Wageningen Academic Publishers

Effect of *Lactobacillus johnsonii* CRL1647 on different parameters of honeybee colonies and bacterial populations of the bee gut

M.C. Audisio[1,2*], D.C. Sabaté[1] and M.R. Benítez-Ahrendts[3]

[1]Instituto de Investigaciones para la Industria Química (INIQUI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Salta, Av. Bolivia 5150, 4402FDC Salta, Argentina; [2]Facultad de Ingeniería, Universidad Nacional de Salta, Avenida Bolivia 5150, 4400 Salta, Argentina; [3]Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Alberdi 47, 4600 Jujuy, Argentina; audisio@unsa.edu.ar

Received: 4 November 2014 / Accepted: 12 January 2015

RESEARCH ARTICLE

Abstract

Lactobacillus johnsonii CRL1647, isolated from the intestinal tract of a worker-bee in Salta, Argentina, was delivered to *Apis mellifera* L. honey bee colonies according to two different administration schedules: 1×10^5 cfu/ml every 15 days (2011) or monthly (2012). The effect of each treatment on the bee-colony performance was monitored by measuring honey production, and the prevalence of varroasis and nosemosis. Worker bees from each assay were randomly captured 3 days after administration and assayed for the following intestinal culturable and defined bacterial populations: total aerobic microorganisms, *Bacillus* spp. spores, *Lactobacillus* spp., *Enterococcus* spp. and enterobacteria. Interestingly, both treatments generated a similar increase in honey production in treated colonies compared to controls: 36.8% (every 15 days) and 36.3% (monthly). *Nosema* index always exhibited a reduction when lactobacilli were administered; in turn, *Varroa* incidence was lower when the lactobacilli were administered once a month. Moreover, the administration of *L. johnsonii* CRL1647 every 15 days produced an increase in the total number of aerobic microorganisms and in bacteria belonging to the genera *Lactobacillus* and *Enterococcus*; at the same time, a decrease was observed in the number of total spores at the end of the treatment. The number of enterobacteria was constant and remained below that of control hives at the end of the assay. On the other hand, the delivery of lactobacilli once a month only showed an increase in the number of bacteria belonging to the genus *Lactobacillus*; meanwhile, viable counts of the remaining microorganisms assayed were reduced. Even though it seems that both treatments were similar, those bee colonies that received *L. johnsonii* CRL1647 every 15 days became so strong that they swarmed.

Keywords: *Lactobacillus johnsonii*, probiotic, apiculture, culturable microorganisms, honeybee

1. Introduction

The honey bee (*Apis mellifera* L.) is one of the most important insects of the terrestrial ecosystem, not only because of its honey production but also because it is the key pollinator of many different crops and flowers (Kremen *et al.*, 2007). The insect is also a host for a large number of living beings like bacteria, moulds, viruses and mites (Engel *et al.*, 2012; Evans and Schwarz, 2011; Gilliam *et al.*, 1988; McFrederick *et al.*, 2012; Newton and Roeselers, 2012; Rosenberg and Zilber-Rosenberg, 2011). It is believed that bees and other insects such as *Bombus* spp., acquire this microbiota through consumption of pollen, food, and through contact with older bees in the same colony or from other environments (Kačániová *et al.*, 2004; McFrederick *et al.*, 2012; Tajabaldi *et al.*, 2013). Other authors have also reported that honey bees (*Apis* spp.) possess a highly specialised gut microbial community comprising about 8 bee-specific phylotypes, that do not come from their food (Martinson *et al.*, 2011; Moran *et al.*, 2012). Interestingly, like in human beings and other animals, the bee microbiota is complex and its health can be seriously affected by disturbances in the balance of the beneficial microbiota or because of ingestion of or contact

ISSN 1876-2833 print, ISSN 1876-2891 online, DOI 10.3920/BM2014.0155

with pathogenic microorganisms (Chambers and Schneider, 2012; Hamdi *et al.*, 2011).

