Engineering the gut microbiota to treat hyperammonemia

(*J Clin Invest*. 2015;125(7):2841–2850. doi:10.1172/JCI79214)

Increasing evidence indicates that the gut microbiota can be altered to ameliorate or prevent disease states, and engineering the gut microbiota to therapeutically modulate host metabolism is an emerging goal of microbiome research.

In the intestine, **bacterial urease** converts host-derived urea to ammonia and carbon dioxide, contributing to hyperammonemia-associated neurotoxicity and encephalopathy in patients with liver disease.

Here, we engineered **murine gut microbiota** to reduce urease activity. Animals were depleted of their preexisting gut microbiota and then inoculated with altered Schaedler flora (ASF), a defined consortium of 8 bacteria with minimal urease gene content.

This protocol resulted in establishment of a persistent new community that promoted a long-term reduction in fecal urease activity and ammonia production. Moreover, in a murine model of hepatic injury, ASF transplantation was associated with decreased morbidity and mortality. These results provide proof of concept that inoculation of a prepared host with a defined gut microbiota can lead to durable metabolic changes with therapeutic utility.

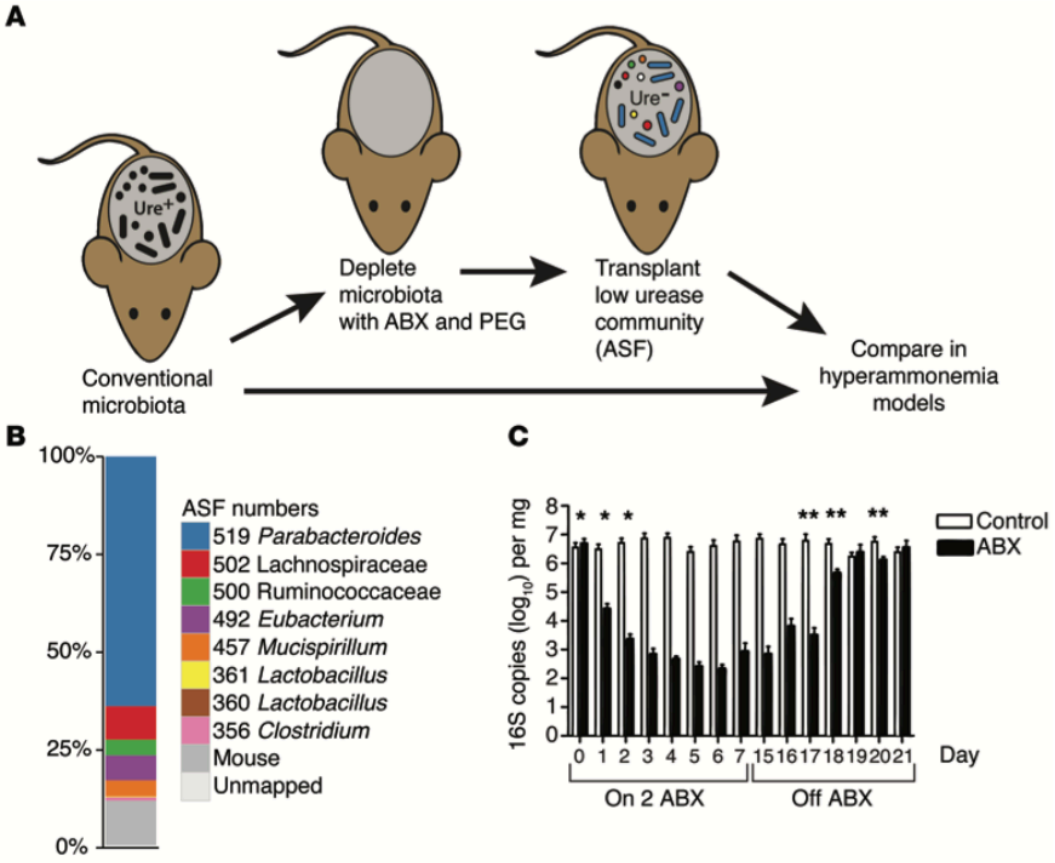


Figure 1. Transfer of ASF into a previously colonized murine host. (**A**) Diagram of the experimental method. Ure, urease. (**B**) Shotgun metagenomic analysis of stool from ASF-colonized animals used for gavage in this study. Proportions of the different ASF lineages and other organisms are indicated by the color key. (**C**) Time course of 16S rRNA gene copy numbers during oral ABX treatment (14 days, vancomycin and neomycin) and upon discontinuation of ABX on day 15 ($n = 3$ per group). *$P < 0.0001$, for days 0–2 compared with the average of days 5–15 in the ABX group; **$P < 0.05$, between the ABX and control groups. Paired-sample t test and 2-tailed Student's t test.

