A gut signature linked with increased ethanol production and liver inflammation contribute to Nonalcoholic fatty liver disease in Indian patients.

PUBLISHED

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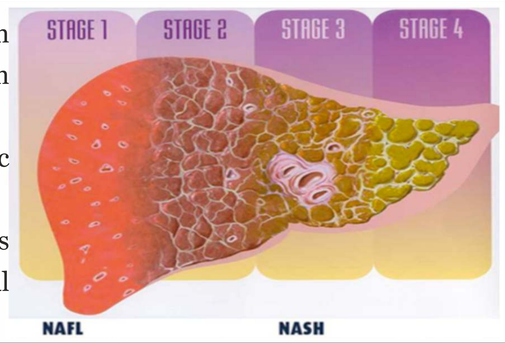
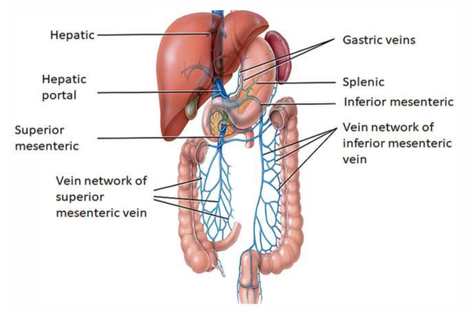
Introduction

- Non Alcoholic Fatty Liver Disease (NAFLD), a metabolic disorder in liver with increase fat accumulation, inflammation and progression to cirrhosis, is the most prevalent liver disease across the world and accounts for 2% of total death worldwide.
- Gut bacteria and its metabolites can permeate the liver via portal circulation and directly or indirectly influence hepatic activity.
- In the present study, we adopted multiple approaches, including metagenomics, isolate bacterial genomics, gas chromatography mass spectrometry (GC-MS), and a combination of in-vivo experiments coupled with functional genomic analysis, to study the role of C. aerofaciens in NAFLD.



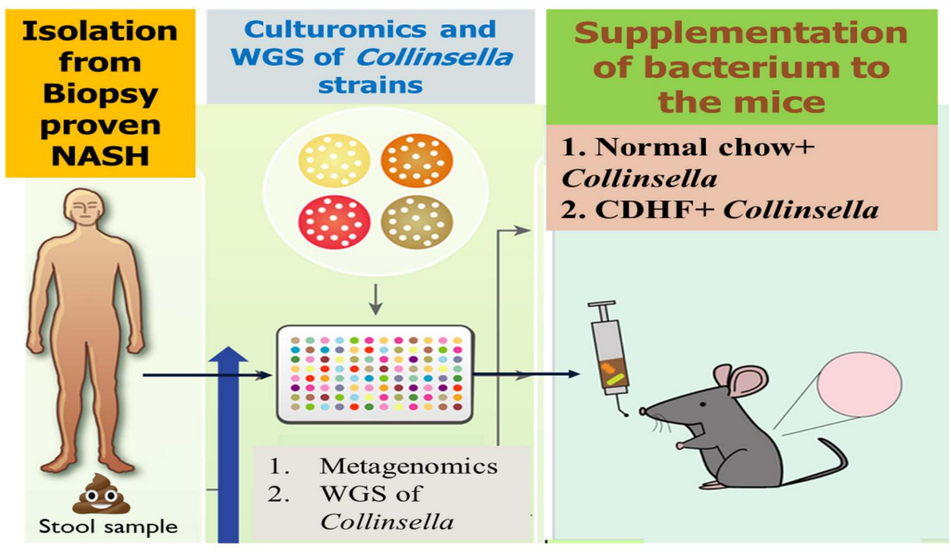


Aim

Identification of gut microbial signature in the Indian NAFLD patients.

