environmental microbiology

Environmental Microbiology (2016) **18**(6), 1720–1729 doi:10.1111/1462-2920.12841

Inoculation of tannin-degrading bacteria into novel hosts increases performance on tannin-rich diets

Kevin D. Kohl,*†‡ Ashley Stengel and M. Denise Dearing

Department of Biology, University of Utah, Salt Lake City, UT 84112, USA.

Summary

It has been hypothesized that herbivores host tannin-degrading bacteria (TDB) to overcome the toxic challenges posed by plant tannins. While TDB have been isolated from the guts of numerous mammals, their functional significance to their hosts has never been explicitly tested. We introduced TDB into lab rats, which do not host TDB, and measured host performance on tannin-rich diets. We first isolated three species of TDB, *Escherichia coli*, *Bacillus subtilis* and *Enterococcus faecalis*, from the guts of the desert woodrat (*Neotoma lepida*), which regularly feeds on tannin-rich plants. Then, we inoculated isolated TDB, as well as full woodrat microbial communities into laboratory rats. A control group was inoculated with sterilized woodrat faeces. Recipient lab rats were fed increasing concentrations of tannic acid, and we monitored tannic acid intake, body mass and liver damage as measured by serum alanine aminotransferase activity. Lab rats given TDB as isolates or full communities exhibited increased tannic acid intake, higher maintenance of body mass and lower indicators of liver damage compared with control animals. These differences were maintained when the trial was repeated after 6 weeks of feeding on tannin-free diets. Our results are the first to demonstrate that TDB significantly increase host performance on tannin-rich diets.

Introduction

Plants produce various plant secondary compounds (PSCs) to deter feeding by herbivores (Dearing *et al*., 2005). One class of PSCs, hydrolysable tannins, act to bind proteins and other macromolecules, limiting nutrient availability to animals (Glick and Joslyn, 1970b). At high doses, tannins can also cause damage to the kidneys, liver and gastrointestinal tract (Dollahite *et al*., 1962; Zhu *et al*., 1992). To overcome these challenges, many mammals host symbiotic microbes that can degrade hydrolysable tannins and are thought to enhance the consumption of tannin-rich diets (McSweeney *et al*., 2001; Goel *et al*., 2005).

The first tannin-degrading bacteria (TDB) were isolated from the guts of koalas (Osawa, 1990) and have since been isolated from the guts of numerous mammals (Osawa, 1992; Osawa and Sly, 1992; Nemoto *et al*., 1995; Osawa *et al*., 2000; Ephraim *et al*., 2005; Shimada *et al*., 2006; Goel *et al*., 2007; Dai *et al*., 2014). It is often speculated that these bacteria allow mammals to consume tannin-rich diets. However, to date there has not been an explicit study investigating whether these bacteria increase host performance on tannin-rich diets. We addressed this knowledge gap by isolating TDB from a wild herbivore that regularly feeds on tannin-rich plants and introducing these TDB into novel hosts, laboratory rats (*Rattus norvegicus*).

First, we isolated and characterized TDB from the faeces of a wild, herbivorous rodent, the desert woodrat (*Neotoma lepida*). The diet of southern populations of *N. lepida* is composed of up to 75% creosote bush (*Larrea tridentata*) (Karasov, 1989). Creosote is heavily defended with various PSCs (Mabry *et al*., 1977), including high levels of tannins (Hyder *et al*., 2002). Additionally, creosote resin induces tannase activity in fungal isolates (Treviño-Cueto *et al*., 2007; Ventura *et al*., 2008) and acts as a selective pressure on the bacterial community of the woodrat gut (Kohl and Dearing, 2012). Further, we have recently demonstrated that gut microbes are crucial in allowing woodrats to consume PSCs (Kohl *et al*., 2014b). Therefore we hypothesized that woodrats would host TDB with high ability to degrade tannins.

Next, we introduced isolated TDB and full woodrat microbial communities into a novel host species, the lab rat. Rats do not harbour TDB (Osawa and Sly, 1992; Nemoto *et al*., 1995), and they experience decreases in nutrient digestibility, loss of body mass, and liver damage when consuming tannin-rich diets (Glick and Joslyn,

Received 15 December, 2014; revised 28 February, 2015; accepted 3 March, 2015. *For correspondence. E-mail kevin.kohl@utah.edu; Tel. (+801) 585 1324; Fax (+801) 581 4668. Present addresses: †Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA; ‡Departamento de Bioquímica y Ciencias Biológicas, Universidad Nacional de San Luis, San Luis, Argentina.

Table 1. Identification of TDB isolates as determined by BLAST analysis.

Host animal number	Isolate	Identification
530	BI122	*Escherichia coli*
542	BI108	*Bacillus subtilis*
542	BI111	*Enterobacter cloacae*
542	BI112	*Bacillus subtilis*
542	BI117	*Escherichia coli*
569	BI120	*Bacillus subtilis*
584	BI136	*Enterococcus faecalis*
584	BI137	*Enterococcus faecalis*
625	BI139	*Enterococcus faecalis*

1970a,b; Galati *et al*., 2006). Laboratory rats and woodrats are in different taxonomic families and are estimated to have diverged roughly 22–25 million years ago (Steppan *et al*., 2004). We predicted that introduction of TDB would increase host performance on tannin-rich diets as measured by food intake, indicators of liver damage and maintenance of body mass. We performed two inoculation treatments by introducing isolated TDB or the full woodrat microbial communities via faecal transplants. If TDB only function in the context of their bacterial community, we predict that rats with the full woodrat microbial transplant would perform better than the group given only TDB isolates.

