

Ligilactobacillus salivarius F14 originated from tribes of Odisha as a potential probiotic

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Abstract

Introduction- Extensive research on probiotics emphasizes the characterization of new potential probiotic strains to aid their benefits in health and disease. Tribes could be unusual source of probiotics due to their unique food habits and least dependence on medications and consumption of antibiotics.

Objectives- Characterization of genetic and probiotic attributes of one of the bacteria (F14) isolated from tribal fecal samples of Odisha, India.

Methods- The probiotic attributes of F14 bacteria was checked through *in vitro* experiments set by ICMR/DBT guidelines and whole genome sequence analysis. The antimicrobial properties was evaluated by *in vitro* co-culture experiment and further confirmed using *in vivo* mice model against *Salmonella typhimurium* BT-XEN33 (ST-Xen 33).

Results- Initial morphological identification and 16S rRNA sequencing confirmed that it is a catalase-negative, Gram-positive bacteria belonging to *Lactobacillus* genus. It showed tolerance to acid and bile stress, as well as cell adhesion and antimicrobial properties. Metabolites like acetate, propionate, and butyrate having immuno-modulating activity were detected in the cell free supernatant of its culture. Co-culture of ST-Xen 33 with F14 for different time point depicted the cytotoxic effect against ST-Xen 33. The *in vivo* experiment showed successive reduction in pathogen load, enteritis and increased survival rate. Whole genome sequence analysis confirmed F14 strain as a member of *L. salivarius* species, having 137 unique genes.

Discussion- F14 bacteria showed all the probiotic properties required for its survival in GI tract and maintaining the gut health. It significantly suppressed pathogenesis of ST-Xen 33 and restored the normal tissue architecture in mice with enteritis.

Conclusion- The probiotic potential of *Ligilactobacillus salivarius* F14 was confirmed through *in vitro* experiments and whole genome analysis. *Ligilactobacillus salivarius* F14 successively alleviates the Salmonella induced enteritis in mice model.

Objectives

- Morphological identification and molecular characterization of F14, isolated from tribal fecal samples of Odisha
- Characterization of the isolate for probiotic attributes as per the ICMR-DBT guidelines
- In vitro* evaluation of anti-Salmonella effect
- In vivo* evaluation of safety and anti-Salmonella efficacy
- Whole genome sequencing for strain level identification

