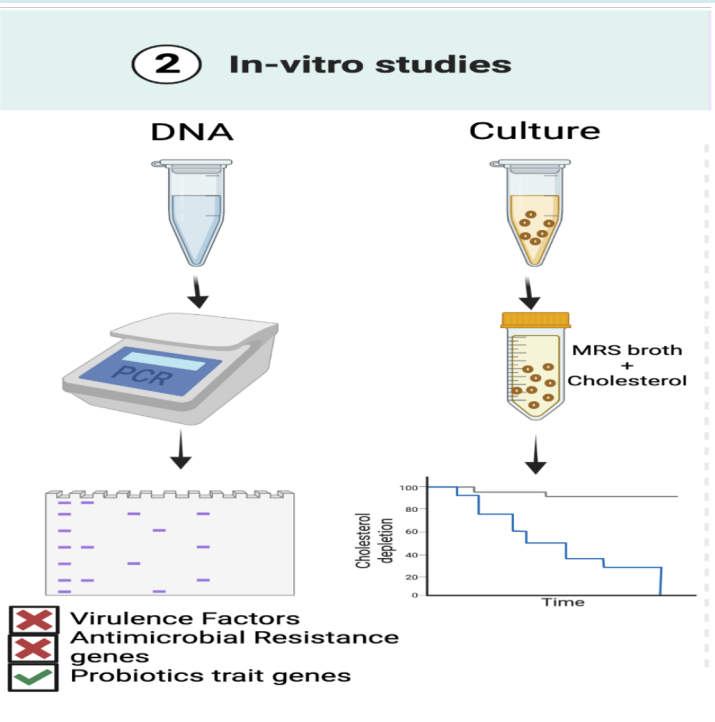
Hypercholesterolemia is a major cause of cardiovascular diseases (CVDs) and therapeutic drugs like statins are used but exhibit several side effects. Probiotics with hypocholesterolemic effects can be effective biotherapeutics for lowering serum cholesterol. Our *in-silico* comparative genomics revealed 21 genes/proteins exclusively present in cholesterol-lowering probiotic *Enterococcus faecium* strains. These genes/proteins were directly/indirectly related to the bacterial cholesterol-assimilation by helping in lipid (sterol) transport and membrane stabilization, producing short chain fatty acids and bile salt hydrolase activity.

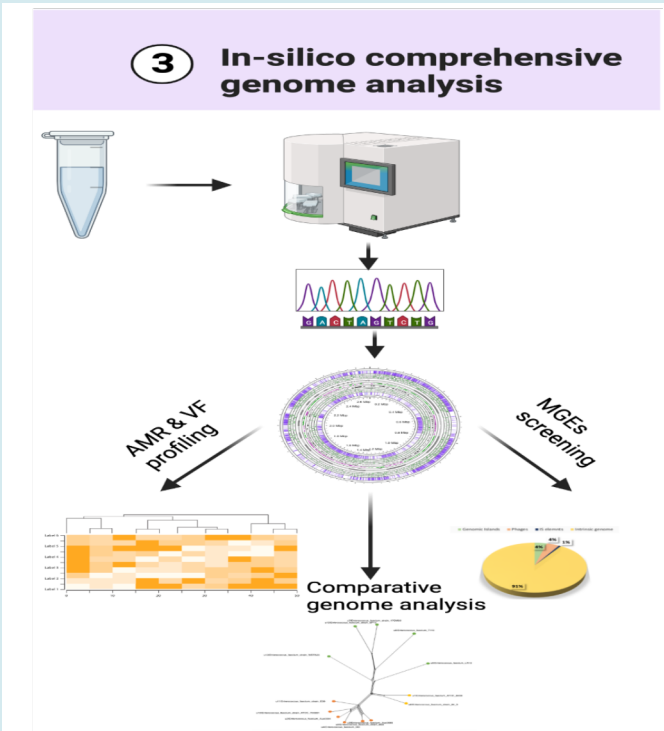
OBJECTIVE

- **Through *in-vitro* and *in-silico* studies reported first indigenous rhizospheric isolate of *E. faecium* LR13 which exhibited several probiotic properties and hypocholesterolemic potential**
- **It was devoid of any virulence factors and antibiotic resistance genes and further unravel the genes/pathways involved in cholesterol assimilation.**