Results

Isolation of TDB from woodrats

We were able to detect TDB in faeces of 7 of the 10 woodrats tested using culture techniques. However, some cultures were lost in the process of subculture, and thus, only nine isolates from five hosts were identified and characterized. These isolates were identified as belonging to four species (Table 1). All isolates had > 99% sequence similarity to public 16S rRNA sequences within GenBank.

Tannase activity of isolates

We were unable to detect positive tannase activity in isolate 111, identified as *Enterobacter cloacae*, and therefore it was not included in statistics comparing tannase activity. Tannase activity of other isolates varied significantly by species identity ($F_{2,16} = 6.54$, $P = 0.008$; Fig. 1). Isolates identified as *Enterococcus faecalis* (isolates 136, 137 and 139) had consistently low but detectable tannase activity. *Bacillus subtilis* (isolates 108, 112 and 120) and *Escherichia coli* (isolates 117 and 122) exhibited consistently higher activity on average than isolates of *E. faecalis*. The activity of *E. coli* was remarkably similar between the two isolates. Within other species, isolates exhibited unique tannase activities [nested analysis of

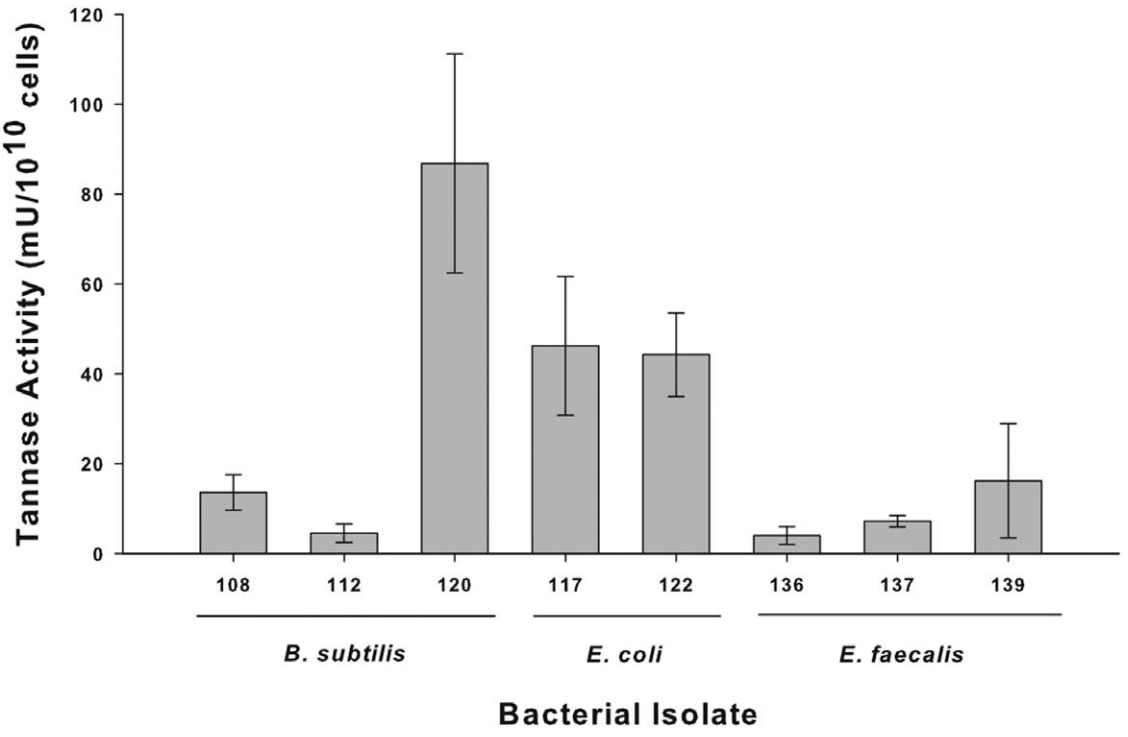

Fig. 1. Tannase activity of bacterial isolates from woodrat faeces. Bars depict means ± standard error of the mean.

variance (ANOVA): $F_{5,16} = 6.02$, $P = 0.003$]. This trend is best illustrated within *B. subitilis*, where isolate 120 demonstrated a tannase activity roughly 20 times higher than isolate 112.

Microbial transplants to novel hosts

We inoculated TDB into laboratory rats to create three experimental groups: a 'control' group with no introduced TDB, an 'isolates' group into which we inoculated three isolated species of TDB and a 'community' group into which we transferred the microbial community from woodrat faeces. For the 'control' group, faeces from two desert woodrats were collected, autoclaved, ground and mixed into a diet of powdered rat chow for a single night. For the 'isolates' group, we grew a mixed culture of bacterial isolates 120, 117 and 139 and introduced the culture to laboratory rats by mixing it in their diet. The 'community' group received ground woodrat faeces integrated into ground rat chow.