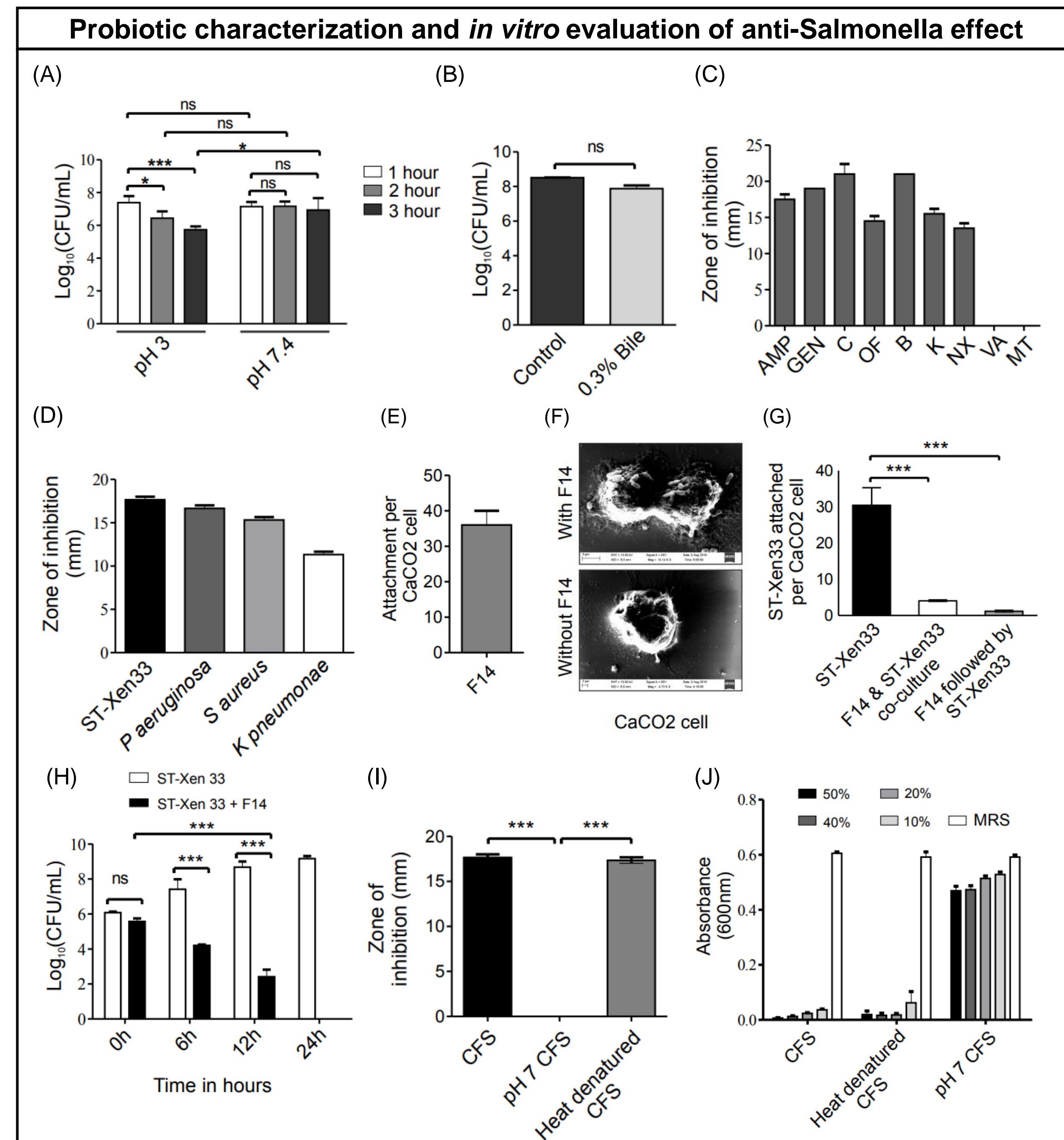
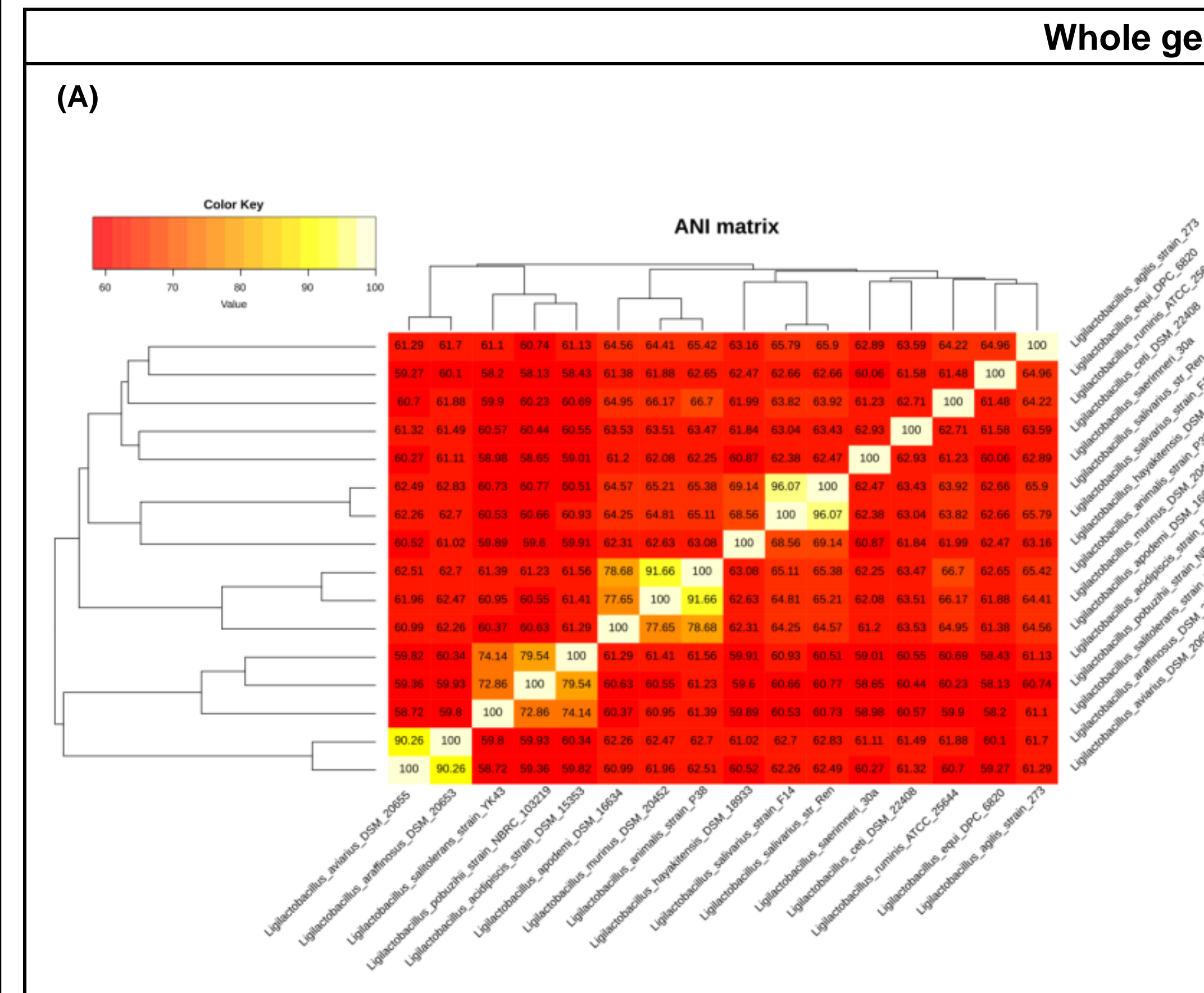