METHODS







RESULTS

- **The isolated colonies from rhizospheric sample were picked up randomly.**
- **Five cultures that yielded pure colonies were presumptively identified as Lactic Acid Bacteria (LAB) on the basis of growth on selective medium, Gram-positive staining, and catalase negative test**
- **The five Gram-positive, catalase-negative LAB isolated from rhizospheric soil were designated as LR2, LR3, ER5, LR13, and VB1.**

Mean percentage cholesterol removal in vitro by E. faecium strains In MRS medium with and without bile salt.

E. faecium strain	Condition	Mean percentage cholesterol removal (%) ± SD
LR2	Cholesterol only	61.63 ± 0.33%
	Bile salt and cholesterol	91.70 ± 0.42%
LR3	Cholesterol only	60.57 ± 0.52%
	Bile salt and cholesterol	91.90 ± 0.15%
ER5	Cholesterol only	58.08 ± 2.10%
	Bile salt and cholesterol	85.70 ± 1.12%
LR13	Cholesterol only	75.97 ± 1.22%
	Bile salt and cholesterol	98.49 ± 0.60%
VB1	Cholesterol only	57.27 ± 0.64%
	Bile salt and Cholesterol	87.49 ± 0.49%

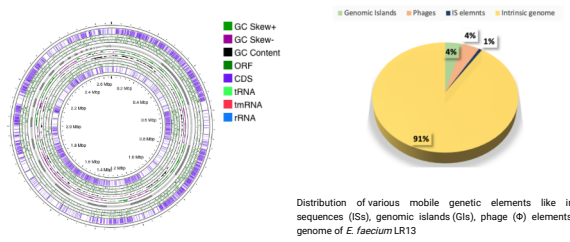
For each sample, the mean of three values are presented ± SD.

Antimicrobial susceptibilities of E. faecium strains.

E. faecium strain	AMX (30)	AMC (30)	CIP (5)	(E) (15)	K (30)	GEN (10)	VA (30)
LR2	S	S	S	R	R	S	R
LR3	S	S	S	R	R	S	S
ER5	R	R	R	R	R	S	R
LR13	S	S	S	R	R	S	S
VB1	R	R	R	R	R	S	R

Amoxycillin (AMX), amoxyclav (AMC), ciprofloxacin (CIP), erythromycin (E), Kanamycin (K), gentamicin (GEN), vancomycin (VA); S, sensitive; I, intermediate; R, resistant. Numbers in parentheses indicate concentration of antibiotic in mcg/disk.

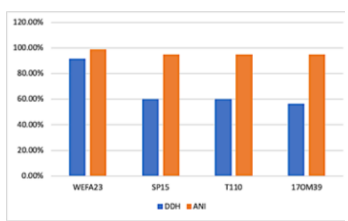
- **Among Five *E. faecium* isolates LR2, LR3, ER5, LR13, and VB1 . LR13 strain showed highest cholesterol assimilation and absence of vancomycin resistance .**
- **Therefore, genome sequencing (Novaseq 6000) 150bp pair-end reads, de-novo assembly and annotation of LR13 was performed as candidate probiotic and hypocholesterolemic strain represented below:**



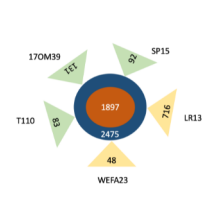
Distribution of various mobile genetic elements like insertion sequences (ISs), genomic islands (GIs), phage (Φ) elements in the genome of *E. faecium* LR13

	Features	E. faecium LR13 draft genome
Assembly	Genome Length	2,665,715 bp (2.66Mbp)
	GC Content (%)	37.79
	N50	123,181
	Largest Contig	282,801bp
Annotations	No. of proteins	2,522
	No. of Pseudogenes	18
	No. of rRNAs	3
	No. of tRNAs	53
	CRISPR array	2
Genomic Signatures	No. of Prophages	10
	IS Elements	14
Reference	GenBank Accession no.	JANRHE000000000

