Journal of Economic Entomology, 2016, 1–7
doi: 10.1093/jee/tow012
Research article

Apiculture and Social Insects

Parasaccharibacter apium, gen. nov., sp. nov., Improves Honey Bee (Hymenoptera: Apidae) Resistance to *Nosema*

V. Corby-Harris,[1,2,3] L. Snyder,[1] C.A.D. Meador,[1] R. Naldo,[1] B. Mott,[1] and K. E. Anderson[1,3]

[1]USDA-ARS Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, Arizona, USA, 85719 (v.corbyharris@gmail.com; lucysnyder@email.arizona.edu; meadorcad@aol.com; rnaldo12@gmail.com; brendon.mott@ars.usda.gov; kirk.anderson@ars.usda.gov), [2]Corresponding author, e-mail: v.corbyharris@gmail.com, and [3]Department of Entomology, University of Arizona, Forbes 410, PO Box 210036, Tucson, Arizona, USA 85721

Received 18 November 2015; Accepted 15 January 2016

Abstract

The honey bee, *Apis mellifera* L., is host to a variety of microorganisms. The bacterial community that occupies the adult worker gut contains a core group of approximately seven taxa, while the hive environment contains its own distribution of bacteria that is in many ways distinct from the gut. *Parasaccharibacter apium*, gen. nov., sp. nov., is a hive bacterium found in food stores and in larvae, worker jelly, worker hypopharyngeal glands, and queens. *Parasaccharibacter apium* increases larval survival under laboratory conditions. To determine if this benefit is extended to colonies in the field, we tested if *P. apium* 1) survives and reproduces in supplemental pollen patty, 2) is distributed throughout the hive when added to pollen patty, 3) benefits colony health, and 4) increases the ability of bees to resist *Nosema*. *Parasaccharibacter apium* survived in supplemental diet and was readily consumed by bees. It was distributed throughout the hive under field conditions, moving from the pollen patty to hive larvae. While *P. apium* did not significantly increase colony brood production, food stores, or foraging rates, it did increase resistance to *Nosema* infection. Our data suggest that *P. apium* may positively impact honey bee health.

Key words: Apis mellifera, honey bee, probiotic, Parasaccharibacter apium, Nosema

The European honey bee (*Apis mellifera* L.) contributes substantially to the worldwide economy because they pollinate much of our food supply (Morse and Calderone 2000). Unfortunately, honey bees are in a period of substantial decline that threatens national and international food security. Their decline is likely owing to a confluence of factors including disease, parasitism, queen failure, malnutrition, pesticides, and anthropogenic factors (Johnson et al. 2010, 2013; van Engelsdorp et al. 2010, 2012; Spleen et al. 2013; United States Department of Agriculture [USDA] 2013; Gallant et al. 2014; Simon-Delso et al. 2014; Steinhauer et al. 2014; Cariveau and Winfree 2015; Vaudo et al. 2015). With respect to parasitism, much of the focus has centered on the mite *Varroa destructor* Anderson & Trueman and *Nosema* disease. Both are consistently among the most important factors leading to colony losses (Higes et al. 2008, Rosenkranz et al. 2010, van Engelsdorp et al. 2012, Spleen et al. 2013, Steinhauer et al. 2014). *Nosema* are intracellular parasites that infect, grow, and reproduce in the gut of the bee, damaging the gut tissue. Nosemosis presents a formidable challenge to beekeepers and may cause even more damage than previously thought, possibly because the heartier, more cryptic, and more virulent species *Nosema ceranae* has nearly or fully displaced *Nosema apis* in bee colonies worldwide (Higes et al. 2007, Huang et al. 2007, Martín-Hernández et al. 2007, Paxton et al. 2007, Chen et al. 2008).

While many of the threats to bee heath will require a multifaceted approach to resolve, nutrition is a common denominator that may positively impact how bees respond to other stressors. The benefits of improved nutrition can be measured by adding dietary supplements when forage is scarce, but these supplements are often a poor substitute for the natural pollens that bees derive their proteins and lipids from (DeGrandi-Hoffman et al. 2015). By improving the nutritional value of bee supplements, we might increase both colony health and food security. This may be achieved by adding back components of natural pollen and nectar that bees might derive a benefit from, including bacteria found in food stores and plant nectar (Anderson et al. 2013, Corby-Harris et al. 2014b).

Parasaccharibacter apium (Acetobacteraceae Alpha 2.2) is a honey bee hive bacterium that occupies niches such as larvae, brood food, hypopharyngeal glands, crop, and food stores (Anderson et al. 2013, 2014; Vojvodic et al. 2013; Corby-Harris et al. 2014a, b). It is also prevalent in the guts of mature queens (Kapheim et al. 2015, Tarpy et al. 2015). *Parasaccharibacter apium* appears to be specific to bees in the genus *Apis* that provision their young and the queen with hypopharyngeal and mandibular gland secretions

(Corby-Harris et al. 2014b). It, therefore, follows the flow of nutrients in the hive beginning with nurse bees that consume *P. apium*-laden food stores to provision larvae with inoculated brood food and queens with inoculated royal jelly. Certain strains of *P. apium* also increase the health of honey bee larvae in vitro by increasing the likelihood that larvae survive to pupation (Corby-Harris et al. 2014b). Because *P. apium* is a natural hive bacterium that might benefit bees, it is ideally suited for further study on its effects at the colony level.

