

Title: Universal adaptive evolution strategy of probiotic *Lactobacillus plantarum* to gut selection pressure from humans, mice and zebrafish

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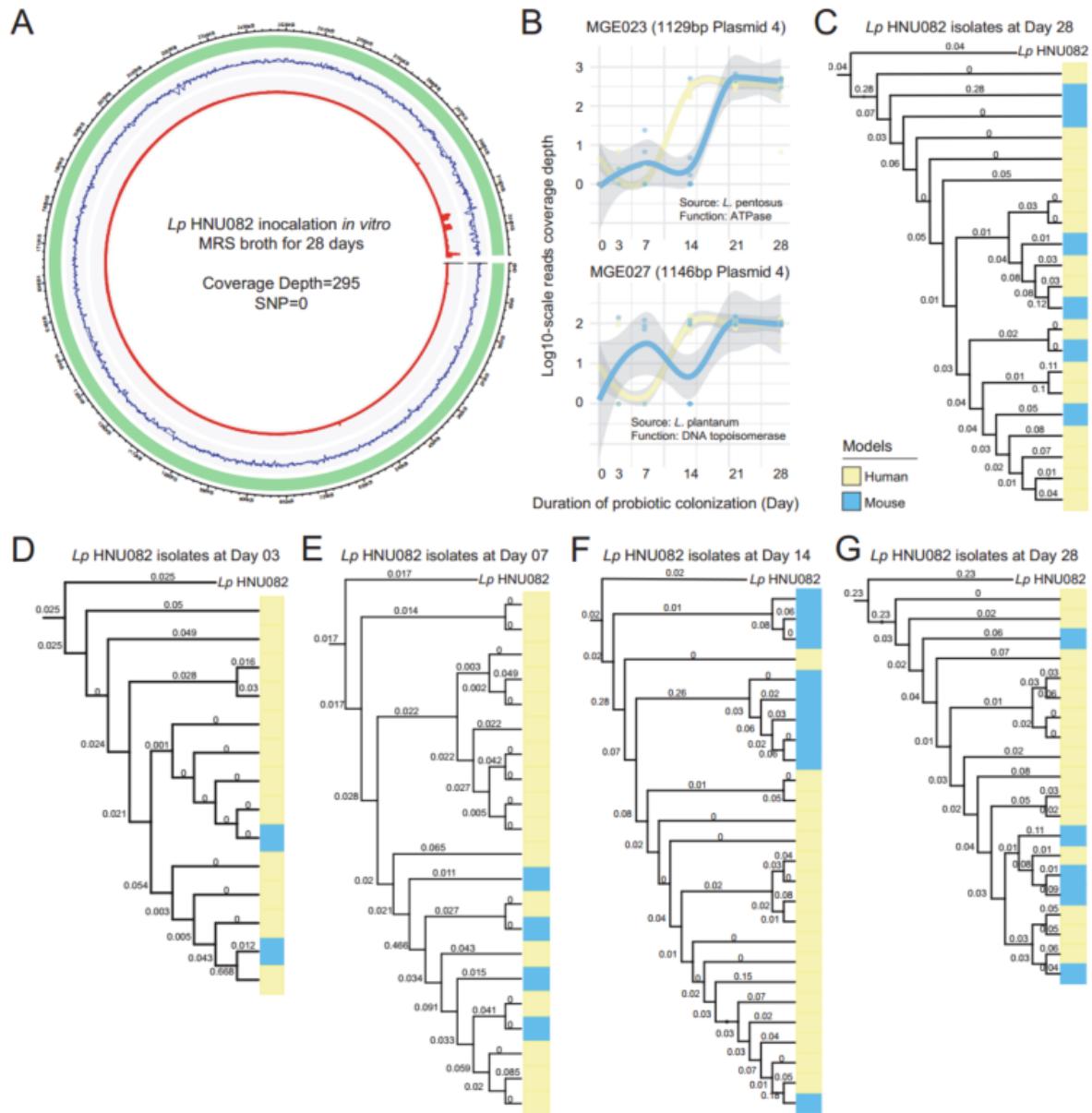
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1 **Supplementary Figures**



