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Effect of bacterial metabolites on microsporidian *Nosema ceranae* and on its host *Apis mellifera*

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Abstract Nosemosis, a disease caused by a microsporidian
infection, is one of the most frequently observed parasitic
pathologies affecting adult honeybees. Presently, *Nosema
ceranae* seems to be the main microsporidian infection in
Apis mellifera. The antibiotic fumagillin is the only
compound available to treat Nosema diseases; however, it
is no longer licensed in most EU member states; therefore,
the need to identify new molecules/substances prevails. The
intent of this paper is to test bacterial metabolites by *Bacillus*
and *Enterococcus* strains, isolated from bee midgut and
honey. The toxicity on bees and the antiparasitic activity on
N. ceranae were assessed under laboratory conditions.
Results did not yield toxicity for the administered surfactin
or bacteriocin concentrations. Spores exposed to direct
contact with a particular surfactin revealed a significant
infectivity reduction when inoculated on bees. This surfactin,
administered ad libitum from the individuals' emergence, led
to a significant reduction in parasitosis development when
bees were infected with untreated spores 7 days postemer-
gence. Based on the results obtained, one of the surfactins is
herein postulated as a molecule capable of reducing *N.
ceranae* development, acting either by direct exposure to
purified spores or incorporated into the digestive tract of the
bee.

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Introduction

Taxonomically, microsporidia are defined as highly
specialized fungi (Sina et al. 2005), parasitizing a wide
range of hosts. When it comes to honeybees, the micro-
sporidian *Nosema ceranae* is one of the etiologic agents of
nosemosis, a current worldwide disease (Klee et al. 2007;
Giersch et al. 2009). This intracellular parasite was first
described for the Asian honey bee, *Apis cerana* (Fries et
al. 1996), and afterward for the European bee, *Apis
mellifera* (Higes et al. 2006). The host cell penetration
occurs through the mechanical injection of the polar
filament, which protrudes from the spore, forced by water
influx (Delbac and Polonais 2008) and takes the infectious
sporoplasm to the host cell cytoplasm, where the organism
replicates.

The antibiotic fumagillin (dicyclohexylammonium),
obtained from *Aspergillus fumigatus*, is the most widely
antimicrobial agent available nowadays to treat Nosema
diseases. Its efficacy has been proven by temporally
reducing the parasitosis caused by *N. ceranae* in *A.
mellifera* (Williams et al. 2008). However, this drug is no
longer licensed in most EU member states. Efforts have been
done in finding alternatives to control this disease, among
them experimenting with different compounds, such as

thimerosal (Gontarski and Wagner 1954; Blatz 1955; Schlüter 1957; Furgala and Bosch 1970; Liu 1988), itraconazole (Liu and Myrick 1988), thymol (Yücel and Doğaroğlu 2005; Maistrello et al. 2008), Sinefungin (Sichtova et al. 1993), organic acids (Forsgren and Fries 2005; Underwood and Currie 2009), herbal hydroalcoholic extracts (Pohorecka 2004), essential oils, lisozima, and phytoalexin resveratrol (Maistrello et al. 2008), among others.

Many bacteria strains are commonly isolated from the hive environment (Gilliam 1979; Gilliam and Prest 1978; Audisio et al. 2005; Sabaté et al. 2009; Alippi and Reynaldi 2006) and bees' digestive tract (Gilliam 1978; Gilliam and Valentine 1976; Audisio et al. 2010). Most bacteria synthesize a wide range of molecules with potential inhibitory effects on bacteria and other organisms (Jack et al. 1995; Katz and Demain 1977; Nielsen and Sorensen 1997). These molecules include toxins, metabolic by-products such as organic acids, hydrogen peroxide, bacteriolytic enzymes, bacteriocin, or surfactin-type molecules, etc.

Surfactant molecules are cyclic lipopeptides with powerful antimicrobial properties including antifungal, antibacterial, antiviral, and antimycoplasma activity (Pooja and Swaranjit 2004). Bacteriocins, in turn, have the ability to interfere with the growth of bacteria phylogenetically related to the producing strain (Jack et al. 1995). Even though great progress has been achieved and extensive efforts are being made in the study of these molecules properties, no reports have yet been made on their anti-microsporidial activity.