Any bacterium that is evaluated for improving colony heath should be naturally found in hives or in bees, distributed throughout the colony, easily administered, and beneficial to colonies. Surprisingly, few candidates have been tested to determine whether they fit all or some of these criteria. Audisio and Benitez-Ahrendts (2011) showed that *Lactobacillus johnsonii* isolated from honey bee guts and fed to colonies in sugar water increased colony brood area, adult population, and honey storage. Bacteria foreign to the bee have also been evaluated, leading to negative or negligible effects on hive health (Johnson et al. 2014, Ptaszyńska et al. 2015), perhaps because they do not normally live with bees. Many more valid candidates have been uncovered with continued study of bee-associated microbial communities (Moran 2015, Schwarz et al. 2015). Here, we investigate the utility of a natural hive bacterium, *P. apium*, for improving supplemental feed and colony health. We test four main hypotheses: 1) *P. apium* survives in supplemental feed, 2) *P. apium*-inoculated feed added to colonies increases the levels of viable *P. apium* in larvae, 3) *P. apium* supplementation increases colony brood area, food stores, and foraging rate, and 4) *P. apium* supplementation increases resistance to *Nosema*. Apart from evaluating *P. apium* in particular, we hope to spark a broader discussion of how bacteria should be evaluated for the purpose of improving bee health.

Materials and Methods

Bees

Forty-nine 3-lb. packages of bees containing one open-mated queen from C.F. Koehnen and Sons in Glenn, CA, were installed in April of 2014. All packages were initially provided with four frames of foundation, two frames of drawn comb, and two food frames containing stored pollen and honey. An inside feeder was also provided. The hives were housed in seven greenhouses at the Carl Hayden Bee Research Center (CHBRC) in Tucson, AZ. Each greenhouse was equipped with a cooler to reduce the temperature in the top of the hive during the hottest part of the year (June and July, average high temperature = 33.6°C, average low temperature = 25.1°C). The entrance of each hive was affixed with a flexible plastic tube to allow bees to enter or exit the hive. The hives were randomly assigned treatments within each greenhouse so that each greenhouse contained an equal number of hives exposed to the experimental and control treatments (Fig. 1).

Parasaccharibacter apium, gen. nov., sp. nov., Culture

Parasaccharibacter apium was isolated as part of a previous study (Corby-Harris et al. 2014b) from second-instar larvae sampled from hives at the CHBRC in June of 2013. Three strains that were tested in Corby-Harris et al. (2014b) were tested for their survival in pollen patty: A29, B8, and C6. Subsequent hive-level experimental treatments consisted only of strain C6. In all cases, the strains grew to log phase in liquid Sabouraud Dextrose growth medium (SDM) at 34°C under low-oxygen (5% CO_2) conditions for 48 h before being used in experiments.

Parasaccharibacter apium Survival in Pollen Patty

Patties of natural pollen were prepared by mixing equal parts of drivert sugar, granulated sugar, and loose dry (unsterilized) corbicular pollen (Great Lakes Pollen, Bulkfoods.com) and water to the consistency of a thick batter. Twelve gram patties were inoculated with ~23,000 colony-forming units (CFUs) of *P. apium* strains A29, B8, or C6 in SDM, *Escherichia coli* strain DH4α in liquid Luria-Bertani medium, or a negative control containing sterile SDM with no bacteria (uninoculated patties). The inoculum was thoroughly mixed into the patty by hand, and the patties were then incubated at either 27 or 35°C under atmospheric conditions. Five replicate patties were made for each temperature × bacteria combination, yielding 50 patties total. At 24 h and again at 48 h, three cores were sampled from each patty using a straw. Each pooled sample containing three cores was ~0.85 g, corresponding to about 1/14th of the 12-g patty. These cores were placed into 5 ml of sterile SDM, vortexed briefly, and spun down at 4,000 rpm for 10 s to remove the pollen patty particulates. Two hundred microliters (1/25th) of the supernatant was then plated and then incubated at 34°C under low-oxygen (5% CO_2) conditions, the preferred growth conditions for *P. apium*. Therefore, 1/350th of the initial inoculum (1/14th of the 12 g patty × 1/25th of the cores washed in SDM) was plated onto each replicate plate. After 48 h, the number of CFUs was counted for each plate, and an average number of CFUs per treatment combination was calculated. There were three plates for each temperature × time × bacteria combination, yielding a total of 100 plates (2 temperatures × 2 time points × 5 bacteria × 5 patties). The experiment was repeated twice. Counts were square root transformed to improve normality and analyzed with an ANOVA model that included the effect of bacteria, time, temperature, and all associated two- and three-way interactions. The two trials were analyzed separately. A Tukey's HSD test was used to determine whether there were significant differences in CFU count between the factors tested.