Microbial transplants significantly altered the resident microbial communities of recipient rats, as revealed by the microbial inventories. We compared microbial community membership (the presence and absence of microbial lineages) and microbial community structure (which takes relative abundances of taxa into account) using unweighted and weighted UniFrac distance metrics respectively. There were no detectable differences in microbial community membership or structure between control and isolate rats [analysis of similarities (ANOSIM) – Bonferroni-corrected *P*-values: community membership – $P = 0.89$; community structure – $P = 0.67$; Fig. 2], likely because of the fact that only three microbial species were introduced to the isolate group. The community group harboured a microbial community that was distinct in terms of membership and structure from both the isolates (community membership – $P = 0.003$; community structure – $P = 0.006$; Fig. 2) and control groups (community membership – $P = 0.009$; community structure – $P = 0.009$; Fig. 2).

The unique microbial community membership and structure of the community group is driven by differences in the relative abundances of several microbial taxa. We identified four bacterial phyla and seven genera that exhibited significant differences in relative abundances (Table 2). The genera *Anaerostipes*, *Bacteroides*, *Butyricimonas*, *Odoribacter* and *Ruminococcus* were enriched in the community group, while *Roseburia* was enriched in the isolates group. Interestingly, the genus *Mucispirillum*, a member of the phylum *Deferribacteres*, was completely absent from the community group (Table 2). We did not detect any differences in the relative abundances of the microbial genera that we introduced to the isolates group (*Bacillus*, *Enterococcus*, *Escherichia*).

The introduction of TDB significantly improved animal performance when feeding on tannic acid. Over the first 10 days of the trial, the isolates and community groups consumed roughly 11% more tannic acid compared with the control group ($F_{2,16} = 3.11$; $P = 0.07$; Fig. 3A). These groups were significantly different from the control group (post-hoc Student's t-tests: $P < 0.05$ for both comparisons; Fig. 3A). Despite higher toxin intake, these groups exhibited lower indicators of liver damage. Across the three groups, there was a trend for differences in measured alanine aminotransferase (ALT) activity ($F_{2,15} = 2.85$; $P = 0.09$; Fig. 3B). The community group exhibited lower ALT activity compared with the control group (Fig. 3B). There were no differences across groups in dry matter digestibility ($P = 0.91$) or water intake ($P = 0.36$).

Overall, groups that received TDB fared better in the trial by persisting longer than the control group on a 12% tannic acid diet (Fig. 4A; control versus isolate: $P = 0.026$; control versus community: $P = 0.021$). There was no difference in persistence between then isolates and community groups ($P = 0.83$). These differences were maintained when the trial was repeated after rats were fed standard rat chow for 6 weeks (Fig. 4B; control versus isolates: $P = 0.037$; control versus community: $P = 0.005$; isolates versus community: $P = 0.48$). Groups did not exhibit any differences in persistence between the two trials ($P > 0.3$ for all groups).

Discussion

Researchers have been isolating TDB from the guts of mammals for nearly 25 years (Osawa, 1990). Despite large interest in bacteria with these capabilities, their functional significance to the host remained unclear. We conducted a number of microbial transplants that demonstrated that TDB allow mammals to consume diets containing high concentrations of tannic acid.

We first isolated TDB from the guts of the desert woodrat. It is notable that we were able to isolate TDB because not all mammals host bacteria with this capability (Osawa and Sly, 1992; Nemoto *et al.*, 1995). A survey of 15 mammalian species was only able to isolate TDB from horses (Nemoto *et al.*, 1995). Although only a limited number of bacterial genera have been documented to exhibit tannase activity (Goel *et al.*, 2005; Aguilar *et al.*, 2007), our results are quite consistent with previous findings. Researchers have isolated tannin-degrading strains of *E. faecalis* from the faeces of goats (Goel *et al.*, 2007), isolates of *E. coli* from the rumen of white-tail deer (Nelson *et al.*, 1998) and species of *Bacillus* from soil (Mondal *et al.*, 2001). While *Bacillus* species are commonly thought of as soil bacteria, they have been isolated from a number of gut environments (Tam *et al.*, 2006; Stenfors Arnesen *et al.*, 2008).