2 **Figure S1. The adaptive evolution of Lp082 under *in vitro* and *in vivo* conditions. (A)**

3 The genome sequence of the original Lp082 strains inoculated in the MRS broth at day 7, 14,

4 21 and 28. During the *in vitro* incubation period, no SNP was annotated in these strains. (B)

5 The temporal pattern of sequencing coverage of two mobile elements inserted in Lp082

6 during its colonization in the gut of human and mouse. The x-axis represents the duration of

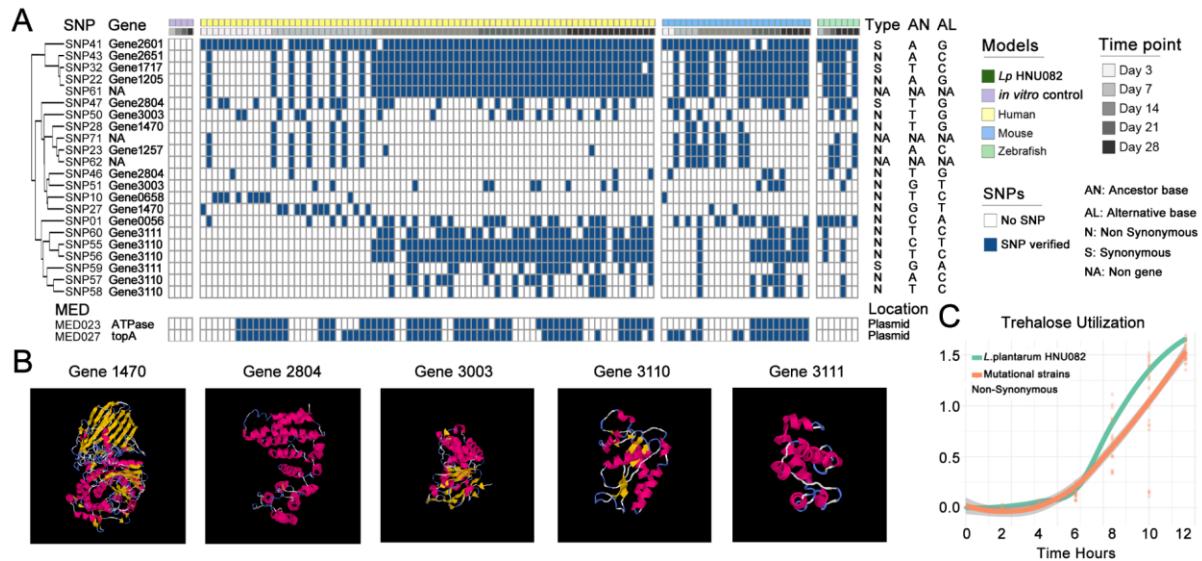
7 probiotic colonization, while the y-axis represents the log10-transformed read coverage of

8 each mobile element that can be inserted into the probiotic genome. Mobile Element 023 and

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10 027 were identified in the plasmid 4 of this probiotic strain. (C-G) Phylogenetic trees
11 constructed based on all Lp082 isolates in each time point. The number in tree branches
12 represent the branch lengths among the isolates.

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15 **Figure S2. The genes underwent *in vivo* evolution in the three hosts. (A)** The heatmap

16 shows the presence or absence of SNPs and mobile elements detected and experimentally

17 verified in all isolates from human, mouse and zebrafish hosts. The probiotic isolates are

18 shown in columns, while the verified SNPs or mobile elements corresponding to a probiotic

19 isolate are shown in the rows. **(B)** The predictive protein structure of five genes underwent *in*

20 *vivo* parallel evolution. **(C)** The phenotypic verification experiments of isolates related to

21 trehalose utilization. No significant difference in trehalose utilization was found between the

22 original probiotic strain and the mutational isolate.