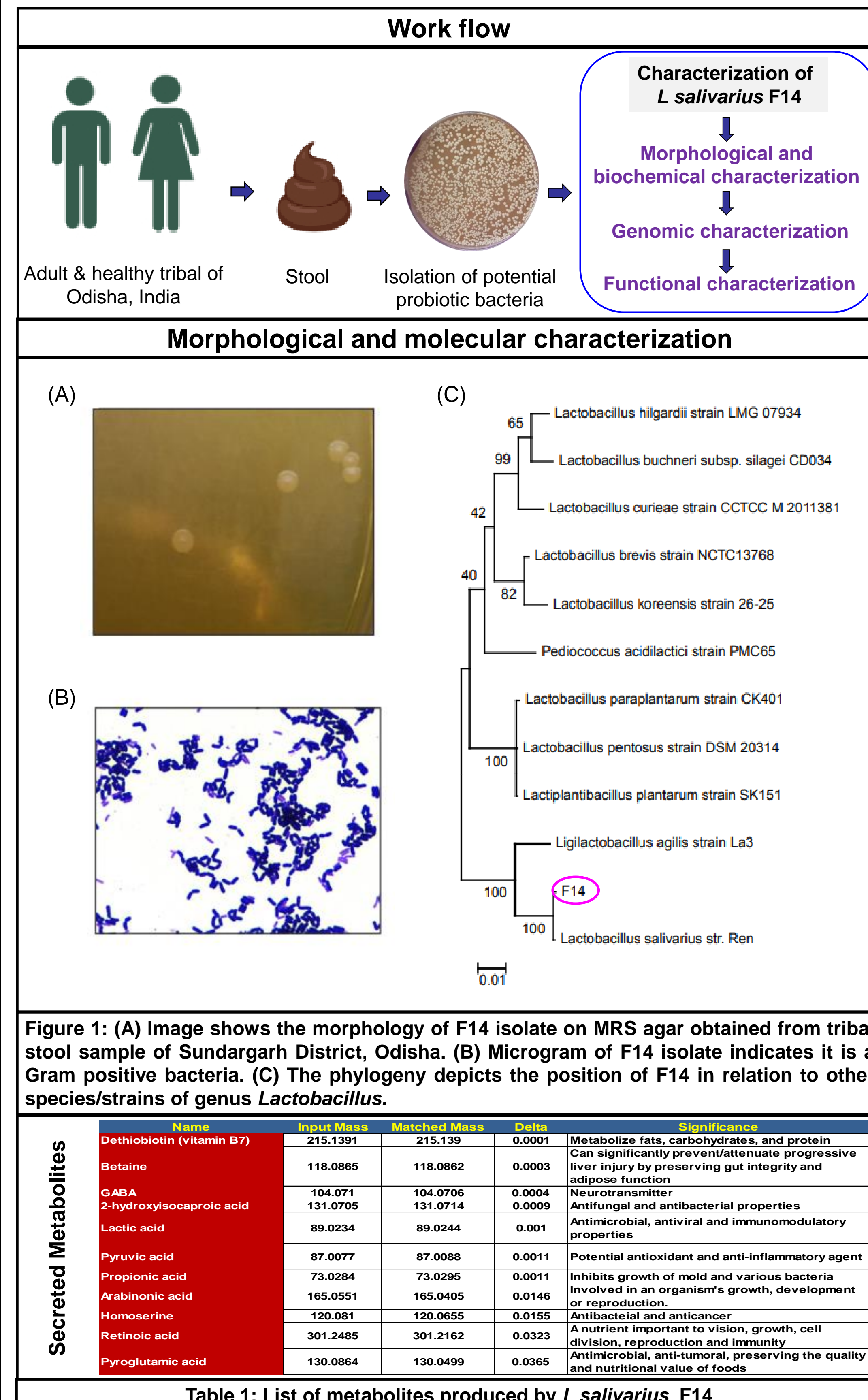