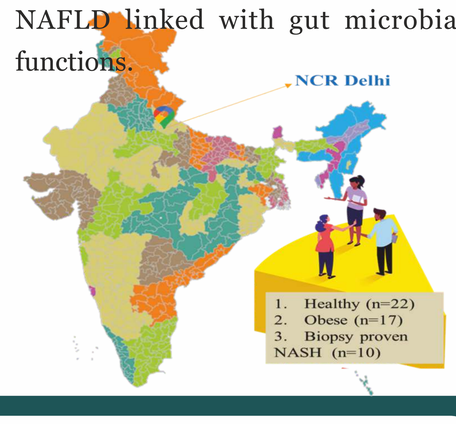
Methods

1. Fecal samples were stored at -80°C before processing DNA extraction and sequencing. Frozen fecal samples were used to extract DNA, decoded the V1-V5 region of 16S rRNA gene sequences and analyzed the composition and diversity of gut microbiota in obese Indian subjects.
2. Whole genome of *Collinsella aerofaciens* was sequenced and analyzed to identify potential inflammatory functions and metabolic functions.
3. Supplementation of bacterium to the mice to examine steatohepatitis by histopathological analysis and mRNA expression by qRT-PCR.



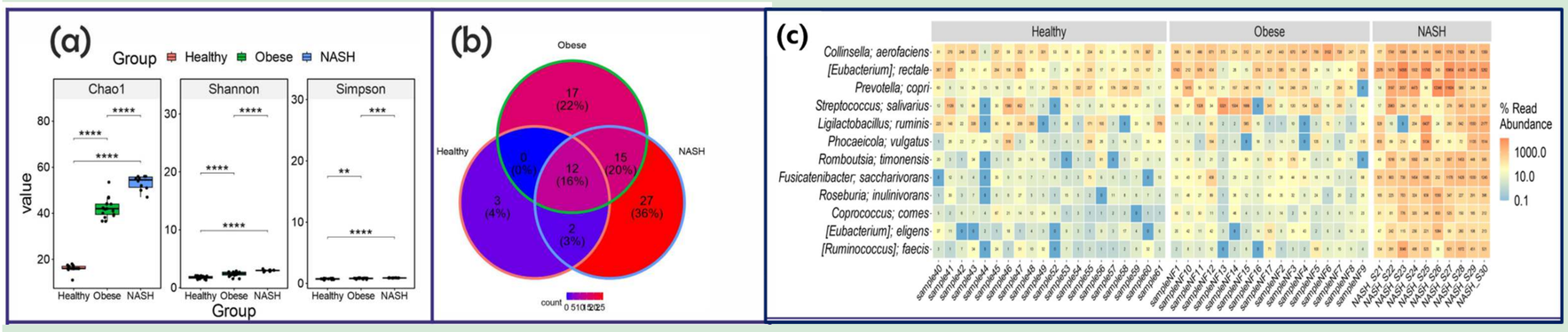
Objectives

- Study the taxonomic and functional changes of gut microbiota in healthy and obese subjects.
- Explore the genomic repertoires of the bacterial taxa associated with NAFLD.
- Understand the mechanistic of NAFLD linked with gut microbial functions.