The aim of the present study was to evaluate the toxicity of bacterial metabolites produced by bacteria isolated from bee's midgut on *A. mellifera* individuals and to determine their antiparasitic properties against *N. ceranae*.

Materials and methods

Bees collection and assays conditions

Experiments were conducted in the Arthropods Laboratory of the UNMdP, Mar del Plata, Argentina, between May and August 2008. Newly emerged *A. mellifera* bees were obtained from a healthy colony located in the experimental apiary J. J. Nagera of the Arthropods Laboratory (Lat.: 38°10′06″ S; Long.: 57°38′10″ W) and kept under incubator conditions during the experiment (33°C±0.79; 60%±3.3 HR).

N. ceranae characterization

The *Nosema* spores used for inoculation were purified from a laboratory strain developed in confined workers. Newly emerged bees were inoculated, supplied with sugar cane syrup 66%, and sacrificed to retrieve fresh spores at the time of assays 2 and 3. Spores were

molecularly characterized following Martin-Hernández et al. (2007). The PCR fragments were purified and sequenced in the Pro-papa Laboratory EEA, INTA, Balcarce, Argentina. Afterward, they were sequenced and matched with respect to those published in the GenBank (NCBI).

Individual infection was achieved using a technique similar to that proposed by Rinderer and Thomas (1976). This methodology enables a uniform handling of bees and obviates trofalaxia among individuals. By means of this technique, consumption is induced by previous starvation, thereby preventing oral structures manipulation to force intake and so minimizing the stress resulting from prolonged handling.

Bacterial metabolites

Surfactins Two cyclic lipopeptides synthesized by *Bacillus subtilis*, were employed S1 (Mori2) and S2 (C4) (Sabaté et al. 2009). They were recovered as precipitates of different cell-free supernatants (CFS), resulting from the acidification with 55 μL of concentrated HCl. Then, lipopeptides were extracted with methanol; the solvent was evaporated, and the precipitate was dissolved in sterile distilled water at pH8 at a final concentration of 10 mg/mL. These solutions were used as the surfactin samples 1 and 2, respectively.

Bacteriocins Two different samples were employed: bacteriocin B1, synthesized by *Enterococcus avium* DSMZ17511 (Audisio et al. 2005) and bacteriocin B2, produced by *Enterococcus faecium* CRL1385 (Audisio et al. 1999, 2001). Both samples were produced by each bacterium in BHI broth (Britania, Argentina) and used as they were present in the CFS applying no further purification steps. The CFS was assayed at the final pH after cell growth (4.5–5.0–5.5) and at pH6.5–7.0, adjusted with NaOH 2 N.

Metabolite concentrations were determined in duplicate by the critical dilution method (Daba et al. 1991), using *Listeria monocytogenes* 01/155 as the indicator culture. The resulting titers were expressed in arbitrary units per milliliter (AU ml^{-1}) and calculated as follows: $(1000)/(V_s \times D)$, where V_s is the bacteriocin volume tested (microliter) and D is the highest dilution still inhibiting cell growth. Also, the lactic acid present in the cell-free supernatant of enterococci cultures was quantified by HPLC.

Assay 1—toxicity of bacteriocin and surfactin on A. mellifera

Metabolites were administered ad libitum either through a sucrose syrup (SSY)–metabolite solution (50:50) or pure solution, providing candy (powdered sugar and glucose) as

carbon source. Individuals were subjected to diets from the first hour of imago, replacing solutions on a daily basis and providing fresh beebread. Diets consisted of surfactin S2 (C4) and bacteriocins B1 (pH5.5, 7) and B2 (pH4.5, 5.5, 7). Distilled water (surfactin was resuspended in this substance) and sterile culture medium (BHI broth, Britania, Argentina) were used as control treatments, since it was in the cultured medium where enterococci produced enterocins. The syrup was prepared at 66% *w/v* of sugar concentration.

Three replicates per treatment were produced, 15 bees in each one. The average solution consumption and mortality were recorded on a daily basis, quantifying the volume loss caused by evaporation.

Since infective spores were detected in pollen samples (Higes et al. 2008), a contamination control was implemented. It was made up of 15 newly emerged individuals, fed on pollen and sucrose syrup (66% *w/v*). Dead and alive individuals were analyzed under microscope at the end of the experiment, and spore presence was determined in the entire abdomen.