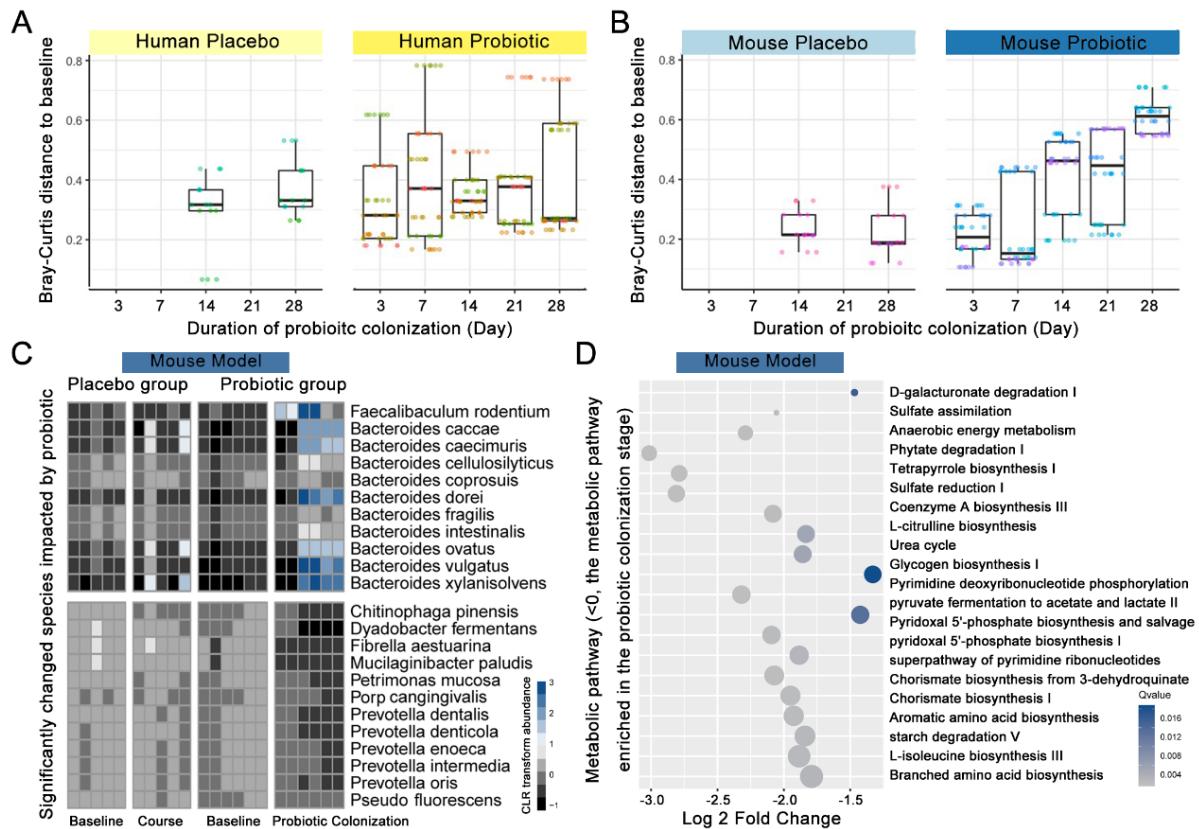
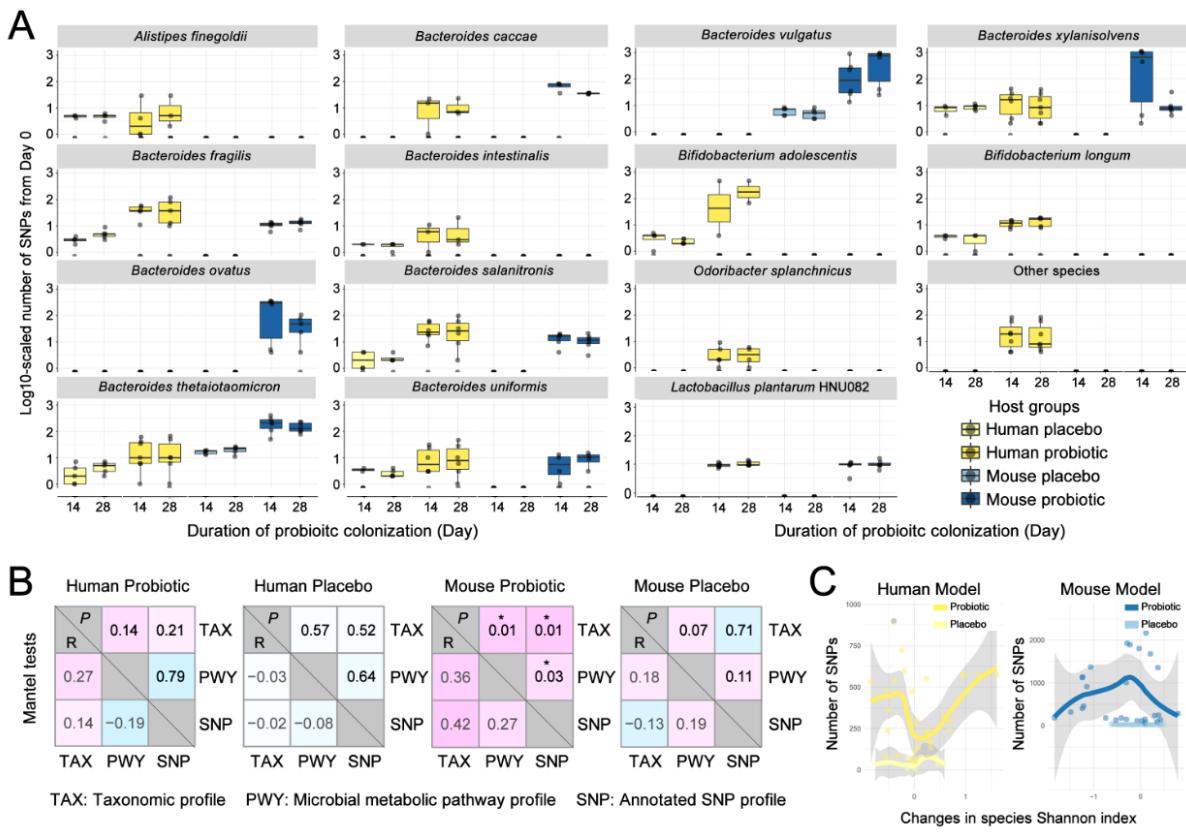