Nosema spp. and *Varroa* spp. are among the most important bee pathogens that still constitute one of the greatest threats for apiculture (Evans and Schwarz, 2011; Higes *et al.*, 2013; Rosenkranz *et al.*, 2010). In order to control or fight these or other diseases, beekeepers frequently use antibiotics and pesticides that not only develop pathogen resistance, but also cause imbalance of the normal bee microbiota (Evans, 2003; Miyagi *et al.*, 2000). This latter factor affects the bees' health and may alter their orientation and consequently may eventually reduce the number of hive members (Barnett *et al.*, 2007). Moreover, the use of antibiotics or chemical products increases the risk of contamination of the hive products because they may persist in the honey and hence affect its quality for human consumption (Martel *et al.*, 2006). Antibiotics such as chloramphenicol have already been detected in honey and other apiculture products in numerous countries (Bogdanov, 2006; Sheridan *et al.*, 2008).

Because of the economic importance of apiculture in Argentina, the second largest honey producer and exporter in the world, it would be useful to develop not only efficient but also sustainable strategies to control honeybee diseases and/or to improve the bee colony health. Therefore, the use of bacterial strains as biological alternatives, such as a bee probiotic, is a promising option. Even though it is well known that bacteria belonging to the genus *Lactobacillus* have been widely used as probiotics for both humans and animals (Lee and Salminen, 2009; Pretzer *et al.*, 2005; Sanders and Levy, 2011; Servin, 2004; Sonnenburg *et al.*, 2006; Vasiljevic and Shah, 2008), only very few studies exist on the use of bacteria as probiotic supplements for honeybees (Audisio and Benítez-Ahrendts, 2011; Evans and Lopez, 2004; Pătruică and Mot, 2012; Pătruică *et al.*, 2012).

In past work, our research group determined that *Lactobacillus johnsonii* CRL1647, isolated from the intestinal tract of a honeybee, when supplemented to beehives mainly favoured open and operculated brood areas, demonstrating a stimulation of egg-laying (Audisio and Benítez-Ahrendts, 2011). The aim of the present study was to determine the influence of the frequency of administration of this *Lactobacillus* on defined and culturable bacterial populations of the bee gut microbiota, honey production and on *Varroa* and *Nosema* prevalence.

2. Materials and methods

Bacterial strains and growth conditions

L. johnsonii CRL1647 (GenBank accession number EU428007; Audisio *et al.*, 2011) was cultured in *Lactobacillus*-selective De Man, Rogosa and Sharpe (MRS) medium (Britania, Buenos Aires, Argentina), at 37 °C during 12 h under microaerophilic conditions, obtained with a candle extinction jar.

Bees, hive location and environmental conditions

The assays were carried out at a commercial apiary in San Antonio, Jujuy province (Argentina) at an approximate altitude of 1,345 m above sea level. Local bees were kept in standard Langstroth hives and ten hives, of ten frames each, were used in the different experiments. The bee colonies used in both trials came from nuclei prepared with an open brood frame, an operculated brood frame including the bees attached to it, and an open frame of honey. Queen cells, obtained from selected hives in the apiary, were introduced into the nuclei 48 h after their generation. It is important to mention that the bee colonies, both treated and control, studied in 2011 were prepared, respectively, from hives that had been treated and used as controls during 2010 (Audisio and Benítez-Ahrendts, 2011). A similar pattern was followed in 2012. Once the new bee colonies were obtained, they were uniformed. Thus, all the hives had a similar size initially and they were located in the same apiary.

Bacterial administration

Lactobacilli viable cells were delivered to the bees by a Doolittle-type feeder in a 125 g sucrose/l syrup as described before (Audisio and Benítez-Ahrendts, 2011). The number of viable *L. johnsonii* CRL1647 cells was determined by a plate count in MRS agar. The plates were incubated at 37 °C for 48-72 h under microaerophilic conditions, as explained above. Five hives were administered with *L. johnsonii* CRL1647 (final concentration of 1×10^5 cfu/ml) and other 5 were used as controls and only received syrup (125 g/l).

Administration schedule

Because the current study was part of the PICTR890/06 and PIP11220100100019 research projects and included a postdoctoral fellowship between 2011 and 2012, the assays were carried out as follows:

- *Assay 1*: was carried out between May and December 2011 and *L. johnsonii* CLR1647 cells were administered twice a month, i.e. every 15 days.
- *Assay 2*: was carried out from May through December 2012 and lactobacilli cells were administered once a month, i.e. every 30 days.