Assay 2—effect of bacteriocin and surfactin on *N. ceranae* spores viability after direct exposure

To analyze metabolites activity on *N. ceranae* spores after direct exposure, fresh spores were obtained (1.54×10^7 spores/mL) from individuals infected with the characterized strain. Spores were stored for 2 h at 4°C in distilled water. One milliliter (for each treatment) of the homogenized initial solution was centrifuged to precipitate the spores, discarding the supernatant. The volume was completed with the pure solutions of bacteriocin B1 (pH5.5 and 7.0), bacteriocin B2 (4.5, 5.5, and 7.0), or surfactin S2, and the spores were resuspended in these solutions. They were placed in darkness at room temperature (25–27°C) and shaken periodically for 40 h. Then, they were centrifuged once again to discard metabolites, completing the volume with distilled water and centrifuging again to wash the remaining substances. The spores were resuspended/homogenized in sucrose solution, and individual inoculation was performed. Ten microliters of solution were administered to each individual (1.54×10^5 spores) on day7 postemergence. In bacteriocins B1 and B2 (at both pH) assays, BHI broth was used as control solution. In the surfactin assay, in turn, distilled water was employed.

During the experiments, individuals were supplied with sugar cane syrup and fresh pollen. To quantify infection development, the surviving individuals were sacrificed 10 days post-infection (p.i.). The digestive tract was removed by pinching the last abdominal segments and cutting the midgut afterward. Spores number (parasitosis intensity) was individually quantified, counting the number of spores in suspension samples with a hemacytometer and light micros-

copy (Cantwell 1970). A contamination control treatment similar to the one described in assay 1 was also adopted.

Assay 3—effect of prolonged consumption of bacteriocins or surfactins on the development of *N. ceranae*

To analyze the effect of a prolonged consumption of metabolites on infection development, metabolites were administered ad libitum, including surfactin S1 in the treatments (not used in previous assays).

Solutions were administered upon individuals' emergence, replacing the volumes on a daily basis until the experiment conclusion. Metabolites were supplied in cane sugar syrup so as to force bees to incorporate them in each feeding. Fresh pollen was also administered ad libitum. The treatments are specified in Table 1. On day 7 (after emergence), bees were individually infected with 10 μL of inoculum. A low spore inoculum was preferred so as to prevent the rapid mortality registered in infected bees subjected to a pollen diet (Rinderer and Thomas 1976; Porrini 2008) and, by so doing, prolonging metabolites consumption. Solutions were replaced daily. Ninety individuals per treatment, confined to experimental wooden boxes with a plastic mesh ($11 \times 9 \times 6$ cm^3) participated in this assay.

On day 20 p.i., the surviving bees were sacrificed in order to individually quantify the number of spores in the midgut just as described in assay 2.

Solution's evaporation was controlled to correct the consumed volume, and a pollen contamination control was adopted, as described in assay 1.

Statistical analysis

The statistical analysis of the results obtained was conducted applying $\alpha = 0.05$. The spore loads recorded upon the experiment conclusion were compared to those of the control group. A *t* test or a Mann–Whitney test was performed if homoscedasticity had not been achieved. ANOVA and Tukey's test were applied to compare the average consumed volumes and the accumulated mortality.

Results

N. ceranae characterization

After PCR multiplex, the fragments obtained from the spores samples only exhibited a band corresponding to 218 bp, in agreement with Martín-Hernández et al. (2007). Sequencing results were entered in the GenBank BLASTn, which yielded 98% homology with *N. ceranae* (accession N° FJ425736).

Table 1 Treatments in assay 3

Surfactins	Bacteriocins	
S1/S2	B1	B2
S1 or S2: SSY (1:1)	B1 4.5 or B1 6.5: SSY (1:1)	B2 4.5 B2 6.5: SSY (1:1)
CTRL S or Cev: SSY (1:1)	CTRL B1 or Cev: SSY (1:1)	CTRL B2 or Cev: SSY (1:1)
Inoculum: 6,000 spores/bee stored 2 h at 4°C	Inoculum: 1,000 spores/bee stored 24 h at 4°C	Inoculum: 1,000 spores/bee stored 2 h at 4°C

Treatments in assay 3 (n=90 bees/treatment)