Hive-Level Bacterial Treatments

We focused on *P. apium* strain C6 for further testing of *P. apium* in the hive. Inocula were prepared ahead of time from the same source culture as above. The number of bacteria in 100 µl of SDM was determined by plating out 100-µl aliquots of the source culture and counting the number of CFUs after 48 h as described earlier. Multiple 500-µl tubes of this source inoculum were prepared at a concentration of ~29 million bacteria per tube and included glycerol at a final concentration of 20%. The tubes were then stored at −80°C. This same source inoculum was used throughout the duration of the hive treatments. The identity and purity of the inoculum was confirmed by sequencing the 16S rDNA gene sequence of the sample as described in Corby-Harris et al. (2014b). Each week between 13 June 2014 and 13 April 2015, one 500-µl tube of inoculum was removed from the −80°C, warmed, and 500 µl of fresh SDM with 20% glycerol was added. Twelve-gram pollen patties were placed in a small weighing dish and were scored with a pipette (Fig. 1). After thoroughly mixing the bacteria and fresh media, 100 µl of this revived inoculum (corresponding to ~2.9 million CFUs) was spread onto the surface of each 12-g pollen patty for the treatment hives ($N = 25$) and 100 µl of sterile SDM was spread onto each 12-g pollen patty for the negative control colonies ($N = 24$). The inoculum was spread onto the patty surface to maximize delivery of the treatment if the bees did not consume the entire patty.

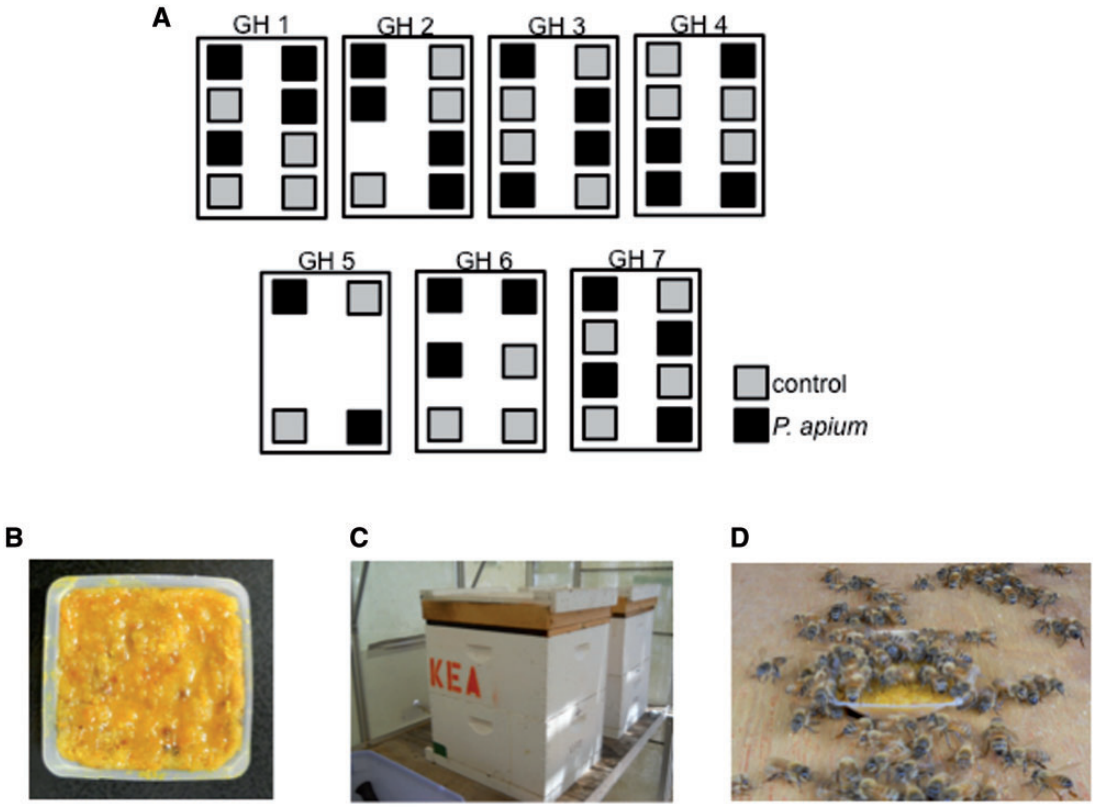

Fig. 1. Experimental design and pollen patty inoculum. (**A**) Forty-nine hives were inoculated with either *P. apium*-laden pollen patty ($N = 25$) or a negative control inoculated with sterile liquid growth culture ($N = 24$). The hives were housed in seven greenhouses, and the treatments were evenly and randomly assigned to hives within each greenhouse. (**B**) Twelve-gram patties containing natural pollen were inoculated on the surface with either *P. apium* or sterile broth. (**C**) The hives were housed in greenhouses, and the treatment was placed in a riser on top of the hive body but below the top cover. (**D**) Bees readily consumed the treatment and the control.

Patties were placed into the hive on a top riser (Fig. 1) for ~96 h and were weighed before and after use to determine how much of the treatment was consumed by the hive each week.

Parasaccharibacter apium in Larvae

Seven weeks into *P. apium* supplementation, the number of *P. apium* in the larvae of a subset of hives was counted. Eleven colonies (seven control and four treated) of only the strongest colonies in the experiment were used. All were of equal size and strength and contained >7 frames of adults and >2 frame faces of brood. From each hive, 100 second- and third-instar larvae were collected and pooled in 500 µl physiological saline (0.9% NaCl in distilled water). Each sample was homogenized, and 100 µl of the solution was plated in triplicate on Sabouraud Dextrose agar plates. The plates were then incubated at 34°C under low-oxygen (5% CO_2) conditions. After 48 h, the number of CFUs on each plate was counted, plates that were deemed too dense with bacteria to count were given a value of 1,000 CFUs per plate, and the three plates per hive were averaged. The mean number of CFUs per plate averaged over the three plates was analyzed using a nonparametric Mann–Whitney or Wilcoxon test.