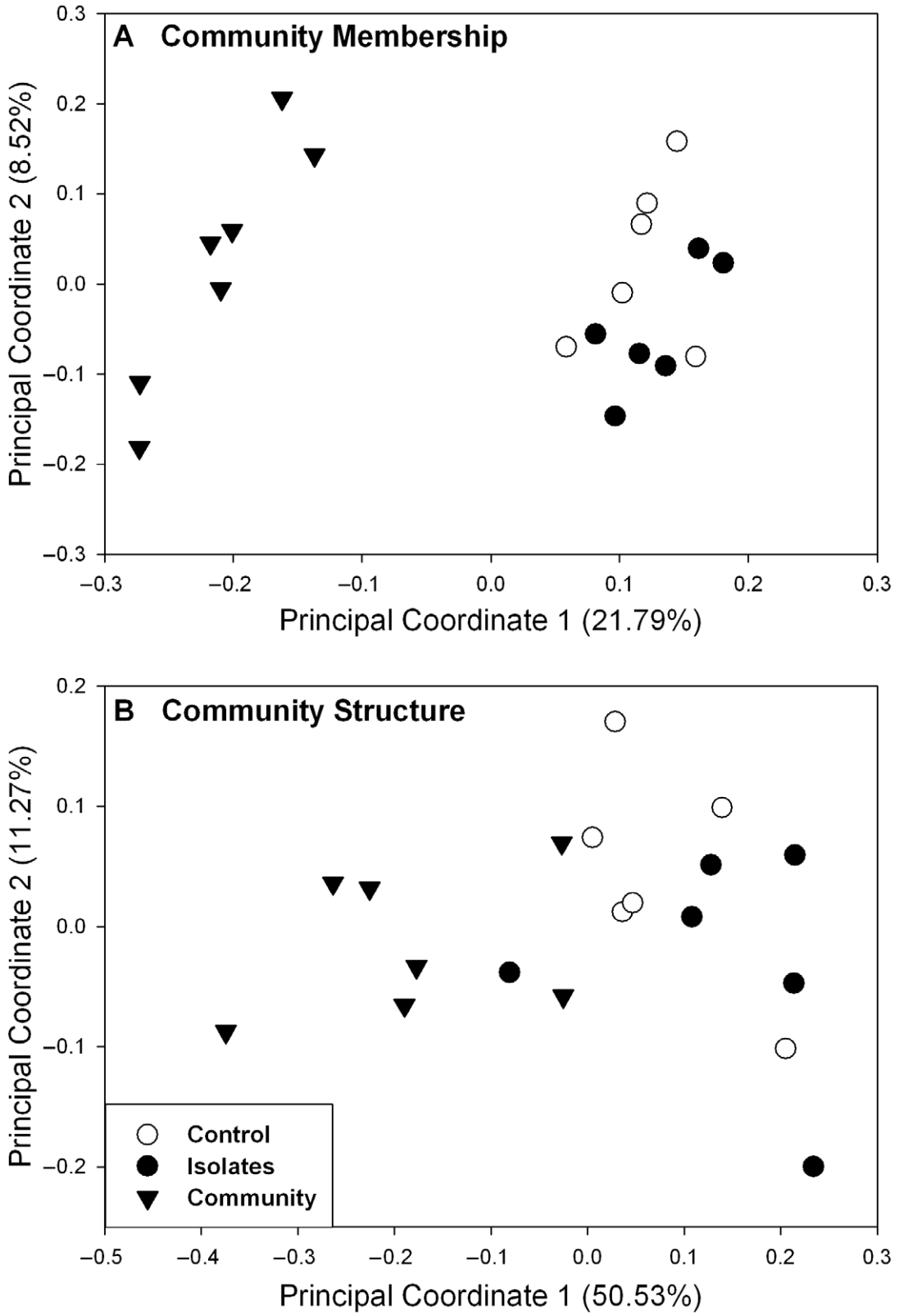

Fig. 2. Microbial community inventories. Principal coordinate analyses of (A) unweighted and (B) weighted UniFrac distances of microbial communities from recipient lab rats. Samples were collected on the third day of the 9% tannic acid diet. Several samples did not amplify properly and thus are not included.

Table 2. Relative abundances (percentage of community) of microbial phyla and genera that exhibited statistically significant differences between groups.

	Control	Isolates	Community	F-ratio	FDR-corrected *P*-value
Phyla					
Bacteroidetes	41.51 ± 4.04	53.13 ± 1.97	36.93 ± 3.54	21.51	0.0001
Deferribacteres	0.01 ± 0.005	0.01 ± 0.003	N.D.	17.96	0.0005
Elusimicrobia	< 0.00001	< 0.00001	0.63 ± 0.11	66.98	< 0.0001
Firmicutes	49.41 ± 5.41	36.85 ± 3.48	51.52 ± 2.16	8.21	0.045
Genera					
Anaerostipes	0.13 ± 0.04	0.10 ± 0.03	0.25 ± 0.04	20.06	0.0006
Bacteroides	3.80 ± 0.78	5.88 ± 1.99	17.04 ± 3.61	24.01	0.0001
Butyricimonas	0.02 ± 0.005	0.02 ± 0.008	0.07 ± 0.012	27.02	< 0.0001
Mucispirillum	0.01 ± 0.005	0.01 ± 0.003	N.D.	17.96	0.0013
Odoribacter	0.01 ± 0.002	0.03 ± 0.01	0.08 ± 0.03	14.56	0.006
Roseburia	0.22 ± 0.08	0.57 ± 0.18	0.09 ± 0.03	11.12	0.028
Ruminococcus	4.38 ± 1.03	4.28 ± 1.47	11.94 ± 3.35	9.71	0.048

F-ratios and FDR-corrected *P*-values were determined using transformed relative abundances.
FDR, false discovery rate; N.D., not detected.

We were unable to detect positive tannase activity in isolate 111, identified as *E. cloacae*. Some strains of *E. cloacae* exhibit tannase activity (Beniwal *et al*., 2010). However, other strains of *E. cloacae* form 'deceptive' zones of clearance during isolation because of alkaline dissociation of tannin-protein complexes (Nishitani and Osawa, 2005). We predict that alkaline dissociation is the mechanism for the formation of zones of clearance by woodrat-hosted *E. cloacae*, given our inability to detect positive tannase activity.

It is unusual that we were unable to isolate tannin-degrading members of the genus *Lactobacillus*. Members of this genus exhibiting tannase activity have been isolated using similar isolation methods from the faeces of humans (Osawa *et al*., 2000) and the Japanese wood mouse (Shimada *et al*., 2006). Moreover, *Lactobacillus* is a highly abundant genus in the woodrat gut (Kohl *et al*., 2011; Kohl and Dearing, 2012). The use of a growth medium that is more selective for *Lactobacillus* might reveal the presence of tannase-producing species.