S1 surfactin 1 (Mori2), *S2* surfactin 2 (C4), *B1* bacteriocin from *E. avium* DSMZ17511 CFS (pH4.5, 6.5), *B2* bacteriocin from *E. faecium* CRL1385 CFS (pH4.5, 6.5), *SSY* sucrose syrup (66% *w/v*), *Ctrl S* substance control (H2Od), *Ctrl B1* and *B2* substance control (BHI broth), *Cev.* evaporation control (H₂Od or BHI)

Bacterial metabolites

Two different sample of bacteriocins and two of surfactins were assayed. Arbitrary units (AU) were used to estimate the substances titers, quantifying the inhibitory effect against *L. monocytogenes*. It was 102,400 AU/mL for CFS B1, 25,600 AU/mL for CFS B2, 2,000 AU/mL for surfactin S1 (Mori2), and 5,000 AU/mL for surfactin S2 (C4). The lactic acid concentration produced by *Enterococcus* and determined by HPLC analyses was close to 32 mM.

Toxicity analyses of bacteriocin or surfactin on *A. mellifera*

No significant mortality was recorded in treated individuals after 30 days of confinement with syrup administration (ANOVA, p=0.60) as well as in metabolites administered with no syrup (ANOVA, p=0.57). The average consumption of sucrose solution–metabolite (50:50) was 23.05 µL/bee/day and 15.3 µl/bee/day for the administration of the pure metabolites and candy. Both values did not diverge from the quantities consumed by the control group (ANOVA, p=0.58 and p=0.47, respectively).

The behavior of the treated individuals was normal, being active during inspections throughout the experiment, and with no excessive dejections during confinement.

It is worth noting that no pollen contamination with spores was reported, as no spores were found in contamination control bees at the end of the experiment.

Bacteriocin and surfactin effect on *N. ceranae* spore viability after direct exposure

Sample 2 of surfactin, synthesized by *B. subtilis* C4, was the only treatment that significantly reduced spores viability after a 40-h exposure, decreasing parasite intensity nearly 50% after 10 days of having infected the individual with the treated inoculum.

No significant bee mortality was accounted during the 17-day trial. Figure 1 depicts the intensity values of spores recorded for each individual, grouped according to an arbitrary scale of intensity assessment (number of spores in midgut): low intensity ($0–1×10^6$), medium intensity ($1–10×10^6$), and high intensity ($>10×10^6$). The general average intensity and intensities for each scale range and both deviations are listed in Table 2.

As it was determined before, no spores of *Nosema* were detected in the control group.

Effect of prolonged consumption of bacteriocin and surfactin on *N. ceranae* development

The antiparasitic effect recorded in assay 2 was also obtained when the diluted solution of surfactin S2 was administered ad libitum for 24 days. Figure 2 illustrates the intensity values registered for each individual, in agreement with the scale described above. Table 2 provides the average intensities for each scale range, the general average, and deviations; also the daily average of consumption and the cumulative mortality at 10 and 20 days p.i. (Table 3). In the contamination control, no spores were detected in individuals.

Discussion

The present study constitutes the first record of administration of "bacteriocin or surfactin" metabolites to *A. mellifera* individuals. It is also the first attempt to test the antiparasitic action of these compounds on Microsporidia in an invertebrate host.