Colony Measures of Brood, Food Stores, and Foraging Rates

For each hive, the brood area, honey stores, and pollen stores were measured every three weeks between June and November 2014 and three times again in March and April of 2015. Brood area and food stores were coarsely estimated as the quarters of a frame face containing brood, honey, or pollen, or that were empty or undrawn. In March, we assessed the survival of the hives from the previous November. At the final hive assessment in April, we also determined which hives contained at least eight frames of adult bees, the number of bees needed to "grade" for pollination services. Foraging rate was determined every other week by counting the number of foragers entering each hive for one minute in the midmorning and again in the midafternoon.

Nosema Challenge

Sealed brood frames from all *P. apium*-inoculated and control colonies were collected into two hive boxes, with each hive box containing frames from either *P. apium*-supplemented or control colonies. The hive boxes were then covered and placed in a temperature-controlled room (33 ± 1°C) and bees from all of the frames emerged overnight and mixed. The <18-h-old newly emerged bees were then collected and subjected to *Nosema* challenge.

The morning of the inoculations, *Nosema*-infected bees were collected from the entrance of a hive that was not part of the experiment (Fries et al. 2013). Their midguts were dissected into distilled water, and the concentration of spores per gut was determined using a hemocytometer (Hausser Scientific, Horsham, PA) according to Cantwell (1970). A subsample of the solution was aliquoted into a separate tube and centrifuged for 60 s to isolate the spores. The supernatant was removed and replaced with 50% sucrose to a