We observed considerable variation in tannase activity between bacterial species and even between isolates of the same species. Previous studies documented similar results, with strains of *Lactobacillus plantarum* exhibiting 10-fold differences in tannase activity (Nishitani and Osawa, 2003). Although we observed this variation, it is difficult to assign biological importance to it for the host. Many physicochemical characteristics such as temperature, aeration, agitation speed, carbon sources and pH can affect tannase production by microbes (Aguilar *et al*., 2001; Banerjee *et al*., 2007). Although conditions were held constant for our analyses, they do not replicate gut conditions of herbivores. Thus, measured activities for species or isolates might not represent how the microbes perform *in situ*.

Introductions of novel microbes into naïve hosts significantly altered gut microbial community membership and

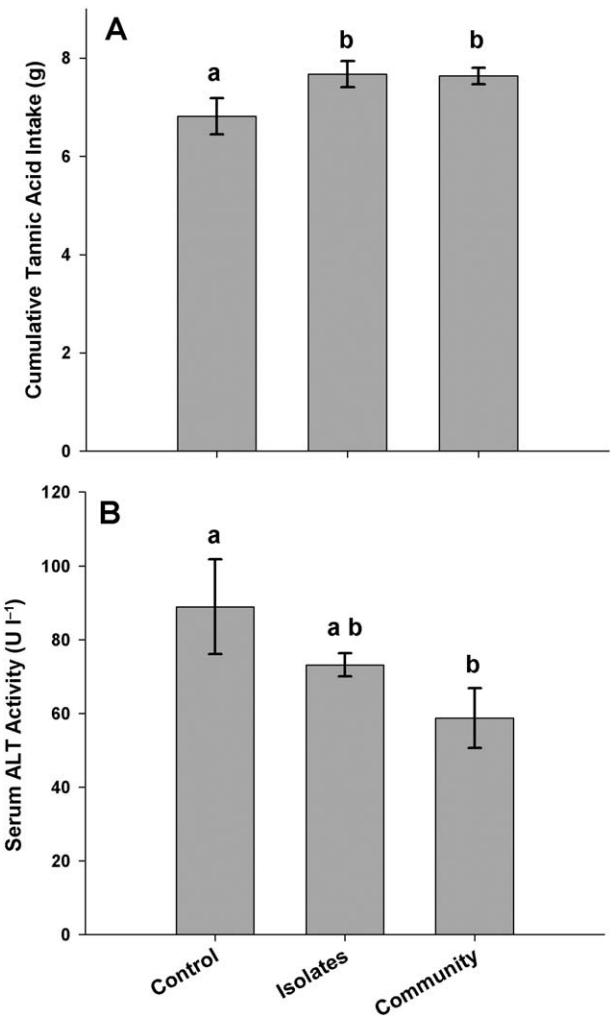

Fig. 3. Performance on tannin-rich diets. (A) Cumulative tannic acid intake over the first 10 days of the trial. (B) Serum alanine aminotransferase activity, measured on the third day of the 9% tannic acid treatment. Bars depict means ± standard error of the mean. Bars labelled with different letters are statically different using post-hoc Student's *t*-tests and corrected *P*-values.