Figure S3. The alterations in the resident gut microbiome responding to the probiotic invasion in humans and mice. A, B) The boxplot indicating Bray-Curtis distance between samples in baseline and other time points both in the human and mouse model. The colored points represent stool samples collected at different time points. We found that there is a relatively large inter-individual variation in the gut microbiome between human hosts as compared to that in mice. **(C)** The heatmap shows intestinal species significantly changed responding to Lp082 ingestion in the mouse model. **(D)** The scatter plot shows the fold change of microbial metabolic pathways from Day 0 responding to Lp082 ingestion in the mouse model. However, no microbial species or functional contents in the human gut showed a significant association with probiotic ingestion potentially due to the large individuality observed in the human gut microbiome.



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36 **Figure S4. The *in vivo* evolution of resident microbial strains in the gut under the**
 37 **selection of probiotic ingestion. (A)** The number of SNPs occurred in the resident intestinal
 38 strains over the 28-days sampling period responding to probiotic ingestion in humans and
 39 mice. **(B)** Mantel tests quantifying the correlation between each pair of measurements
 40 (taxonomic profile, functional profile and SNP profile) on each sample collected from
 41 humans and mice. Each matrix showed results on the Mantel tests on a sample group
 42 (probiotic or placebo) from humans or mice. In each matrix, the values in the lower diagonal
 43 indicate the R values of the Mantel test, which range from -1 to 1, representing the correlation
 44 between a pair of measurements. The corresponding P values of the correlations are shown in
 45 the upper diagonal of the matrix. Results showed that probiotic ingestion resulted in a tighter
 46 coupling between SNP profiles and taxonomic or functional profiles in the gut of mice. **(C)**
 47 The relationship between the changes in the species-level Shannon diversity and the number
 48 of SNPs. The probiotic intake did lead to more fluctuations in the Shannon diversity than

49 usual (placebo group) yet no significant correlation between change in Shannon diversity and
50 mutation frequency was observed in both humans and mice.

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