Growth of the colonies was monitored and any change was compared with control hives that did not receive the lactic acid bacterium. All other conditions (weather, geographical location, nourishment and supervision) were identical.

Bee colony performance

Honey production was used as a parameter to describe the general condition of the colonies during the study, and it was compared with control hives without bacteria supplement. Honey was harvested in December.

To evaluate the sanitary status of the colonies, *Varroa* and *Nosema* indices were monitored using the standard protocols by De Jong (1980) for *Varroa* and Cantwell (1970) to quantify the number of mature spores of *Nosema* spp.

It is important to mention that the study was carried out following standard protocols according to sanitary regulations of the province of Jujuy, Argentina, where the hives were located; the protocols were defined by SENASA (the National Service for Agro-Alimentary Public Health, Safety and Quality). The products to treat *Varroa* spp. were administered to all the hives used in the experiments (i.e. control and treated), but they were different from one year to the other in order to rotate the active components and avoid resistance. The first assay (2011) used 1 (one) band of Amivar (6.25 g amitraz in slow-release plastic strips; Apilab SRL, Tandil, Argentina) per hive, whereas the assays carried out in 2012 used Flumevar (36 mg flumethrin/strip; also 1 (one) strip per hive; Apilab SRL).

Evaluation of defined culturable bacterial populations of bee gut microbiota

This analysis was performed during spring (September to December). Three days after each lactobacilli cell administration, three of the five hives, both in the treated and in the control groups, were tested and worker bees from the brood frame were randomly captured to analyse their gut microbiota. From each tested hive, a pool of 10 bees was prepared. Bee guts were aseptically dissected, the contents pooled and diluted with sterile distilled water. Decimal dilutions were made and immediately sown on the corresponding culture media for analysis of the following microorganisms:

- *Lactobacillus*: MRS agar (1.5% w/v) at pH 5.0.
- *Enterococcus*: MSS agar (trypsin 0.14 g/l; glucose 0.05 g/l; sodium citrate 0.01 g/l; sodium chloride 0.04 g/l; sodium azide 0.0022 g/l; meat peptone 0.05 g/l; sodium sulfite 0.0022 g/l; L-cysteine 0.0021 g/l; agar 1.5 g/l (w/v)) according to Audisio *et al.* (2005).
- Total microorganisms and bacterial spores: brain heart infusion (BHI, Britania).
- Enterobacteria: Eosin Methylene Blue (EMB, Britania) agar for cell counts.

All media were incubated for 24 to 48 h at 37 °C; MRS cultures were incubated under microaerophilic conditions, obtained with a candle extinction jar as explained above. The remaining cultures were grown under uncontrolled atmospheric conditions.

Statistical analysis

The results of *Varroa* and *Nosema* indexes, and honey yield were expressed as mean ± standard deviation of the groups and subjected to Student's t test for independent samples with 5 repetitions at a significance level of $P<0.05$. The results of culturable microbiota assays were expressed as mean ± standard deviation and were subjected to Student's t test for independent samples with 3 repetitions at a significance level of $P<0.05$. In both situations, one repetition represents one hive. Data were analysed using the statistical program InfoStat (2008; National University of Córdoba, Córdoba, Argentina).