Comparative genome analysis of cholesterol-lowering probiotic *E. faecium* strains (LR13 and WEFA23) and general *E. faecium* probiotic strains (T110, 170M39 and SP15)

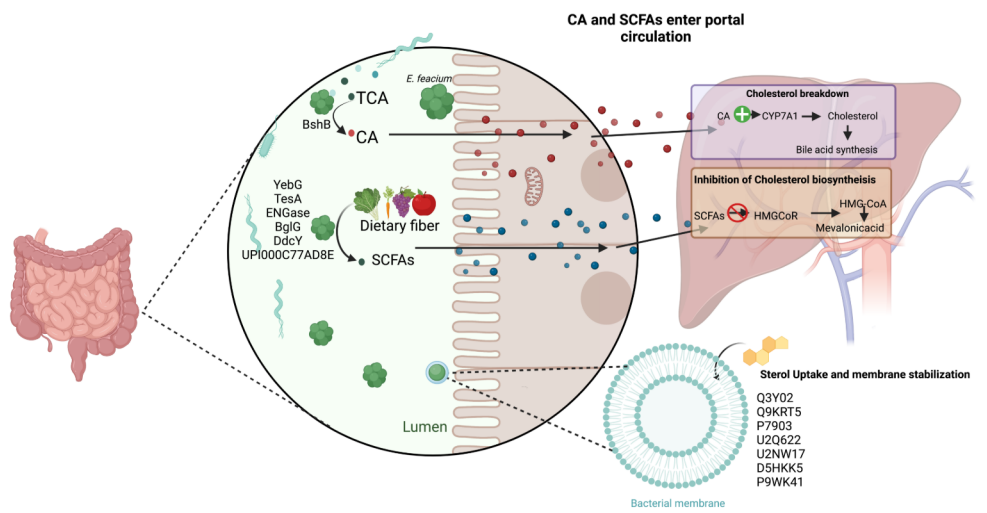


Pan-genomic comparison of *E. faecium* LR13 with other probiotic strains using DNA-DNA Hybridization (DDH) and average nucleotide identity (ANI)



***Enterococcus faecium* LR13 genome show number of core, accessory and unique proteins.** (Orange – Core genes , Blue – accessory genes, Green - unique genes with general probiotic strains and Yellow - unique genes with cholesterol lowering probiotic strains)

CONCLUSION



COG analysis between LR13 and WEFA23 hypocholesterolemic strain reveal 21 genes/proteins in common and out of which 14 were found to be directly involved in cholesterol reduction. These genes/proteins we found to be absent in other general probiotic strains (T110, 170M39 and SP15)

- Proteins involved in bile salt hydrolysis: bacterial bile salt hydrolases (BshB) converts taurine/glycine cholic acid (T/GCA) to cholic acid (CA) that aids in the activation of cholesterol 7α-hydroxylase (Cyp7a) in the liver resulting in degradation of cholesterol into bile acids (126).
- Proteins involved in production of short chain fatty acids (SCFAs): the bacterial proteins YebG, TesA, ENGase, BglG, DdcY and UPI000C77AD8E are involved in the uptake and breakdown of dietary fibers into short chain fatty acids (SCFAs) like propionate, acetate and butyrate. In the liver, inhibits HMG-CoA reductase enzyme (3-hydroxy-3-methylglutaryl-CoA) which converts HMG-CoA to mevalonic acid resulting in inhibition of cholesterol biosynthesis
- Proteins involved in lipid (sterol) transport, membrane stabilization and binding of cholesterol to the bacterial cell walls: bacterial proteins Q3Y02, Q9KRT5, P7903, U2Q622, U2NW17, D5HKK5 and P9WK41 aids in uptake of sterols(lipids) from the intestinal lumen and incorporating in their cell membranes/walls.

ACKNOWLEDGEMENTS

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2. Aswal et al."Comprehensive genomic analysis of hypocholesterolemic probiotic *Enterococcus faecium* LR13 reveals unique proteins involved in cholesterol-assimilation" (ManuscriptID: 1082566) (Journal: Frontiers in Nutrition- Nutrition and microbe) (Under final review)