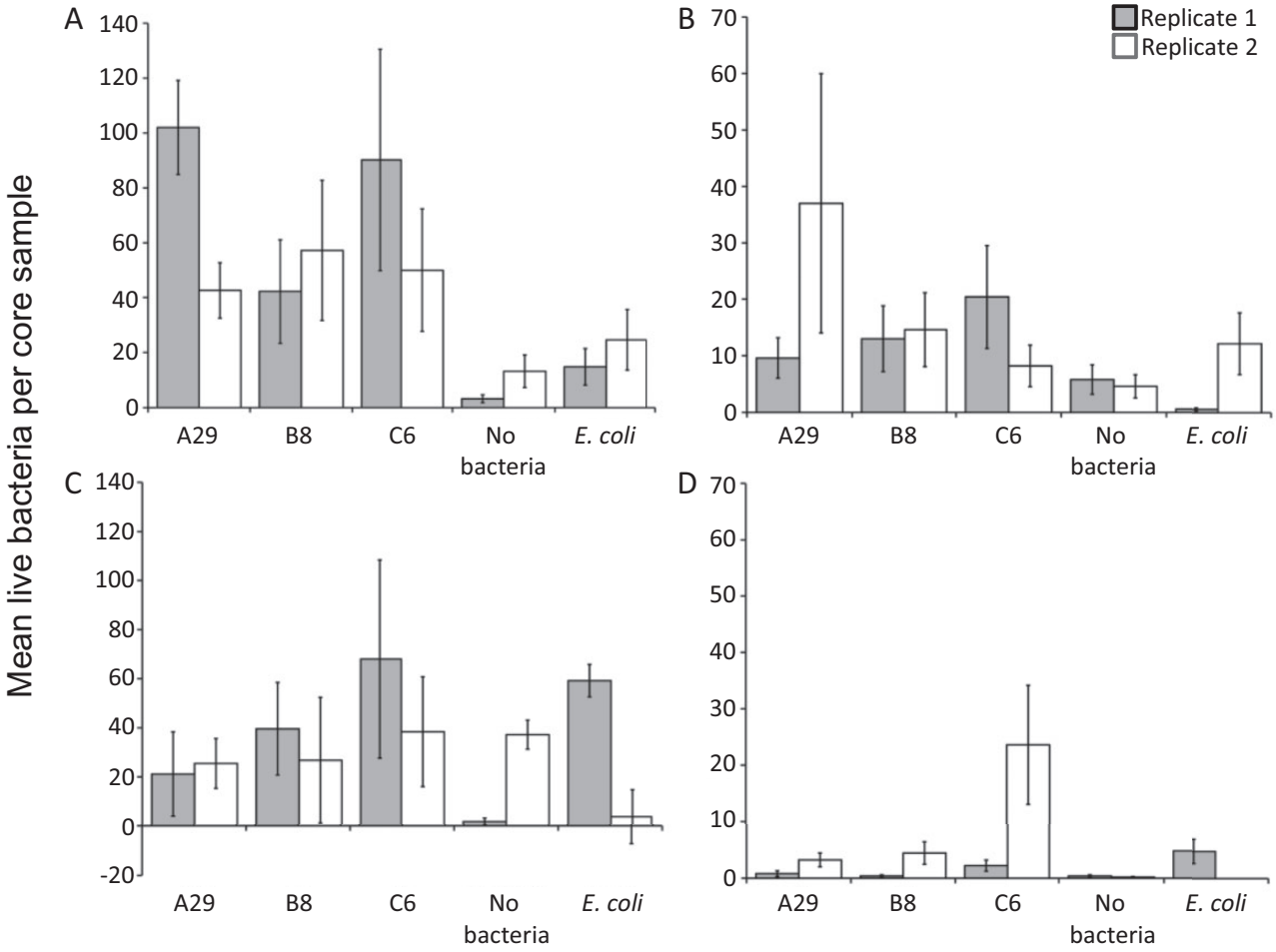

Fig. 2. The number of live bacteria recovered from pollen patty cores supplemented with three types of *P. apium* (A29, B8, or C6), *E. coli*, or sterile broth. Patties were incubated at two different temperatures for 24 or 48 h. Approximately 23,000 live bacteria were initially added to each patty, which corresponds to ~66 bacteria per sample of the core (1/350th of the 23,000 = 66). Replicates 1 (gray bars) and 2 (white bars) are shown separately. Please note the difference in scale of the y-axes. Panel **A** shows growth at 27°C for 24 h. Panel **B** shows growth at 27°C for 48 h. Panel **C** shows growth at 35°C for 24 h. Panel **D** shows growth at 35°C for 48 h.

concentration of 5,000 spores/μl. These spores were used to challenge the newly emerged bees.

Individual bees were challenged by placing them into 1,000 μl pipette tips with the tips removed so as to restrain the bees and allow only their forelegs and head to be free. A 2 μl drop (10,000 spores) of spore-laced inoculum in 50% sucrose or a 2 μl drop of a control dose containing 50% sucrose and no spores was fed to each individual bee using a 10-μl pipette. All bees were restrained individually for 30–45 min to prevent cross-contamination between bees (Fries et al. 2013) before placing them into plexiglass cages that allowed air flow through wire mesh panels. Infected or uninfected bees were placed into cages separated by treatment with ad libitum access to pollen patty, water, and 50% sucrose. The cages were housed for 10 d at 33 ± 1°C, and dead bees were removed daily. At 10 d postchallenge, the remaining live bees were removed from the cage. Each bee's midgut was dissected into 1 ml of distilled water, and the number of spores per bee was determined as in Cantwell (1970). In total, 50 *Nosema*-challenged guts (28 bees from *P. apium*-supplemented hives and 22 bees from nonsupplemented hives) and 50 control guts (19 bees from *P. apium*-supplemented hives and 31 bees from nonsupplemented hives) were assessed for spore load. The number of spores per bee was normally distributed, and so the data were analyzed with a *t*-test to compare the spore load between bees *from P. apium*-supplemented and uninoculated (control) hives. *Nosema*-challenged and unchallenged bees were analyzed separately.

Results

Parasaccharibacter apium Survival in Pollen Patty

Some but not all *P. apium* persisted in the pollen patty. Live bacteria were recovered from the patty after 24 and 48 h, but their numbers decreased with time and compared with the starting inoculum for both trials (rep 1: $F_{1,80} = 16.469$, $P = 0.0001$; rep 2: $F_{1,79} = 41.276$, $P < 0.0001$; Fig. 2). The effect of the inoculum type was significant in both trials (rep 1: $F_{4,80} = 15.711$, $P < 0.0001$, rep 2: $F_{4,79} = 5.013$, $P = 0.0012$) and post hoc comparisons indicated that strain C6 consistently yielded more CFUs per patty than those inoculated with the negative control or *E. coli* (Fig. 2). Heat was increasingly detrimental to *P. apium* survival in pollen patty (rep 1: $F_{1,80} = 92.667$, $P < 0.0001$; rep 2: $F_{1,79} = 41.276$, $P < 0.0001$). Patties kept at 35°C yielded lower numbers of viable bacteria than those kept at 27°C (Fig. 2). Interaction effects were not significant in either trial.

Parasaccharibacter apium in Larvae

Given the results of the above experiment and because *P. apium* C6 is most beneficial to larvae in vitro (Corby-Harris et al. 2014b), further experiments on *P. apium* focused specifically on strain C6. *Parasaccharibacter apium*-laden patty was readily consumed by the bees, but consumption was not affected by the treatment. *Parasaccharibacter apium*-supplemented hives had higher numbers of viable bacteria in the second- and third-instar larvae compared with hives that were not supplemented ($\chi^2_1 = 4.401$, $P = 0.0359$).

Colony Measures of Brood, Food Stores, and Foraging Rates

Brood area, frames of food, and foraging rate were not affected by the treatment. Overall, there was no effect of the treatment on the survival of hives from the beginning of the experiment through the spring. Although the trend was not significant, it was notable that *P. apium*-supplemented hives were 60% more likely to be strong in the spring (≥8 frames of adults) than bees fed the control (*P. apium*-supplemented: 7 out of 25 hives; control: 4 out of 24 hives; $\chi_1^2 = 0.914$, $P = 0.34$).