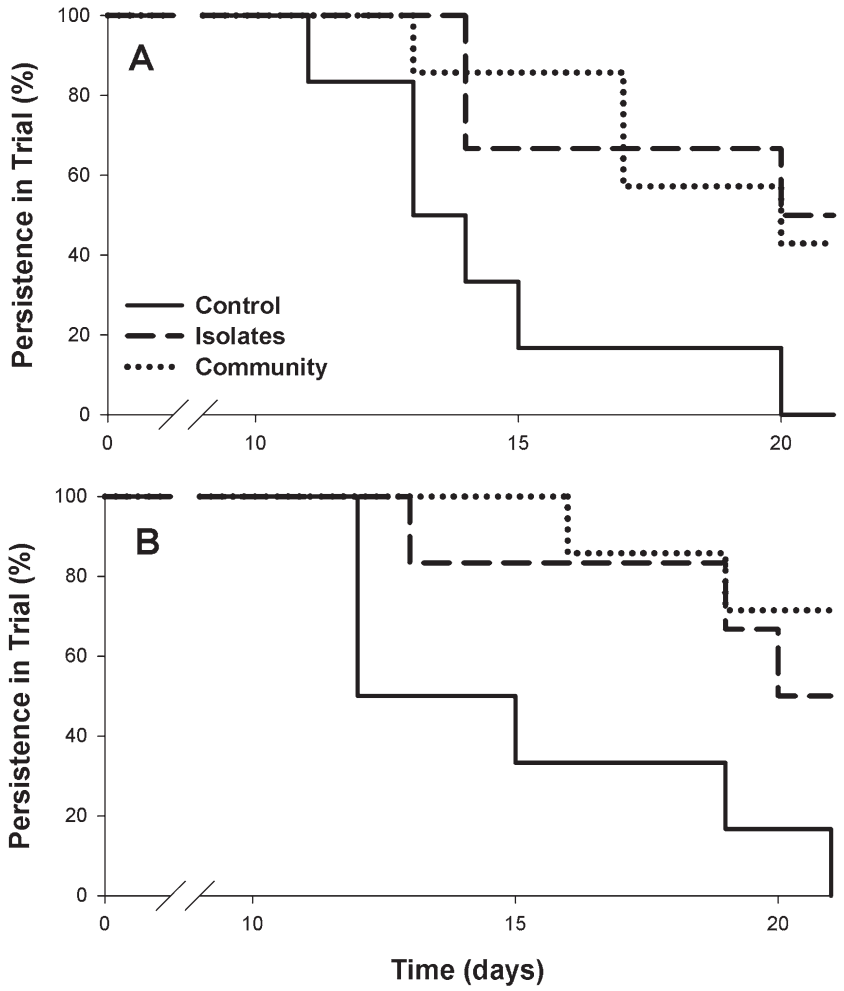

Fig. 4. Persistence of lab rats on tannin-rich diets. (A) Persistence following microbial transplants. (B) Persistence on tannin-rich diets after 6 weeks on a tannin-free diet. The rats were fed powdered rat chow with increasing amounts of tannic acid (3%, 6% and 9% for 3 days each from days 0–9) and then held on a diet of 12% tannic acid for 12 days (days 10–21).

structure. A majority of the differences we observed were taxa that were present in higher abundances in the community group. These differences are likely because of the introduction of a novel, complex, herbivore microbial community. For example, the genus *Ruminococcus*, which was roughly twice as abundant in the community group compared with others, is a dominant fermentative genus in herbivores (Nelson *et al*., 2003) and a common member of the woodrat faecal community (Kohl *et al*., 2011; 2014a). Interestingly, the microbial community of the isolates group was enriched in the phylum Bacteroidetes and the genus *Roseburia*. These results suggest that introduction of TDB can alter the microbial community dynamics in the gut. Similarly, the phylum Deferribacteres and the genus *Mucispirillum* were completely absent from the community group, suggesting that the complex woodrat microbiota resulted in extirpation of *Mucispirillum* from the guts of recipient rats, perhaps through competition or interference. Microbial transplants may be a useful system in which to study microbe–microbe interactions, community stability and other community dynamics.

Inoculations of TDB into novel hosts provided a thorough test of their effect on host performance. Rats receiving TDB as bacterial isolates or the full woodrat community were able to consume higher doses of tannic acid and exhibited lower indicators of liver damage. Further, rats with TDB persisted in the trial longer than control rats. These results demonstrate that TDB significantly increase host performance on diets containing tannic acid. Our results are the first test of this function since TDB were isolated nearly 25 years ago and suggest that the wide array of mammals that host TDB are aided in the consumption of tannin-rich diets (Osawa, 1992; Osawa and Sly, 1992; Nemoto *et al*., 1995; Osawa *et al*., 2000; Ephraim *et al*., 2005; Shimada *et al*., 2006; Goel *et al*., 2007; Dai *et al*., 2014). These symbiotic relationships would benefit hosts by allowing them access to tannin-rich plants in the absence of competitors. Further, it suggests that TDB may be useful in agricultural systems

to enhance animal performance on tannin-rich diets. However, it should be noted that our study focused on tannic acid, a hydrolysable tannin. Tannins can have a large diversity of chemical structures (Zucker, 1983; Khanbabaee and van Ree, 2001). Future studies could investigate the breadth of tannin diversity that TDB are able to assist mammalian hosts in consuming.

The rats in our experiments may have used various mechanisms to recognize and regulate the intake of tannic acid. One possibility is through bitter taste rejection, where compounds interact with bitter taste receptors and cause the animals to suppress feeding (Torregrossa and Dearing, 2009). We find it unlikely that bacteria interact and suppress the bitter taste response to allow feeding on tannins. Rather, we hypothesize that degradation of tannins by TDB limits systemic effects and allows animals to continue feeding. At high doses, tannins cause damage to the kidneys, liver and gastrointestinal tract (Dollahite *et al*., 1962; Zhu *et al*., 1992), which may cause animals to stop feeding due to nausea or gastrointestinal illness (Torregrossa and Dearing, 2009). This idea is supported by the fact that rats hosting TDB exhibited lower indicators of liver damage despite consuming higher doses of tannins.

Our results suggest that TDB are able to enhance animal performance in isolation and do not need to be introduced with an ecologically relevant microbial community. Gut microbes perform different functions for hosts in the context of various microbial communities and will often utilize the products of other microbes for their own metabolism (Samuel and Gordon, 2006). Because of the many ecological interactions that take place within the gut system, it could be hypothesized that TDB rely on the thousands of other bacterial members that reside in the woodrat gut to successfully degrade tannins for the benefit of the host. However, our functional tests did not detect differences between the rats given only bacterial isolates versus the more diverse collection of bacteria in the community group. These results hold promise for the possibility of developing a probiotic containing isolated TDB.

Additionally, the functions provided by bacteria transplanted into novel hosts were maintained over long time periods even in the absence of the selective force. The ability of lab rats to ingest tannic acid was unchanged 6 weeks after the microbial transplants. This result is even more remarkable because the animals were fed a diet free of tannins during that 6 week interval. The population dynamics of the gut are highly dynamic and many gut bacteria can be lost over time (Blaser and Falkow, 2009; Kohl and Dearing, 2014). If TDB are quickly lost from the gut, it would limit their practicality for use as a probiotic in agricultural animals. Our results demonstrate that these bacteria are retained in the guts of lab rats for at least 6 weeks post-inoculation. This result holds promise for future investigations into the function and retention of TDB in agricultural mammals.