3. Results

Administration of probiotic bacteria to bee colonies every 15 days

Effect on selected and culturable bacterial populations

Administration of *L. johnsonii* CRL1647 to the bee colony produced an increase of about 1 order of magnitude in the total number of microorganisms in October and November ($P=0.0185$), but at the end of the trial no significant differences were observed between treated and control bee colonies (Figure 1A). During the first months of the experiment, the number of bacterial spores present in the gut of treated bees was slightly higher than that of controls, but at the end of the assays, the number decreased about two orders of magnitude in bees that received *L. johnsonii* CRL1647 ($P=0.0023$) (Figure 1B). Interestingly, the number of bacteria that potentially belong to the genus *Lactobacillus* varied between 1.5×10^3 and 1×10^4 cfu/ml for control bees and between 1.5×10^3 and 1×10^5 cfu/ml for treated bees. However, at the end of the assays, the final number of this microorganism in treated and untreated bees was the same (Figure 1C). *Enterococcus* species were found at an initial density of *ca.* 1×10^7 cfu/ml and this concentration diminished during the treatment until a final value of 3×10^3 cfu/ml in treated bees compared with 3×10^6 cfu/ml in control colonies (Figure 1D). Finally, the number of enterobacteria remained almost constant during the entire experiment for treated colonies (ca. 1×10^6 cfu/ml) and in December this value was even lower than that registered for the control hives (ca. 1.5×10^7 cfu/ml) ($P=0.0135$) (Figure 1E). In this sense, the control bee-colonies started with an enterobacteria population closes to 1×10^4 cfu/ml in September, and their number progressively increased throughout the treatment until it reached the aforementioned final value (Figure 1E).

