Receptor Targeted Next-Generation Probiotics Ameliorate Inflammation and Promote Gut Health

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Abstract

Background: Intestinal barrier dysfunction, inflammation, and elevated expression of heat shock protein 60 (Hsp60) are features of the dysbiotic gut. Probiotics can alleviate inflammation but are ineffective due to poor adhesion and adaptation to the inflamed bowel. We hypothesize that enhancing probiotic adhesion to intestinal cells may augment the immunomodulatory response, mucosal healing, and tight junction restoration. Earlier, we identified *Listeria* adhesion protein (LAP; 94-kDa) that aids *Listeria* attachment to the epithelial cells by interacting with Hsp60. Next-generation bioengineered *Lactobacillus casei* (Lbc) probiotics (BLP) expressing LAP from *L. innocua* showed strong interaction with epithelial Hsp60, high immunomodulatory response, and epithelial barrier integrity while reducing inflammation.

Method: We fed BLP for 10 d to mice pretreated with dextran sulfate sodium (DSS, 2% for 7 d)- as a chemically-induced inflamed gut model.

Results: BLP-fed DSS-treated mice gained 5% body weight compared to the DSS- treated mice that did not receive any probiotics during that period. BLP feeding conferred a 67% reduction in disease activity index compared to the control and 50% to LbcWT- treated mice. BLP treatment restored fecal consistency to Type 3- 4 (Bristol scoring) within 9 d of feeding, while the control and LbcWT treatment groups failed. BLP-fed mice showed improved gut barrier; >50% reduction in FITC-labeled 4-kDa dextran (FD4) permeability compared to LbcWT or the control group. DSS-induced shortened colon length, abdominal adhesions, and mucus accumulation were substantially improved by BLP feeding relative to the control and LbcWT groups. Colon pathology, including neutrophil infiltration of the BLP-fed group, was 50% lower than the LbcWT group. BLP-fed mice also showed reduced Hsp60 expression than the control groups.

Conclusions: BLP feeding ameliorated gut inflammation, thus offering a potential dietary supplement for reducing gut-associated inflammation and improving gut health in livestock.

Keywords: next-generation probiotics, Listeria adhesion protein, inflammation

Materials and Methods

- Mice* (6-10 weeks old (♀, ♂) C57BL/6) were given 2% DSS *ad-libitum* in drinking water for 7 days to induce colitis.
- Daily metrics of body weight, hemoccult, fecal pellet physiology and health (fur ruffling, movement, behavior were assessed.
- After 7 days, DSS was pulled. The probiotic was fed (9-10 log) for 10 d.
- Water for the control groups (Naïve, DSS+H_2O) was given.
- On Day 17, mice were euthanized. Blood, organs, and feces were collected for analysis.

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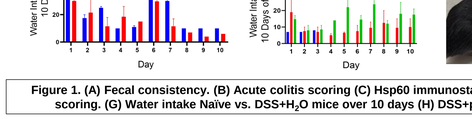
Timeline of DSS Treatment



DAI Scoring System

Disease Activity Index (DAI) Scoring System			
Score	Weight Reduction (%)	Stool Consistency	Hemoccult
0	0	Normal	Normal
1	1-5	Normal	Blood +
2	5-10	Loose	Blood ++
3	10-15	Loose	Blood +++
4	20 ≥ 15	Diarrhea	Gross bleeding

Treatment Groups

Treatment Groups	7 Days	10 Days
Naïve	H_20	H_20
DSS+H_20	2.0% DSS	H_20
DSS+LbcWT/Lbc^{Vec}	2.0% DSS	9-10 Log in H_20
DSS+LbcLap^{Lin} (BLP)	2.0% DSS	9-10 Log in H_20

Results

BLP Alleviates DSS-Induced Pathologies

Acute Colitis Score (ACS)	
Group	**Tissue Fragility**
Naïve	-
DSS+H_2O	+++
DSS+Lbc^{Vec}	++
DSS+LbcLAP^{Lin}	+

Figure 1. (A) Fecal consistency. (B) Acute colitis scoring (C) Hsp60 immunostaining. (D) Fecal tracking. (E) Fecal Lipocalin-2 ELISA. (F) Bristol Stool scoring. (G) Water intake Naïve vs. DSS+H_2O mice over 10 days (H) DSS+probiotic mice water intake over 10 days. (I) Mouse health tracking