Experimental procedures

Isolation of TDB from woodrats

Woodrats were collected in October 2010 near Lytle Ranch Preserve, Washington, UT (37°07′N, 114°00′W), using Sherman live traps. Animals were brought to the University of Utah Animal Facility, housed in individual cages ($48 \times 27 \times 20$ cm) and acclimated to laboratory conditions for 4 months (12:12 light : dark cycle, 22–28°C, 20% humidity). During this time, animals were fed rabbit chow (Harlan Teklad formula 2031). All procedures were approved by University of Utah's IACUC Protocol No. 12-12010.

Isolation of TDB was conducted following previously established protocols (Osawa, 1990). Ten animals were placed in empty, ethanol-sterilized cages for approximately 1 h, after which faeces were collected. Faeces were plated on Brain Heart Infusion agar (BHI agar; BD Diagnostics, Franklin Lakes, NJ USA) treated with tannic acid. Tannin-treated plates were prepared by adding 5 ml of a 2% tannic acid solution (tannic acid; Mallinckrodt Baker Chemical product 0380) to the surface of the BHI agar plates. The tannin solution was allowed to sit for 1 h, causing the agar to become opaque because of the formation of tannin-protein complexes. The tannin solution was removed, and plates were rinsed with 20 ml of water. Tannin-treated BHI agar plates were prepared immediately before plating faecal solutions. Faeces were weighed and homogenized for 30 s with nine parts saline (mass : mass). Faecal homogenate was diluted 10^{-3} using saline, and 200 µl of this solution was plated on tannin-treated BHI agar. Plates were then incubated in anaerobic conditions using the AnaeroPack system (Mitsubishi Gas Chemical, Tokyo, Japan) at 37°C for 5 days. Colonies that had created zones of clearance (by degrading tannin-protein complexes) were subcultured twice to ensure isolation of single species.

Identification of TDB

We classified bacterial isolates by sequencing the 16S rRNA gene. Isolates were grown overnight in BHI broth at 37°C, and DNA was isolated using a DNeasy Blood and Tissue Kit (QIAGEN). Bacterial 16S rRNA sequences were amplified using polymerase chain reaction (PCR) and universal primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), and PCR products were purified using a GeneJET Gel Extraction Kit (Fermentas). Purified DNA was Sanger sequenced at the University of Utah Core DNA Sequencing Facility using the 27F primer. Bases with Phred-quality values (Q) < 20 were trimmed from the ends. Sequences were compared with existing databases using BLAST, and archived in GenBank under Accession Numbers JX456079–JX456087.

Tannase activity of isolates

Tannase activity of isolates was measured using previously established techniques (Nishitani and Osawa, 2003).

Isolates were grown overnight at 37°C in BHI broth. Density of bacteria was estimated by diluting overnight cultures and counting cells with a Petroff-Hauser slide (the average of 15 viewing fields). Twenty-five microlitres of undiluted overnight culture was placed into 2.5 ml of 5 mM methyl gallate in 33 mM NaH_2PO_4 (pH 5.0), and these tubes were incubated at 37°C for 24 h. Then, 300 μl of the methyl gallate reaction mixture was added to 300 μl of saturated $NaHCO_3$ and incubated at 37°C for 2 h. The mixtures were centrifuged at 8000 × g for 20 s, aliquots were transferred to a 96-well plate and absorbencies were measured at 450 nm using a BioTek PowerWave HT microplate spectrophotomer (Broadview, IL, USA). Tannase activity (in $mU/10^{10}$ cells) was calculated by comparing absorbency values to a standard curve created using similar techniques and purified tannase enzyme from *Aspergillus ficuum* (Product 42395, Sigma-Aldrich, St Louis, MO, USA). Activity was measured three times for each isolate, using two technical replicates per measurement. Tannase activity was compared between isolates using a nested ANOVA using the identified species and isolate number as independent effects, with isolate number being nested within species.

Transplantation of TDB to novel hosts

We investigated the role of TDB in allowing hosts to feed on tannin-rich diets by transferring them to novel hosts. Outbred, female Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN, USA). Rats were housed in individual cages (48 × 27 × 20 cm), with a 12:12 light : dark cycle, average temperature of 22–28°C and 20% humidity. Rats were maintained a diet of standard rat chow (Harlan Teklad formula 2018).

We generated three groups of rats: a 'control' group with no introduced TDB, an 'isolates' group into which we inoculated three isolated species of TDB and a 'community' group into which we transferred the microbial community from woodrat faeces. For the 'control' group, faeces from two desert woodrats were collected, autoclaved, ground and mixed into a diet of powdered rat chow at a ratio of 15% w/w, and fed to control recipients for a single night. The autoclaved faeces were sterile, and thus this group did not receive any TDB. For the 'isolates' group, we grew a mixed culture of bacterial isolates 120, 117 and 139. These isolates exhibited the highest tannase activity for each bacterial species, as measured in the tannase assay. The culture was grown overnight at 37°C in 100 ml BHI broth. The cells were pelleted and reconstituted in 15 ml of phosphate-buffered saline and added to 150 g of powdered rat chow. The resulting diet was fed to the 'isolate' recipients for a single night to transfer these bacteria. The 'community' group received ground woodrat faeces integrated into powdered rat chow at a ratio of 15% w/w for a single night. Woodrat faeces contain a complex community with thousands of bacterial members (Kohl and Dearing, 2014). Sample sizes for the treatment groups were as follows: control – 6; isolates – 6; community – 7. The rats were then fed powdered rat chow with increasing amounts of tannic acid (3%, 6% and 9% for 3 days each) and then held on a diet of 12% tannic acid for 12 days.