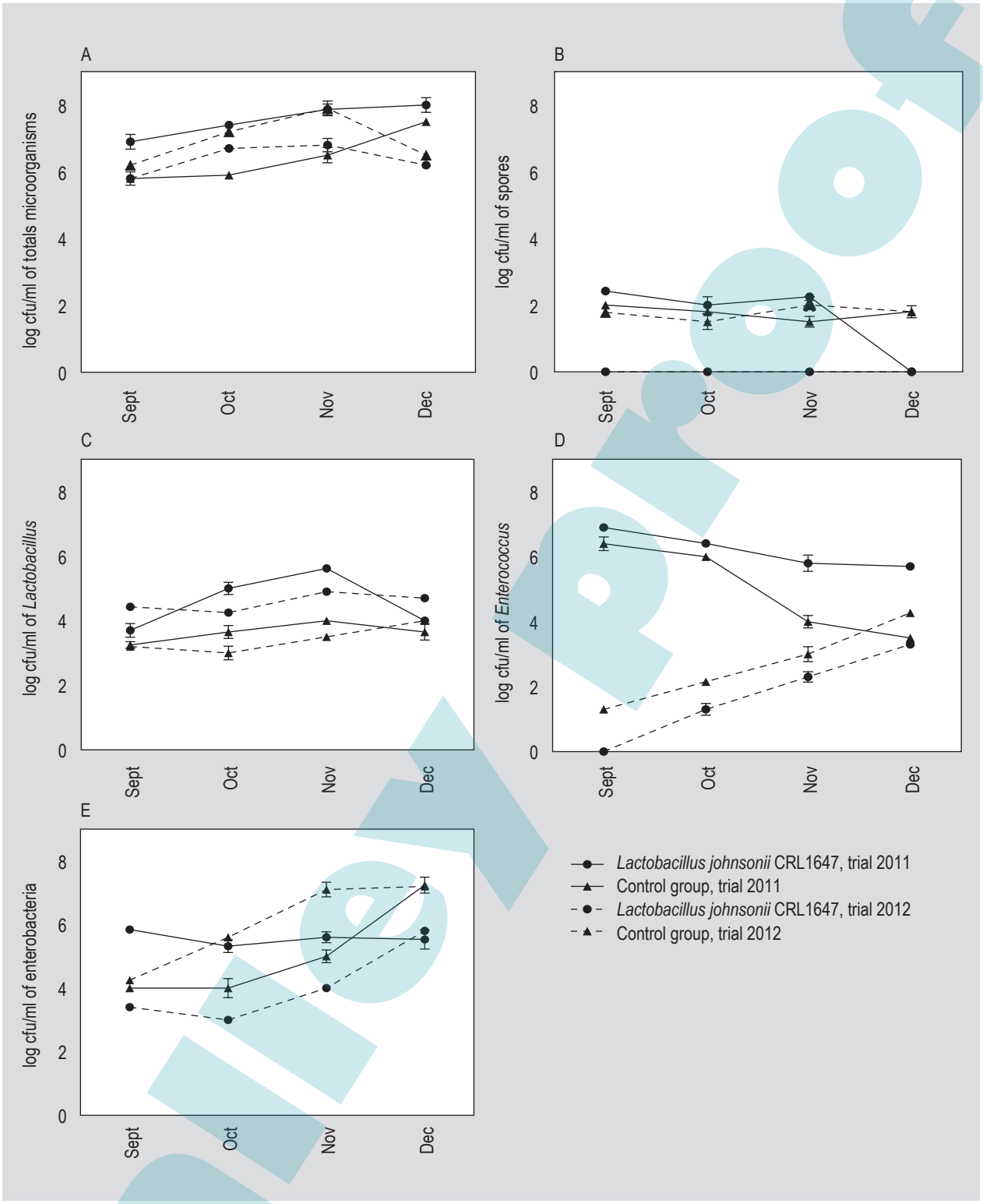

Figure 1. Viable number of culturable and defined bacterial populations studied in the *Apis mellifera* L. gut: (A) total count; (B) spores; (C) *Lactobacillus* spp.; (D) *Enterococcus* spp.; (E) *Enterobacteriaceae*. Samples were taken from worker bees.

Impact on honey yield

Honey production was used as a bee-colony performance marker. Honey production in untreated colonies was 11.41±0.90 kg of honey/hive. Administration of *L. johnsonii* CRL1647 to the hives yielded a production of 18.08±1.06 kg of honey/hive, 37% higher than the controls. This difference was significant ($P<0.05$).

Varroa and *Nosema* prevalence

The incidence of phoretic *Varroa* was registered in June, when bee colonies start wintering, and it was determined as 9.07±1.34% in control hives and 2.78±0.09% in *Lactobacillus*-treated colonies. Taking these values into account, and according to 2011 acaricide administration protocols in Argentina, all the hives were treated with Amivar in July. For that reason, when the next samples were taken in August, the index of *Varroa* in all the hives was below the detection limit. From September till the end of the assay, the *Varroa* index in control hives was lower than that registered for the treated hives (Figure 2A). However, in December, the difference between the groups was not significant.

As the intensity of *Nosema* was low (around 28,000 spores in both groups) this bee pathogen was not treated with chemicals. Yet, at the end of the winter, the number of *Nosema* spores in control colonies was 15,000±1,340 and in treated colonies 10,000±2,230 (Figure 2).

It is worth to mention that five from the five bee colonies treated with *L. johnsonii* CRL1647 under this schedule (i.e. every 15 days) a high number of bees was also observed and became so strong that they swarmed.

Administration of probiotic bacteria to beehives every 30 days

Influence on defined and culturable bacterial populations

During the assay, the number of total microorganisms present in bee guts from control hives was slightly higher than those from treated colonies (Figure 1A). The number of spores in bee guts from control hives and after administration of lactobacilli every 15 days (2011) was similar and oscillated between 3×10^1 and 1×10^2 spores/ml. However, in bee guts from hives treated once a month (2012), the number of spores was below the detection limit of the technique used (i.e. plate count) (Figure 1B).

In the bee gut from colonies treated with *L. johnsonii* CRL1647, the number of culturable *Lactobacillus* spp. increased about 1 order of magnitude compared with control hives throughout the study ($P<0.0001$) (Figure 1C), whereas the number of *Enterococcus* spp. diminished nearly 1 order which was lower than that of control colonies (Figure 1D). In the treated colonies the number of enterobacteria kept significantly lower than in the control hives throughout the experiment ($P=0.027$); in particular, during October and November the number was nearly 3 orders of magnitude lower (Figure 1E).

Impact on honey production/yield

The average amount of honey obtained from the colonies treated once a month with CRL1647 was 20.40±1.09 kg, about 7 kg higher than the control that produced 12.98±1.33 kg. This difference was significant ($P<0.05$).

Varroa and *Nosema* prevalence

In June, a *Varroa* spp. index of 7%±1 was observed in control hives, while *Lactobacillus*-treated colonies showed a 2%±0.1 index. This difference was significant ($P<0.05$). In July, as explained before, all the hives were treated with Flumevar, the antibiotic indicated by SENASA for 2012. Bee colonies initiated September, at the beginning of spring, with a lower