Nosema Challenge

Bees from *P. apium*-supplemented hives that were challenged individually with 10,000 *Nosema* spores had considerably lower *Nosema* titers compared with hives fed uninoculated patties (*P. apium*-supplemented: mean = 951,355,000 ± 120,993,000 SE spores per bee, control: mean = 1,646,636,000 ± 122,907,000 SE spores per bee; $t_{48} = 3.980$, $P = 0.0002$; Fig. 3). The observed variance in spore load was comparable with other studies where individuals were infected (Paxton et al. 2007, Maistrello et al. 2008). Bees fed a control solution of sucrose without *Nosema* had a lower level of infection (mean = 3,800,000 ± 916,515 SE spores per bee) compared with the *Nosema*-challenged bees. *Parasaccharibacter apium* treatment did not impact *Nosema* titers in the bees fed the control. Mortality up to 10 d was minimal and equivalent in all cages.

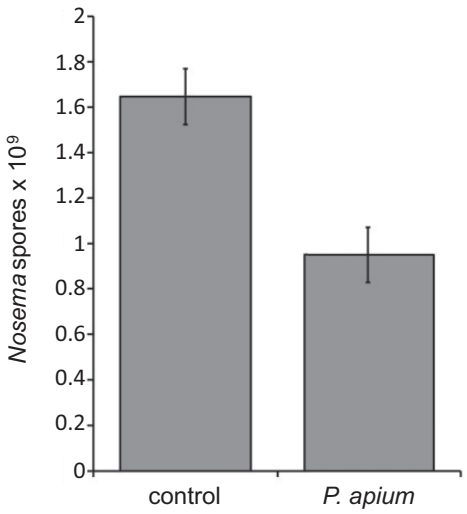

Fig. 3. *Nosema* levels in bees from *P. apium*-inoculated colonies. Newly emerged bees from either *P. apium*-supplemented or control (uninoculated) colonies were challenged with 10,000 *Nosema* spores. Ten days later, the *Nosema* spores in the guts of the challenged bees were counted.

Discussion

The goal of this study was to determine whether the benefits conferred by *P. apium* in the lab (Corby-Harris et al. 2014b) translated to colonies. We found that *P. apium* persisted in pollen patty and was distributed from the patty to the larvae. Although *P. apium* did not result in greater brood area, food stores, or foraging rate, *Nosema* infection was lower in bees from hives fed pollen patty inoculated with *P. apium*, and there was a slight but nonsignificant effect of *P. apium* on colony overwintering. *Parasaccharibacter apium*, therefore, shows some promise as a probiotic for increasing colony health.

None of the three bacterial strains that were tested were able to reproduce in pollen patty, but one strain of *P. apium*—strain C6—lived well enough to yield consistently more CFUs after 24 and 48 h compared with both of the controls. *Parasaccharibacter apium* strain C6 may be more suited to withstand sugar-rich niches such as bee bread, honey, and pollen patty. Importantly, strain C6 persisted long enough in the patty to travel from the patty to the larvae. This confirmed that when *P. apium* strain C6 is added to patty, it is distributed throughout the hive. It also supports previous work suggesting that *P. apium* follows the flow of hive nutrients, particularly between nurses who consume the pollen and larvae who consume nurse-secreted brood food (Corby-Harris et al. 2014b).

Hives supplemented with *P. apium* were more resistant to a fixed dose of *Nosema* under controlled conditions. Although the mechanism underlying this phenotype is unclear, we envision three possibilities. First, *P. apium* may strengthen gut integrity or immunity. In other insect systems, commensal Acetobacteraceae affect tissue development (Chouaia et al. 2012, Mitraka et al. 2013) and *Commensalibacter intestinii* primes gut immunity in *Drosophila melanogaster* (Ryu et al. 2008). Second, if *Nosema* reproduction depends on physiological cues from the host (Terry et al. 1997), *P. apium* may alter gut physiology and block these cues. Thirdly, like many lactic acid bacteria (Cabo et al. 2002), *P. apium* may secrete antifungal compounds, such as acetic acid, that directly harms *Nosema*.

Parasaccharibacter apium increased *Nosema* resistance but not the other measures of hive health such as brood area, food stores, or foraging rate. Colony growth rate is highly dependent on queen egg-laying rate and the number of workers in the hive and is sensitive to the number of workers present when the colony is established (Bodenheimer 1937, McLellan et al. 1980, Brian, 1983). Our measurements were performed on colonies started as packages in the spring, and their size was highly variable at the start of the experiment, perhaps owing to forager drift that occurs among colonies during this frenzied time. It is therefore possible that brood area, food stores, and foraging rate were not impacted by *P. apium* simply because these colonies were so variable from the very beginning. That being said, *P. apium* did have a slight impact on overwintering in that hives fed the bacterium were more likely to be stronger in the spring compared with uninoculated hives. This difference in spring hive strength was small, and the sample size was not sufficient to demonstrate significance. But it is tempting to speculate that *P. apium* keeps *Nosema* levels low, leading to stronger buildup in the spring. While this hypothesis should be tested, it is worth noting that other types of microbes fed to hives can also increase *Nosema* levels (Ptaszyńska et al. 2015). Many microbes fluctuate between pathogenic and nonpathogenic depending on factors such as the genetic background of the host and abiotic factors such as geography and weather (Daskin and Alford 2012). Before *P. apium* is

distributed to beekeepers, it is worth investigating whether its benefit is consistent across geographic locations.