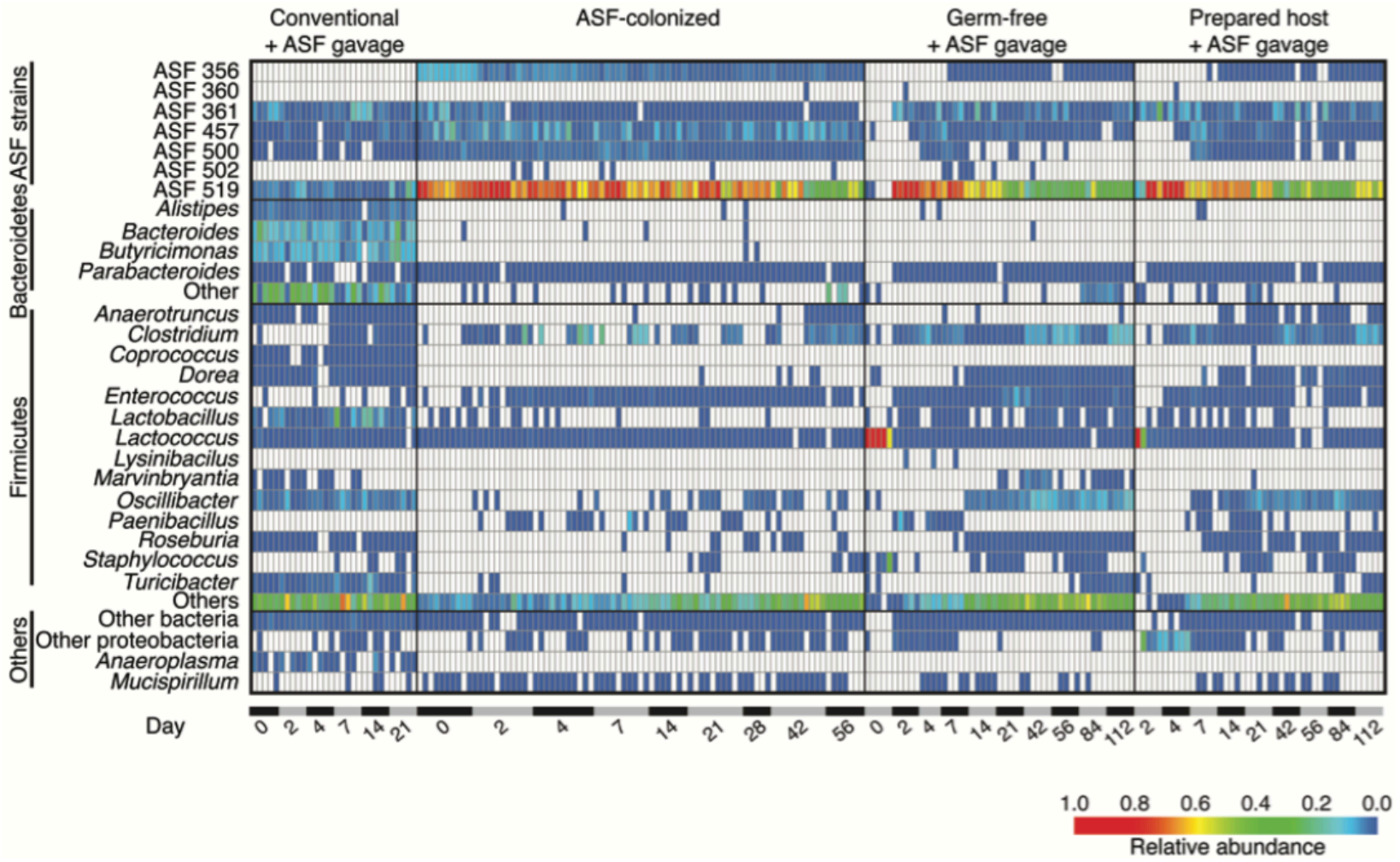


Figure 2. Heatmap showing the relative abundance of bacterial lineages over time in ASF-colonized mice and controls. Rows indicate bacterial lineages as annotated on the left. Relative abundance is indicated by the color key at the bottom of the figure. Columns summarize the sequencing results from individual fecal specimens. Elapsed time in days is shown along the bottom. The groups studied are indicated at the top of the heatmap and include (from the left) conventional mice that were gavaged with ASF stool without preparation (Conventional + ASF gavage); mice that were ASF colonized from birth, then transferred to a nonsterile SPF facility (ASF-colonized); mice that were germ-free, then gavaged with ASF (Germ-free + ASF gavage); and conventional mice that were prepared with ABX and PEG treatment, then gavaged with ASF (Prepared host + ASF gavage).