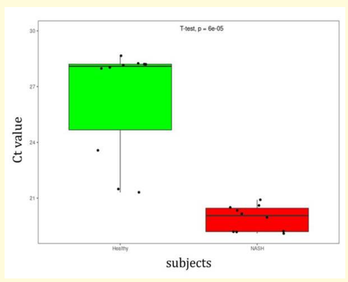
Result

1. Diversity measurement and statistical analysis of the gut microbiota in healthy, obese, and NASH subjects.



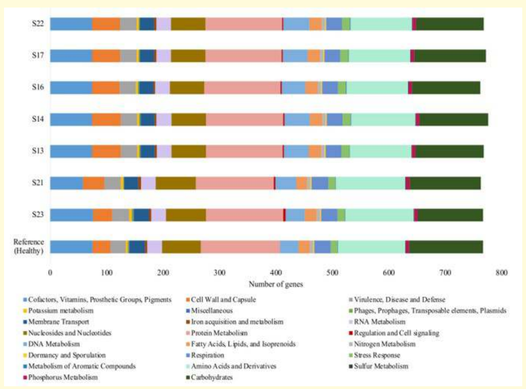
(a): Statistical comparison of alpha diversity indices (i.e., Chao1,Shannon and Simpson).
(b) Venn Diagram depiction of 80% core OTUs in the Healthy, Obese and NASH groups. Each group has shown by circle that have been surrounded by fade red, green and blue circles respectively
(c) The distribution of OTU numbers of 80% core OTUs in Healthy, Obese and NASH groups in heatmap plot.

2. Relative abundance of C. aerofaciens in the stool sample of healthy and NASH subjects



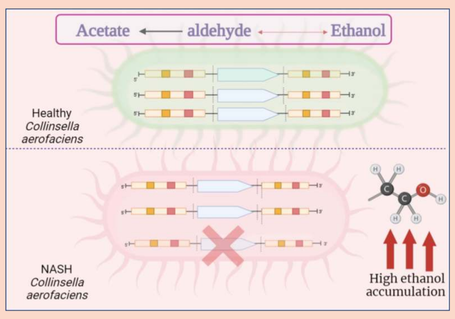
The relative abundance was measured by quantitative PCR (qPCR) using specific primers. The abundance of *C. aerofaciens* is significantly high in NASH patients compared to the healthy people.

Subsystems analysis of the *Collinsella aerofaciens* genomes

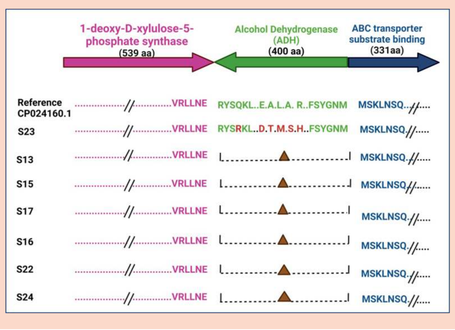


The subsystems present in the *C. aerofaciens* genome isolated from NASH patients and healthy were predicted by RAST annotation pipeline.

3. Differences in the genomic repertories and Genetic analysis of the alcohol dehydrogenase encoding functions of *Collinsella aerofaciens* isolated from healthy and NASH subjects.

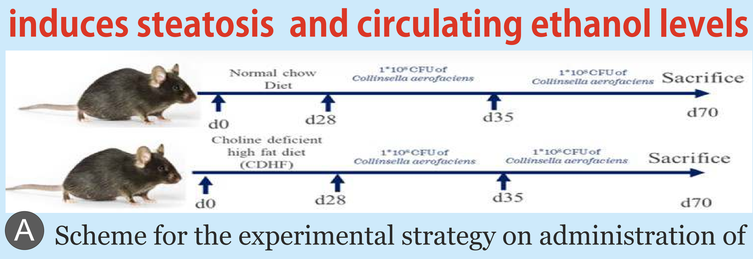


Genetic background of genes encoding for alcohol dehydrogenase function as depicted from the strain isolated from a heathy individual and NASH subjects

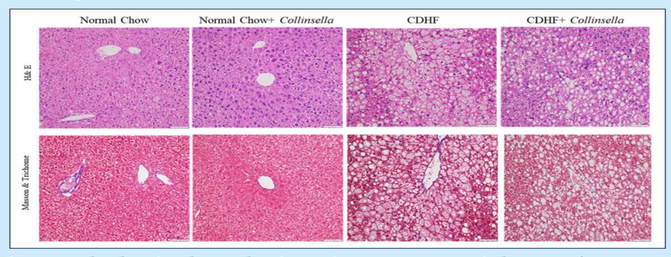


Amino acid sequence alignment of the alcohol dehydrogenase (ADH) gene along with the upstream and the downstream genes in C. aerofaciens chromosome.