Results

BLP Promote Mouse Recovery, Anti-Inflammatory Response and Extend Protective Benefits To DSS-Treated Mice

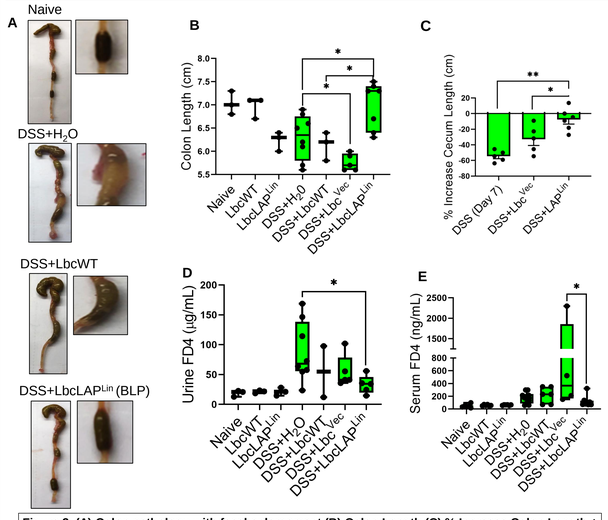


Figure 2. (A) Colon pathology with fecal enlargement.(B) Colon Length (C) % Increase Colon Length ± SEM. (D) FITC Translocation in urine ± SEM. (E) FITC translocation in serum ± SEM

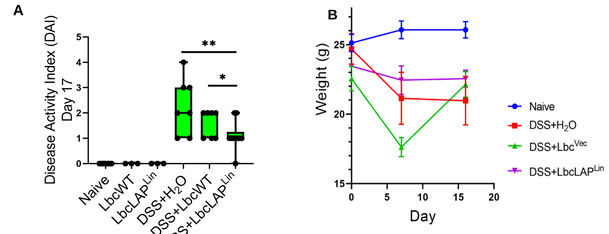


Figure 3. (A) Disease Activity Index. (B) Day 17 final mouse weights. ± SEM

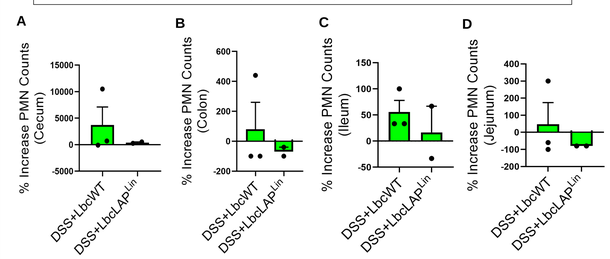


Figure 4. (A) % Increase PMN in Cecum. (B) % Increase PMN in Colon. (C) % increase PMN in Ileum. (D) % Increase PMN in Jejunum. ± SEM

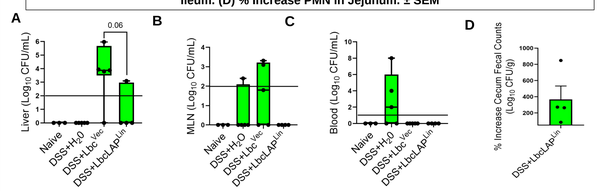


Figure 5. (A) Liver bacterial recovery. (B) MLN bacterial recovery. (C) Blood cultures. (D) Cecum fecal bacterial recovery. ± SEM

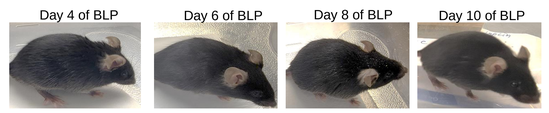


Figure 6. Progression of IBD mouse fed with BLP.