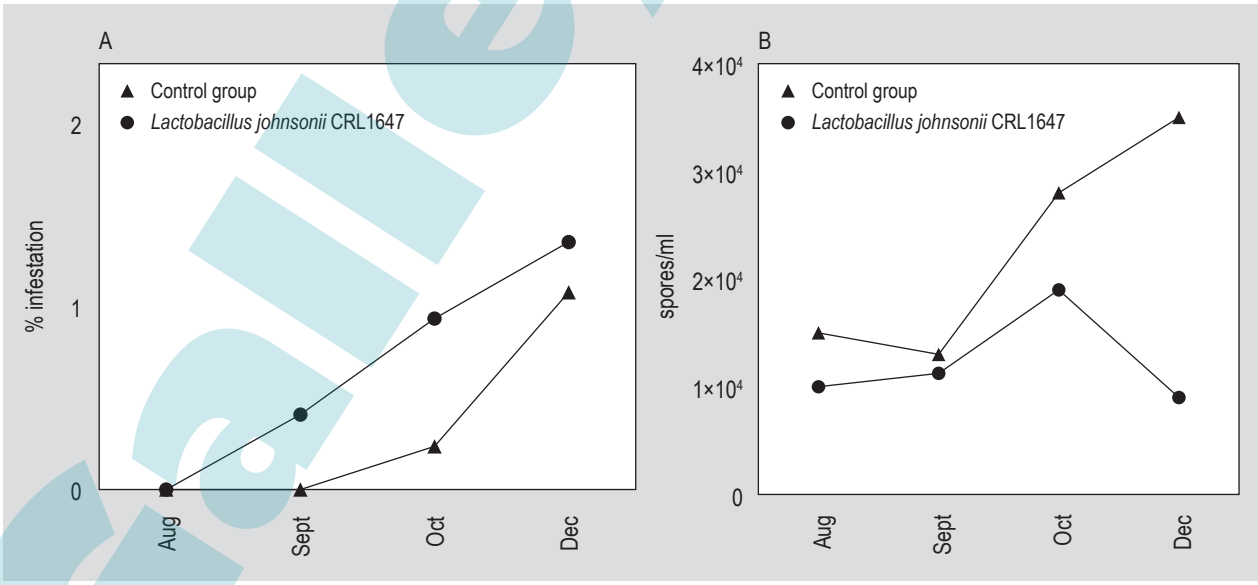

Figure 2. Average values of monthly-determined infestation indexes for the 2011 trial: (A) *Varroa destructor*, in phoretic phase; (B) *Nosema apis* spores.

Varroa percentage (2% for control and below detection limit for treated hives).

Nosema spp. infection intensity was also evaluated demonstrating significant differences between control and treated hives ($P<0.05$). At the beginning of the winter, in June in our country, 49,000±4,591 spores were determined in control hives versus 10,000±620.4 in *Lactobacillus*-treated hives. In September, the number of *Nosema* spores was 86,000±7,434 in control hives and only 16,000±991 in treated colonies.

4. Discussion

Lactic acid bacteria, mainly from the genus *Lactobacillus*, are generally considered beneficial bacteria and they have become a major ingredient in probiotic supplements or formulae for humans and numerous animals (Kesarcodi-Watson *et al.*, 2008; Lee and Salminen, 2009; Sanders and Levy, 2011; Quigely, 2010). Even though the mechanisms of the positive effects produced by probiotic microorganisms have not been completely elucidated yet, they most likely include the following features: the ability of the bacteria to synthesise metabolites with antagonistic properties against surrounding microbiota, competition for nutrients, stimulation of the immune system and competitive exclusion (Oelschlaeger, 2010; Pretzer *et al.*, 2005; Sonnenburg *et al.*, 2006). If this idea is translated to apiculture, it can be an interesting trend to develop natural and non-contaminant alternatives to maintain a beehive strong and healthy using this type of bacteria.

The gut microbiota of the honey bee, is receiving increasing attention as a potential determinant of the bees' health and their efficacy as pollinators (Hamdi *et al.*, 2011). The current study was designed to evaluate the impact of the bee-probiotic bacterium *L. johnsonii* CRL1647 on the bee colony performance/evolution (i.e. honey production and health) and on the following defined and selected culturable bacteria present in the bee gut microbiota: *Lactobacillus*, *Bacillus*, *Enterococcus* and microorganisms of the *Enterobacteriaceae* family. An important and relevant result of the present study is that the honey yield in colonies treated with *L. johnsonii* CRL1647 was higher, independently if bacteria were administered every 15 days or monthly. A significant increase close to 35% was observed in both assays, although they were carried out in two different years, which reflects that the effect of this lactic acid bacterium on the honeybee 'life cycle' is reproducible.