4. Animal model showing *Collinsella aerofaciens* induces steatosis and circulating ethanol levels

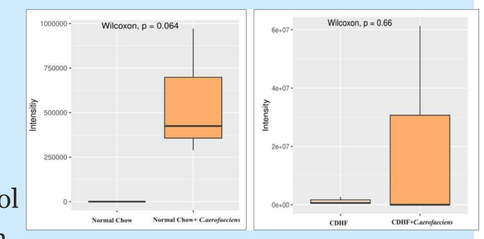


A Scheme for the experimental strategy on administration of *Collinsella aerofaciens*- with normal chow diet and choline deficient high fat diet (CDHF).

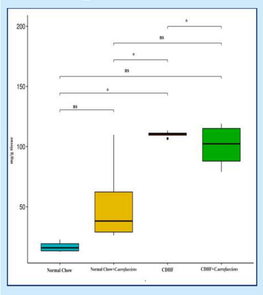


B Histopathological analysis using mason trichome for fibrosis and hematoxylin eosin for steatosis, inflammation and ballooning according to NAS score.

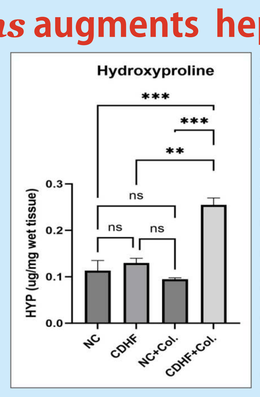
C The Box plot depicted the intensity of ethanol production a) The production of ethanol was found to be high when normal chow plus *C. aerofaciens* diets was given to mice models whereas no ethanol production was observed in case of normal chow diet (Wilcoxon, p-value = 0.064. **(ii)** The production of ethanol was found to be high when CDHF (choline deficient high fat diet) plus *C.aerofaciens* diets was given to mice models whereas less ethanol production was observed in case of CDHF diet. (Wilcoxon, p-value = 0.066).



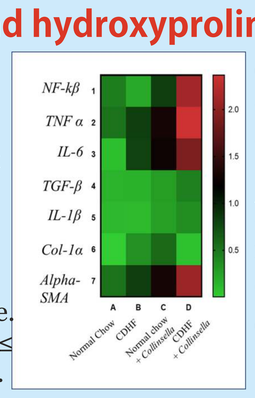
D *Collinsella aerofaciens* augments hepatic triglycerides and hydroxyproline levels



Box plot depicting the level of triglycerides in hepatic tissue of mice, as compared to normal chow fed mice group, CDHF groups has higher level of triglycerides. In the figure * represents p ≤ 0.05.



Bar plot showing the concentration of hydroxyproline in mice hepatic tissue of distinct diet induce NAFLD model. In comparison to the normal chow CDHF + *Collinsella* is showing significant higher abundance of hydroxyproline. In the figure ** represents p ≤ 0.01, *** represent p ≤0.001.



qPCR analyses of **(a) inflammation, (b) fibrosis and the mRNA** levels in two groups mice challenged by *Collinsella aerofaciens*.

Discussion

- We study the gut microbiome of NAFLD, obese, and healthy subjects.
- We observed a higher abundance of *Collinsella aerofaciens* in obese and NASH patients.
- *C. aerofaciens* contributes to increase the level of systemic ethanol in mice.
- Increased ethanol, hydroxyproline, and triglycerides induce hepatic inflammation

Conclusion

The insights from the current findings will help us to understand the importance of Collinsella aerofaciens in the progression of NAFLD. It could be a potential geospecific biomarker for early prediction of NAFLD.

Acknowledgements :
Authors like to acknowledge **Dr. Shalimar and Dr. Rajini Yadav** from All India Institute of Medical Science (AIIMS),New Delhi for scientific assistance .

The present study is funded from Department of Biotechnology (DBT) Department of Science and Technology (DST)

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