Colony failure is a complicated issue that requires a multifaceted plan of attack. This plan may include increased focus on improving nutritional supplements by adding back aspects of the bees' natural diet, such as beneficial microbes. The data presented here suggest a possible benefit of *P. apium*, and provide an interesting contrast to other studies of probiotics and honey bee health. We stress that no matter the bacteria in question, each candidate should be 1) naturally found in hives, 2) distributed throughout the hive, 3) easily administered to the hive, and 4) beneficial to the bees. If this is not the case, the candidate may have no effect or even a negative effect on hive health. *Parasaccharibacter apium* fits the above criteria. We hope that the framework developed here provides a framework for testing how probiotic feed additives are developed and evaluated in the future.

Acknowledgments

We acknowledge the staff of the Carl Hayden Bee Research Center and Gloria DeGrandi-Hoffman and Mark Carroll for comments on a previous version of the manuscript. This research was funded by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) and the National Honey Board. The ARS-USDA is an equal opportunity employer and provider.

References Cited

Anderson, K. E., M. J. Carroll, T. Sheehan, M. C. Lanan, B. M. Mott, P. Maes, and V. Corby-Harris. 2014. Hive-stored pollen of honey bees: Many lines of evidence are consistent with pollen preservation, not nutrient conversion. Mol. Ecol. 23: 5904–5917.

Anderson, K. E., T. H. Sheehan, B. M. Mott, P. Maes, L. Snyder, M. R. Schwan, A. Walton, B. M. Jones, and V. Corby-Harris. 2013. Microbial ecology of the hive and pollination landscape: Bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). PLoS ONE 8: e83125.

Audisio, M. C., and M. R. Benitez-Ahrendts. 2011. *Lactobacillus johnsonii* CRL1647, isolated from *Apis mellifera* L. bee-gut, exhibited a beneficial effect on honeybee colonies. Benef. Microbes 2: 29–34.

Bodenheimer, F. S. 1937. Population problems of social insects. Biol. Rev. 12: 393–430.

Brian, M. V. 1983. Social insects: Ecology and behavioural biology. Chapman and Hall, London, New York, NY.

Cabo, M. L., A. F. Braber, and P. M. F. J. Koenraad. 2002. Apparent antifungal activity of several lactic acid bacteria against *Penicillium discolor* is due to acetic acid in the medium. J. Food Prot. 65: 1309–1316.

Cantwell, G. E. 1970. Standard methods for counting *Nosema* spores. Am. Bee J. 110: 222–223.

Cariveau, D. P. and R. Winfree. 2015. Causes of variation in wild bee responses to anthropogenic drivers. Curr. Opin. Insect Sci. 10: 104–109.

Chen, Y., J. D. Evans, I. B. Smith, and J. S. Pettis. 2008. *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. J. Invertebr. Pathol. 97: 186–188.

Chouaia, B., P. Rossi, S. Epis, M. Mosca, I. Ricci, C. Damiani, U. Ulissi, E. Crotti, D. Daffonchio, C. Bandi, et al. 2012. Delayed larval development in *Anopheles* mosquitoes deprived of *Asaia* bacterial symbionts. BMC Microbiol. 12: S2.

Corby-Harris, V., P. Maes, and K. E. Anderson. 2014a. The bacterial communities associated with honey bee *Apis mellifera* foragers. PLoS ONE 9: e95056.

Corby-Harris, V., L. A. Snyder, M. R. Schwan, P. Maes, Q. S. McFrederick, and K. E. Anderson. 2014b. Origin and effect of Alpha 2.2 Acetobacteraceae in honey bee larvae and description of *Parasaccharibacter apium* gen. nov., sp. nov. Appl. Environ. Microbiol. 80: 7460–7472.

Daskin, J. H., and R. A. Alford. 2012. Context-dependent symbioses and their potential roles in wildlife diseases. Proc. R. Soc. Lond. B Biol. Sci. 279: 1457–1465.

DeGrandi-Hoffman, G., Y. Chen, R. Rivera, M. Carroll, M. Chambers, G. Hidalgo, and E. de Jong. 2015. Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. Apidologie 1–11.

Fries, I., M.–P. Chauzat, Y.–P. Chen, V. Doublet, E. Genersch, S. Gisder, M. Higes, D. P. McMahon, R. Martín-Hernández, M. Natsopoulou, et al. 2013. Standard methods for *Nosema* research. J. Apic. Res. 52: 1–29.

Gallant, A. L., N. H. Euliss, Jr, and Z. Browning. 2014. Mapping large-area landscape suitability for honey bees to assess the influence of land-use change on sustainability of national pollination services. PLoS ONE 9: e99268.

Higes, M., P. García-Palencia, R. Martín-Hernández, and A. Meana. 2007. Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). J. Invertebr. Pathol. 94: 211–217.

Higes, M., R. Martin-Hernandez, C. Botias, E. G. Bailon, A. V. Gonzalez-Porto, L. Barrios, M. J. Del Nozal, J. L. Bernal, J. J. Jimenez, P. G. Palencia, et al. 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environ. Microbiol. 10: 2659–2669.

Huang, W.-F., J. –H. Jiang, Y.-W. Chen, and C.-H., Wang. 2007. A *Nosema ceranae* isolate from the honeybee *Apis mellifera*. Apidologie 38: 30–37.

Johnson, B. R., W. Synk, W. C. Jasper, and E. Müssen. 2014. Effects of high fructose corn syrup and probiotics on growth rates of newly founded honey bee colonies. J. Apic. Res. 53: 165–170.