It is well known that the presence of *Varroa* and *Nosema* weakens a bee colony due to loss in weight, malformation and weakening of the bees (Traver and Fell, 2011). These bee pathogens have also been associated with winter colony mortality and they are important vectors of several honey bee viruses. Many researchers believe that *Nosema* spp. is also a possible cause of Colony Collapse Disorder. However, the specific causes of most losses are still undetermined (Ratnieks and Carreck, 2010). In the present work it was found that the number of *Nosema* spp. spores in both trials was lower in treated hives than in controls. However, a different situation was observed with the index of phoretic *Varroa.* At the beginning of both trials, before delivering *L. johnsonii* CRL1647, the index of 'basal' *Varroa* was significantly higher in the control hives. These lower initial *Varroa* indexes in the bee colonies belonging to the treated group can be seen as a potential 'residual protective effect' produced by the previous *L. johnsonii* CRL1647 administration. It is important to remark that independently of colony origin, at the beginning of each assay all of them were uniformed, i.e. all the nuclei had a similar size initially and were located in the same apiary. However, if *Varroa* females kept 'hidden' in the operculated brood area, they were perhaps not detected when preparing the different nuclei.

Perhaps the fact that the study comprises two different years may suggest many variables that may be difficult to be handled, but the assay protocol of the present analysis revealed interesting results. First of all, independently of the fact that the study was carried out during two different years using two distinct *Lactobacillus* administration schedules (i.e. every 15 or 30 days), the development of the culturable populations assayed was similar, except for enterococci and sporulating bacteria. At the start of the 2nd assay in September 2012, the *Enterococcus* spp. population in the hives that received *L. johnsonii* CRL1647 was below the detection limit of the technique used (plate cell counts), but at the end of the assay the number had become similar to that registered in December 2011 (ca. 7 log in the cfu/ml). For both years the value was lower than that observed for enterococci in control hives. Regarding sporulating bacteria, the number of *Bacillus* spores was low and almost the same for the control groups in both years (ca. 10^2 spores/ml), but practically none of the samples from *Lactobacillus*-treated colonies contained spores, particularly in 2012.

A plausible explanation for the uniformity observed on the studied culturable bacterial population of the bee gut could be related to the fact that the bee colonies studied inhabited the same geographical space, so they could have had a similar feeding pattern. It is known that the microbiota in bees is acquired by consumption of pollen and other food stuffs, interaction with flowers and through contact with other bees in the colony (McFrederick *et al.*, 2013; Tajabadi *et al.*, 2013). In turn, the differences observed regarding *Enterococcus* and the behaviour of culturable spore populations might be associated with the aseptic conditions in which the samples were taken and the repeated visits to the apiary (twice a month in 2011). Interestingly, the weather conditions seem not to have an important effect on the results because in both years the

average temperature from May to December (ca. 17 °C in May and 24 °C in December) and the average annual rainfall (508.2 in 2011 and 506.7 in 2012) were similar.

The culturable bacterial population evaluated in this work can be present in the bee gut or in the honey stomach of honey bees. For example, Endo and Salminen (2013) reported that honeybees are a rich source of fructophilic lactic acid bacteria, mainly *Lactobacillus kunkeei* that predominates in bee products and in larvae. Other researchers have even demonstrated the presence of new *Lactobacillus* species in the bee gut and honey stomach (Kwong *et al.*, 2014; Olofsson and Vásquez, 2008; Olofsson *et al.*, 2014; Tajabadi *et al.*, 2013). Audisio *et al.* (2011) reported the presence of culturable *Lactobacillus* spp., mainly *L. johnsonii*, as well as the presence of the genus *Enterococcus*, predominantly *Enterococcus faecium*. Other authors have informed about the honey bee and another insect gut microbiome, but those studies were non-culturable (Martinson *et al.*, 2011; McFrederick *et al.*, 2012; Mohr and Tebbe, 2006; Moran *et al.*, 2012;). Even though no ecological studies of the bee gut microbiome were done in this work, the results obtained do make a relevant contribution to the knowledge about culturable bacteria from the bee gut. Indeed, references about this topic are limited and only few researchers have been able to isolate or cultivate the same bacterial group. For example, previous work about the genus *Enterococcus* as a member of the honeybee gut microbiota is scarce (Audisio *et al.*, 2011; Kačániová *et al.*, 2004, 2009; Mohr and Tebbe, 2006). In the present study, this group of bacteria was detected and cultured.