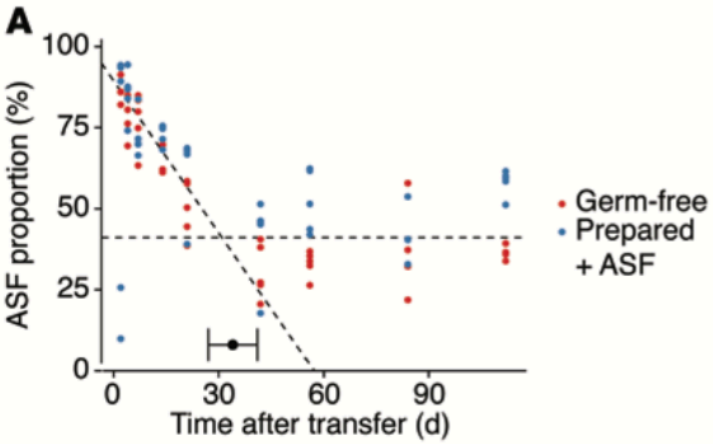


Figure 3. Development of a stable gut microbial community nucleated by inoculation with ASF. (**A**) Segmented regression analysis of communities in mice that were either germ-free or prepared conventional mice subjected to ASF gavage. The *y* axis shows the proportion of ASF lineages inferred from 16S rRNA gene tag pyrosequencing data. The *x* axis shows the number of days after transfer. Segmented regression analysis showed 2 phases, indicating a slow decline in the ASF proportion up to about day 30, followed by establishment of a new steady state consisting of approximately 40% ASF lineages.