Figure 2: (A) Viability of F14 isolate at pH 3 and 7.4, indicates the tolerance to acidic pH. (B) Tolerance to bile salt was evident from the viable growth after 4 hours exposure to 0.3% bile in the growth media. (C) Isolate F14 is susceptible to most of the commonly used antibiotics to various degrees. (D) Agar well diffusion assay shows good zone of inhibition indicating the antimicrobial effect of F14 against different enteric pathogens. (E) Attachment efficiency of F14 after co-culturing with Caco2 intestinal epithelial cell. (F) Scanning Electron Microscopy images of the Caco2 cells co-cultured with F14 isolate shows the cell adhesion efficiency of the isolate. (G) Competitive exclusion of ST-Xen33 effectively by F14 after co-cultured with intestinal epithelial cells. It was enhanced further when caco2 cells were pre-treated with the probiotic F14 prior to pathogen challenge. (H) Co-culture of ST-Xen 33 with F14 in different time point demonstrates the cytotoxic effect of F14 towards the pathogen. (I) Agar well diffusion assay shows the effect of normal, pH neutralized and heat denatured CFS of F14 on growth of ST-Xen 33. (J) ST-Xen33 was grown in different percentage of normal, pH neutralized and heat denatured CFS of F14 to get the minimum inhibitory percentage (MIP) of the CFS of the isolate.