The results obtained with the group of cultured enterobacteria are significant, because there is little information about their presence or role in the bee gut. *Escherichia, Enterobacter, Proteus, Hafnia, Klebsiella*, and *Erwinia* were detected in the bee intestine (Gilliam *et al.*, 1988; Lyapunov *et al.*, 2008). Pătriucă and Mot (2012) studied the effect of two commercial probiotics designed for human consumption, Enterobiotics (*Lactobacillus acidophilus* LA-14 and *Bifidobacterium lactis* BI-04) and Enterolactis Plus (*Lactobacillus casei*), on the gut microbiota of honey bees. They found that after administration for 21 days the number of pathogenic bacteria in newly emerged worker bees had diminished. Our study shows similar results when *L. johnsonii* CRL1647, a honeybee-associated probiotic, was administered. The lactobacilli reduced the total amount of enterobacteria in treated hives compared with control hives. However, whether these results are positive or not is not easy to determine, because thus far scientific studies have only assayed the presence of enterobacteria; whether their presence is beneficial or not has not yet been mentioned or even studied (Lyapunov *et al.*, 2008). In this sense, Grobov and Likhotin (2003) report a negative effect of enterobacteria because they are involved in the aetiology of bacterial diseases in honey bees, and in the microbiological quality of the pollen that affect honey properties (Khismatullin *et al.*, 2004). Consequently, it can be suggested that a reduction in the number of enterobacteria is a positive result, even though neither phylogenetic nor identification studies have been carried out.

In principle, it may seem that both treatments were similar, due to the effects observed on the sanitary status of the bee colony, on the impact in the culturable microbiota studied and on honey production. However, after three months of administration, the bee colonies that had received *L. johnsonii* CRL1647 every 15 days showed such an increase in the number of bees, due to stimulation of egg-laying, that they swarmed. The fact that the *L. johnsonii* CRL1647 administration stimulates egg-laying was observed and reported before (Audisio and Benítez-Ahrendts, 2011); however, in that work no 'swarming' effect was registered. It is known that honeybees will start a new colony by swarming when the old hive becomes crowded or when a new queen emerges (Getz *et al.*, 1982). Thus, swarming may indeed be an indicator of bee colony strength due to an increase in the number of bees, a situation observed in our work. This biological phenomenon, in which one honeybee hive splits into two almost equally-sized hives, occurs suddenly and quickly (Lin *et al.*, 2003). Depending on the hive-handling technique applied, swarming may have disparate impacts on beekeepers. On one hand, it may be considered negative because it means loss of the bee colony; however, some may regard it positive, since a ready-to-swarm colony can be handled in order to obtain a new one. In designing a bee probiotic it is thus important to determine the right dose and to select the one that modulates the positive effect on the host under analysis.

Finally, and in agreement with new trends to improve colony health such as studies of honey bee gut symbionts (Crotti *et al.*, 2013) or paratransgenesis (Rangberg *et al.*, 2013), it is highly encouraging that a microorganism without genetic modifications from the bee gut environment produces a positive effect on bee colonies.

5. Conclusions

Our results reveal that, in order not to disturb bee colony normal development, administration of 1×10^5 cfu/ml of *L. johnsonii* CRL1647 to honeybees once a month can produce a positive effect on the sanitary status of the bee colony and on honey production. A deeper study on the impact of honey bee physiology due to *L. johnsonii* CRL1647 administration will be performed in our group in future assays.

Acknowledgements

The authors would like to thank CIUNSa (PI 1974), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT2011-0767) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 11220100100019) of Argentina for the financial support. M.C. Audisio and D.C. Sabaté are members of the Research Career of CONICET.

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