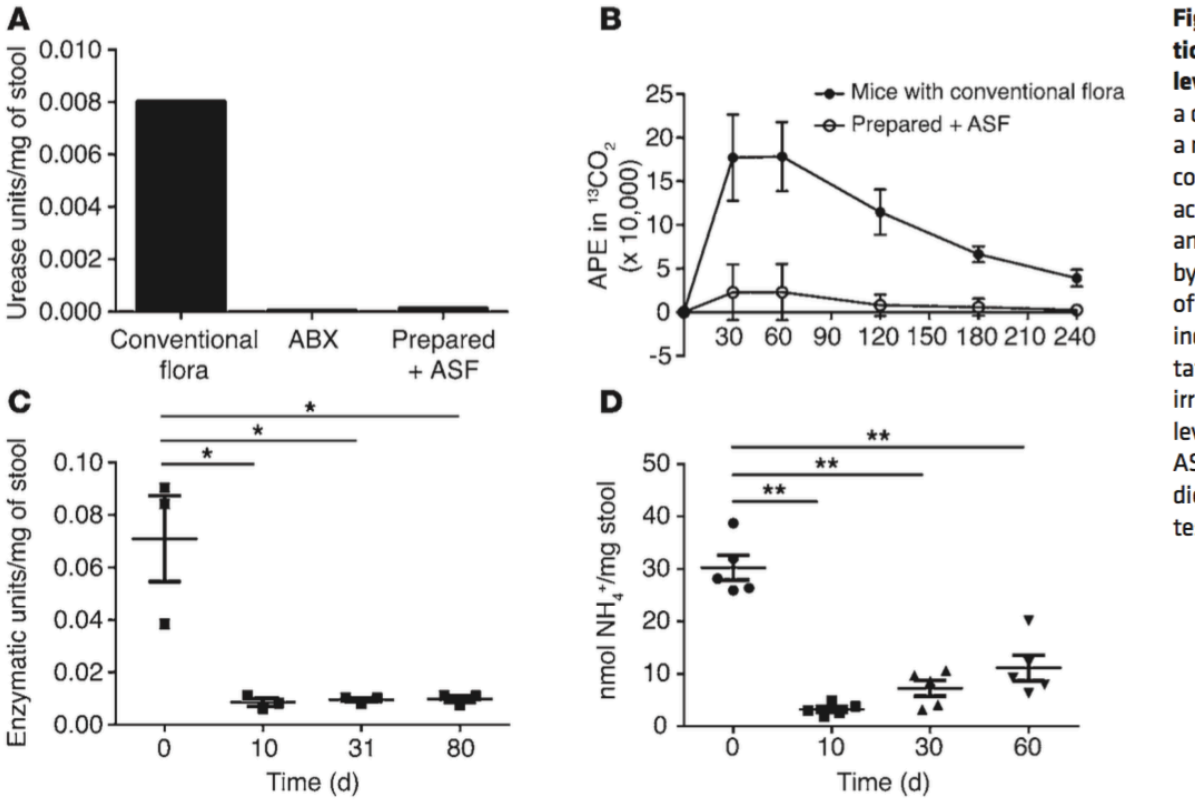
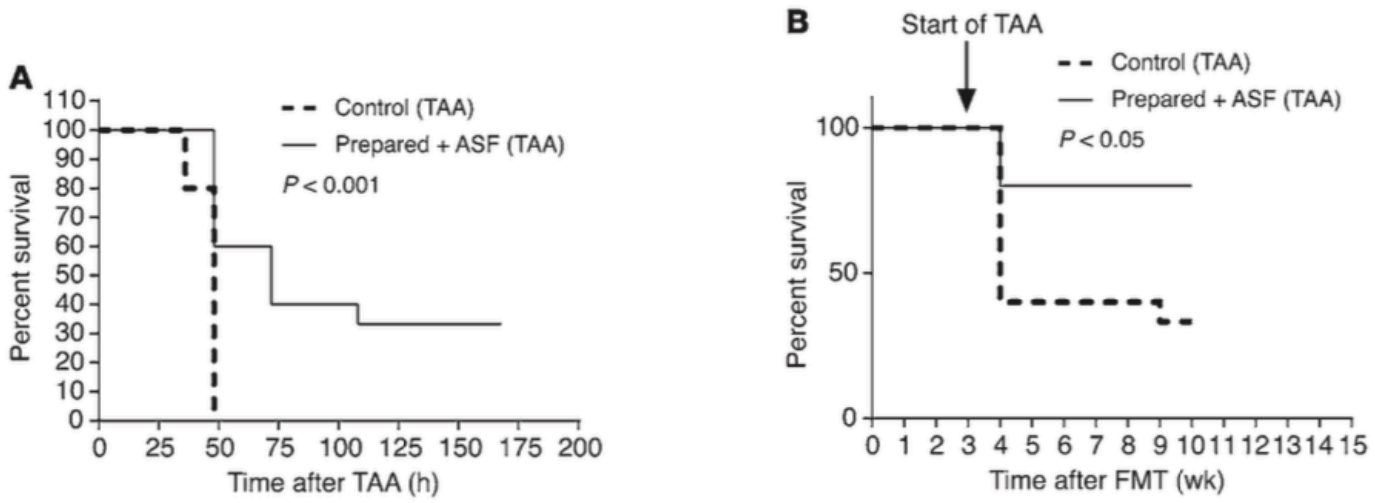


Figure 5. Transfer of ASF leads to a reduction in urease activity and fecal ammonia levels. (**A**) Urease activity in the feces of a conventionally housed mouse versus a mouse treated with ABX and a mouse colonized with ASF. (**B**) In vivo urease activity in conventionally housed (n = 5) and ASF-colonized mice (n = 5) quantified by the release of $^{13}CO_2$ after i.v. injection of ^{13}C-urea. (**C**) Fecal urease activity at the indicated time points after transplantation of ASF into prepared mice fed an irradiated diet (n = 3). (**D**) Fecal ammonia levels before and after transplantation of ASF into prepared mice fed a nonirradiated diet (n = 5). *P < 0.01; **P < 0.001. Tukey's test for multiple comparisons.

ASF transplantation reduces mortality and cognitive impairment in murine models of acute and chronic liver injury. A major cause of morbidity and mortality associated with acute liver injury is the development of HE. Since hyperammonemia is associated with the development of HE in patients with impaired hepatic function



ASF transplantation into prepared mice reduces mortality after thioacetamide-induced hepatic injury and fibrosis. (A) Kaplan- Meier survival curves of high-dose TAA-induced acute hepatic injury in conventional versus prepared/ASF mice (n = 15 per group). (B) Kaplan- Meier survival curves of chronic, thrice weekly TAA administration at low, escalating doses, initiated 3 weeks after ASF transplantation (n = 15 per group). Survival curves were analyzed by the Kaplan-Meier method using the log-rank test.