Johnson, R., M. Ellis, C. Mullin, and M. Frazier. 2010. Pesticides and honey bee toxicity — USA. Apidologie 41: 312–331.

Johnson, R. M., L. Dahlgren, B. D. Siegfried, and M. D. Ellis. 2013. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). PLoS ONE 8: e54092.

Kapheim, K. M., V. D. Rao, C. J. Yeoman, B. A. Wilson, B. A. White, N. Goldenfeld, and G. E. Robinson. 2015. Caste-specific differences in hindgut microbial communities of honey bees (*Apis mellifera*). PLoS ONE 10: e0123911.

Maistrello, L., M. Lodesani, C. Costa, F. Leonardi, G. Marani, M. Caldon, F. Mutinelli, and A. Granato. 2008. Screening of natural compounds for the control of Nosema disease in honeybees (*Apis mellifera*). Apidologie 39: 436–445.

Martín-Hernández, R., A. Meana, L. Prieto, A. M. Salvador, E. Garrido-Bailón, and M. Higes. 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl. Environ. Microbiol. 73: 6331–6338.

McLellan, A. R., C. M. Rowland, and R. H. Fawcett. 1980. A monogynous eusocial insect worker population model with particular reference to honeybees. Insectes Soc. 27: 305–311.

Mitraka, E., S. Stathopoulos, I. Siden-Kiamos, G. K. Christophides, and C. Louis. (2013). *Asaia* accelerates larval development of *Anopheles gambiae*. Pathog. Global Health 107: 305–311.

Moran, N. A. 2015. Genomics of the honey bee microbiome. Curr. Opin. Insect Sci. 10: 22–28.

Morse, R. and N. Calderone. 2000. The value of honey bee pollination in the United States. Bee Cult. 128: 1–15.

Paxton, R., J. Klee, S. Korpela, and I. Fries. 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 38: 558–565.

Ptaszyńska, A., G. Borsuk, A. Zdybicka-Barabas, M. Cytryńska, and W. Małek. 2015. Are commercial probiotics and prebiotics effective in the treatment and prevention of honeybee nosemosis C? Parasitol. Res. 115: 397–406.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103: S96–S119.

Ryu, J.-H., S.-H. Kim, H. –Y. Lee, J. Y. Bai, Y.–D. Nam, J.-W. Bae, D. G. Lee, S. C. Shin, E.-M. Ha, and W.–J. Lee. 2008. Innate immune homeostasis by the homeobox gene *caudal* and commensal-gut mutualism in *Drosophila*. Science 319: 777–782.

Schwarz, R. S., Q. Huang, and J. D. Evans. 2015. Hologenome theory and the honey bee pathosphere. Curr. Opin. Insect Sci. 10: 1–7.

Simon-Delso, N., G. San Martin, E. Bruneau, L.-A. Minsart, C. Mouret, and L. Hautier. 2014. Honeybee colony disorder in crop areas: The role of pesticides and viruses. PLoS ONE 9: e103073.

Spleen, A. M., E. M. Lengerich, K. Rennich, D. Caron, R. Rose, J. S. Pettis, M. Henson, J. T. Wilkes, M. Wilson, J. Stitzinger, et al. 2013. A national survey of managed honey bee 2011–12 winter colony losses in the United States: Results from the Bee Informed Partnership. J. Apic. Res. 52: 44–53.

Steinhauer, N. A., K. Rennich, M. E. Wilson, D. M. Caron, E. M. Lengerich, J. S. Pettis, R. Rose, J. A. Skinner, D. R. Tarpy, J. T. Wilkes, et al. 2014. A national survey of managed honey bee 2012-2013 annual colony losses in the USA: Results from the Bee Informed Partnership. J. Apic. Res. 53: 1–18.

Tarpy, D. R., H. R. Mattila, and I. L. G. Newton. 2015. Characterization of the honey bee microbiome throughout the queen-rearing process. Appl. Environ. Microbiol. 81: 3182–3191.

Terry, R. S., A. M. Dunn, and J. E. Smith. 1997. Cellular distribution of a feminizing microsporidian parasite: A strategy for transovarial transmission. Parasitology 115: 157–163.

(USDA) United States Department of Agriculture. 2013. *Report on the National Stakeholders Conference on Honey Bee Health*, p. 62: USDA. http://www.usda.gov/documents/ReportHoneyBeeHealth.pdf (accessed May 2015).

vanEngelsdorp, D., J. Hayes, Jr. R. Underwood, and J. S. Pettis. 2010. A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. J. Apic. Res. 49: 7–14.

vanEngelsdorp, D., D. Caron, J. Hayes, R. Underwood, M. Henson, K. Rennich, A. Spleen, M. Andree, R. Snyder, K. Lee, et al. 2012. A national survey of managed honey bee 2010-11 winter colony losses in the USA: Results from the Bee Informed Partnership. J. Apic. Res. 51: 115–124.

Vaudo, A. D., J. F. Tooker, C. M. Grozinger, and H. M. Patch. 2015. Bee nutrition and floral resource restoration. Curr. Opin. Insect Sci. 10: 133–141.

Vojvodic, S., S. M. Rehan, and K. E. Anderson. 2013. Microbial gut diversity of Africanized and European honey bee larval instars. PLoS ONE 8: e72106.