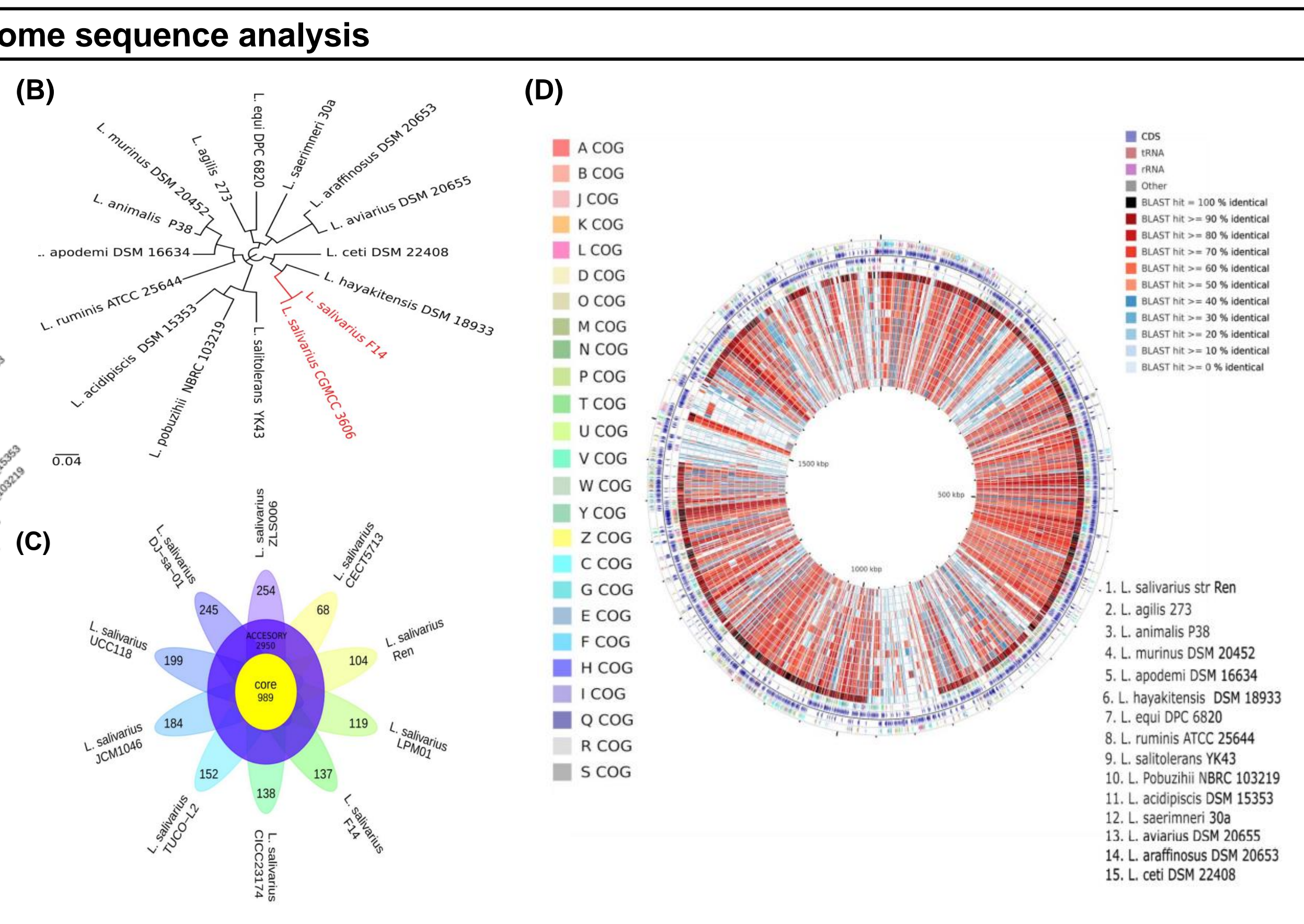


Figure 4: (A) ANI matrix showing genomic relatedness performing multi-genome comparison among *Ligilactobacillus* species from LPSN (B) Phylogenetic tree based on WGS data compared to other *Ligilactobacillus* species from LPSN (C) Venn diagram showing number of orthologous gene clusters of the core genome and the unique genes specific to genome of individual strains compared from LPSN (D) Circular genome map of *Ligilactobacillus salivarius* compared to other type strains (percentage similarity is represented by different color code)

Results

In vivo evaluation of the anti-Salmonella effect

(A) Treatment of F14 did not show any harmful effect on the survival of the animal. (B) Pathogen ST-Xen 33 dose optimisation. (C) Schematic diagram showing the experimental workflow. (D & E) Animals treated with F14 in pathogen infected mice had increased survival rate and body weight as compared to the infected group. (F-I) Bioluminescent image, pathogen load and tissue image of different groups of experimental mice showed that in ST-Xen 33 infected mice, F14 significantly reduced pathogen load and enteritis. (J & K) The percentage of Treg population in spleen of control and F14 treated mice. (L-N) Graphs showing cytokines expression in lymphoid organs of F14 treated mice and IL-10 expression in intestinal flush (O) and serum (P) of F14 treated ST-Xen 33 infected mice.

Conclusions

- Isolate F14 is a Gram positive and catalase negative bacteria upon preliminary microscopic observation. Further, the 16S r-RNA sequencing results confirmed that it belongs to genus *Lactobacillus* genus
- The probiotic characterization (ICMR-DBT guidelines) revealed that F14 has good acid, bile tolerance properties, potential antimicrobial activity against different pathogens and is also sensitive to most of the commonly used antibiotics
- The isolate is also capable of adhering to intestinal epithelial cell surface and excluding the adherence of pathogen from the intestinal surface
- Metabolites like acetate, propionate, and butyrate having immuno-modulating activity were detected in its cell free supernatant of its culture
- Further, *in vitro* evaluation of the antimicrobial effect of F14 by co-culture experiment indicates its anti-Salmonella effect. The effect of CFS of F14 against ST-Xen 33 is bactericidal or bacteriostatic depending upon percentage of CFS in culture i.e., up to 20% CFS the effect is bactericidal and at 10% CFS effect is bacteriostatic
- F14 significantly suppressed pathogenesis of ST-Xen 33 *in vivo* and induced increase in systemic/splenic immunosuppressive immune cells (Treg) population.
- Whole genome analysis confirmed F14 strain as a member of the *L. salivarius* species, having 137 unique genes including 25 proteins with known functions and 112 hypothetical proteins of unknown function

Ongoing/Future directions

Characterisation of unreported bacterial isolates from the tribal population for their probiotics attributes and possible health benefits

Acknowledgement

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