16S rRNA inventories to monitor efficacy of microbial transplant

We inventoried the microbial communities of recipient rats to ensure success of microbial transplants. Faeces were collected on the third day of the 9% tannic acid diet treatment. Whole DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD, USA). The V4 region of the 16S rRNA gene was amplified with primers *515F* and *806R* and sequenced on an Illumina MiSeq platform (Caporaso *et al*., 2012). Sequences were analysed using the QIIME software package (Caporaso *et al*., 2010). Sequences underwent standard quality control and were split in to libraries using default parameters in QIIME. Sequences were grouped into operational taxonomic units (OTUs) using UCLUST (Edgar, 2010) with a minimum sequence identity of 97%. The most abundant sequences within each OTU were designated as a 'representative sequence' and aligned against the Greengenes core set (DeSantis *et al*., 2006) using PyNAST (Caporaso *et al*., 2009) with default parameters set by QIIME. A PH Lane mask supplied by QIIME was used to remove hypervariable regions from aligned sequences. FastTree (Price *et al*., 2009) was used to create a phylogenetic tree of representative sequences. OTUs were classified using the Ribosomal Database Project classifier with a standard minimum support threshold of 80% (Wang *et al*., 2007). Sequences identified as chloroplasts or mitochondria were removed from the analysis. We compared community membership (presence or absence of lineages, and not their relative abundances) and community structure (which incorporates relatives abundances of taxa) by calculating unweighted and weighted distances respectively. UniFrac distances measure diversity shared between samples by determining the fraction of shared branch length in the phylogenetic tree created from all representative sequences (Lozupone and Knight, 2005). We then conducted principal coordinates analysis on unweighted and weighted UniFrac scores using a random subset of 30 000 sequences per sample to investigate similarities. Group differences were tested using the pairwise comparisons of the three groups using the ANOSIM function within QIIME. *P*-values were corrected using the Bonferroni correction. Relative abundances of microbial phyla and genera were normalized using a variance stabilizing transformation of $\arcsin(\text{abundance}^{0.5})$ (Shchipkova *et al*., 2010; Kumar *et al*., 2012) and compared using ANOVAs. We corrected *P*-values with the false discovery rate correction. All sequences were deposited in the Sequence Read Archive under Accession SRP045299.

Measuring host performance on tannin-rich diets

We monitored various physiological parameters of recipient lab rats challenged with tannic acid. Animals were placed in metabolism cages that allowed collection of urine and faeces. As stated earlier, rats were exposed to increasing concentrations of tannic acid in the diet (0%, 3%, 6% and 9% for 3 days each, and then held on a diet of 12% tannic acid for 12 days). We measured food intake and water intake daily. Faeces were also collected daily and dried at 45°C overnight and weighed. We calculated dry matter digestibility as the (grams food intake – grams faeces output)/grams food intake. Cumulative tannic acid intake was calculated by multiplying the

amount of food consumed each day by the concentration of tannic acid in the diet and summed over the first 10 days (up to one day on 12% tannic acid, i.e., before any animals dropped out of the trial). We measured body mass daily and removed animals from the trial when they lost more than 10% of their original body mass.

We also measured the activity of ALT in the serum as an indicator of liver damage. This enzyme is primarily produced in the liver, but as liver cells rupture the activity is detectable in the serum. Blood samples were collected on the last day of the 9% tannic acid diet. Blood samples were allowed to sit for 30 min, after which they were centrifuged for 15 min at 3000 × g. The serum was removed and frozen at −80°C. ALT activity was measured using a commercial ALT activity assay kit (Sigma-Aldrich MAK052). We were unable to obtain a sufficient blood sample from one individual in the community group, and thus the sample size for ALT activity was $n = 6$ instead of $n = 7$.

Water intake and dry matter digestibility were compared using repeated measures ANOVA. Cumulative tannic acid intake and ALT activity were compared using one-way ANOVAs. Differences between groups were tested with Student's *t*-tests and correcting *P*-values. Persistence in the trial was compared using a log-rank Kaplan–Meier survival analysis.

Maintenance of microbial function

We investigated the maintenance of microbial function by placing all rats on a control diet of basic rat chow for 6 weeks. Then, we conducted the trial as before without repeating the microbial transplant. Rats were fed increasing concentrations of tannic acid, and we monitored body mass. Animals were removed from the trial when they lost more than 10% of original body mass. Persistence curves were compared using a log-rank Kaplan–Meier survival analysis.

Acknowledgements

This study was funded by grants from the National Science Foundation (Graduate Research Fellowship to K.D.K, IOS 0817527 to M.D.D., DEB 1210094, to M.D.D. and K.D.K., DEB 1342615 to M.D.D. and DBI 1400456 to K.D.K.).

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