Background

Probiotic Interactions With Intestinal Epithelial Cells

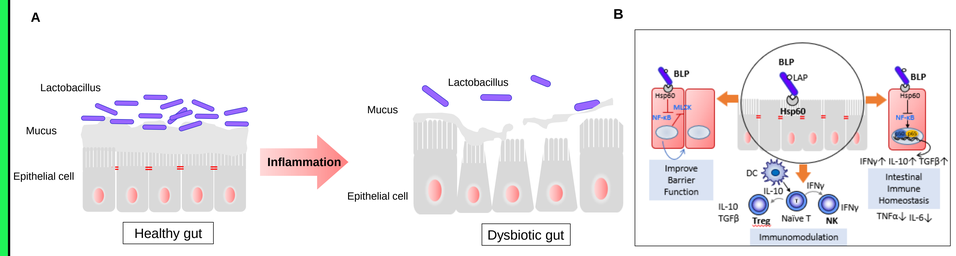
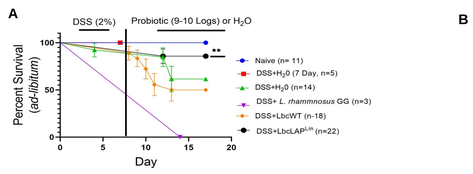


Figure 7. (A) Healthy vs. inflamed gut. (B) BLP-mediated healing.

Results

Survivability of DSS and Probiotic Treated Mice



B

BLP Prevents DSS (2%, *ad-libitum*)- Induced Mortality in Mice				
Group	**Total**	**Perished**	**Survived**	**% Survival**
Naïve	11	0	11	100
DSS+H_2O (Day 7)	5	0	5	100
DSS + H_2O	14	5.39	8.61	61.5
DSS+LGG	3	3	0	0
DSS + LbcWT	18	9	9	50
DSS + LbcLAP^{Lin} (BLP)	22	3.146	18.85	85.6

Figure 8. (A). Survivability of *ad-libitum* DSS and Probiotic fed mice. (B). *Ad-libitum* survival table.

Results

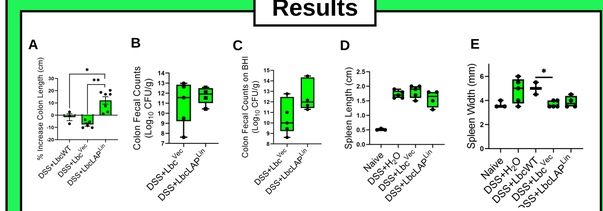


Figure 9. (A). % Increase in colon length from DSS+H_2O. (B). MRS colon fecal counts. (C). BHI colon fecal counts. (D). Spleen length. (E). Spleen width. (F) MLN invasion. (G). Blood cultures. (H). % Increase Bristol Score. (I) Fecal Isolation of probiotic. ± SEM

Caco-2 IBD *In-Vitro* Model

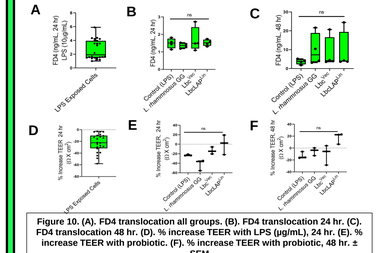


Figure 10. (A). FD4 translocation all groups. (B). FD4 translocation 24 hr. (C). FD4 translocation 48 hr. (D). % increase TEER with LPS (µg/mL), 24 hr. (E). % increase TEER with probiotic. (F). % increase TEER with probiotic, 48 hr. ± SEM

Microbiome Analysis

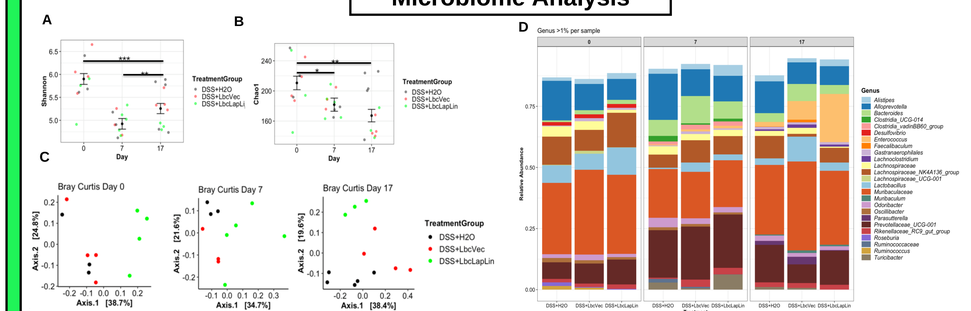


Figure 9. (A).Shannon Diversity. (B). Chao1 Index. (C) Bray Curtis. (D). Genus Level Taxonomy.

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