The administered metabolites revealed no toxic effect on worker bees after 30 days of consumption, thereby demonstrating that the surfactins and bacteriocins interaction with the luminal environment and the microbiota incorporated with pollen had no negative effect on bees. Particularly worth of mention is the fact that the compounds safety was obtained, either by administering pure metabolites (resuspended surfactin precipitate), with no prior purification (bacteriocins and lactic acid), or by administering sugar syrup to both solutions. Metabolites adminis-
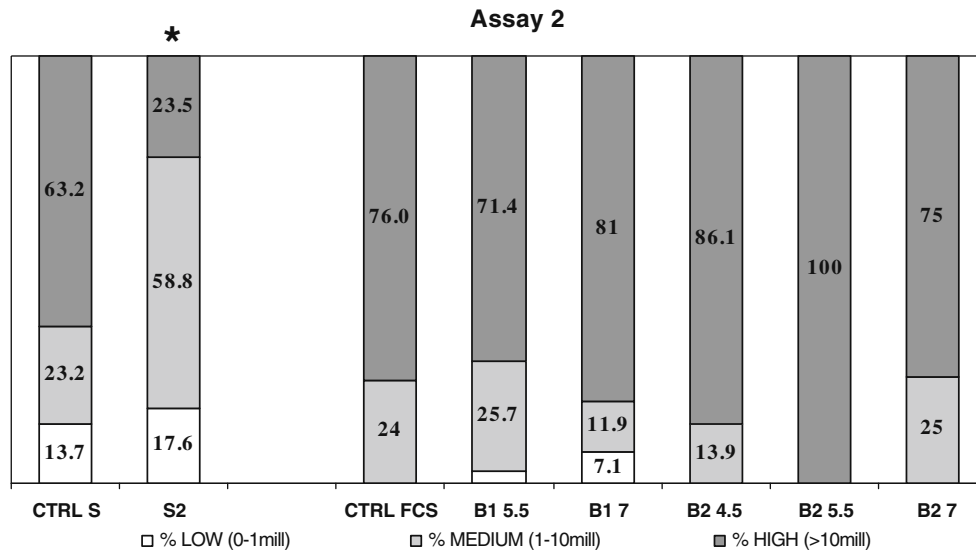
PERCENTAGES OF NOSEMA INFECTION
Assay 2

Fig. 1 Assay 2 ($n=90$ bees/treatment). Percentage of individuals at each level of infection on the arbitrary scale for each treatment (day 10 p.i.). %LOW, %MEDIUM, %HIGH: percentage of individuals with low ($0–1\times10^6$), medium ($1–10\times10^6$), or high ($>10\times10^6$) intensity of spores in midgut. *Bars* indicate percentages above 3%. *S2* surfactin 2 produced by C4, *B1* bacteriocin from *E. avium* DSMZ17511 (pH5.5, 7), *B2* bacteriocin from *E. faecium* CRL1385 (pH4.5, 5.5, 7), *CTRL* control groups of CFS and S2. *Asterisk* indicates significant differences between spore intensities when compared to the control group (Mann–Whitney $p<0.001$)

tration did not play a key role in bees behavior nor in the solutions' consumed volumes. Surfactin S2, the cyclic lipopeptide produced by *B. subtilis* C4, was the only compound administered that decreased parasitosis intensity after 40 h of metabolite–spore contact. This antiparasitic effect, obtained with 10 mg/ml of surfactin concentration, was observed once again when a metabolite concentration of 5 mg/ml was supplied ad libitum before and after bees infection. In this last experiment, the suppression in the parasitic load was significant, even when individuals were inoculated with a spore dose six times greater than that administered in bacteriocins treatments. As stated by Mayack and Naug (2008), who demonstrated an increased hunger level in *N. ceranae* infected bees, S2 was the only solution less consumed than the control solution during the post infection period.

Surfactin possesses antimicrobial and antifungal properties (Katz and Demain 1977; Joshi et al. 2008; Sabaté et al. 2009), one of the main effects being the detergent action on the lipid bilayer (Peypoux et al. 1999; Pooja and Swaranjit 2004). Therefore, one possible mechanism of antiparasitic action can be through a disruption in the integrity of the spore's coat. If proteinaceous exospore and chitinous endospore are permeable to metabolites, the plasmatic membrane integrity and germination or replication mechanisms are likely to have been affected. In our experiments, these surfactant properties could have affected not only the spores exposed to metabolite solutions before being inoculated on bees (assay 2) but also the untreated spores inoculated in assay 3 and then exposed to metabolite contact in the digestive tract. Also the spores generated in the midgut after parasite replication could become affected in this last experiment.

Table 2 Average values of intensities in assay 2

Average intensity	CTRL S2	S2	CTRL CFS	B1 5.5	B1 7	B2 4.5	B2 5.5	B2 7
GRAL	15.20±11.09	7.89±6.78[a]	18.25±10.99	16.30±9.68	20.97±11.99	20.88±10.35	34.32±11.20	16.99±9.25
% Low	0.32±0.38	0.10±0.08	–	0.46±0.23	0.08±0.04	–	–	–
% Medium	5.02±2.65	6.35±2.02	6.23±2.09	5.84±3.00	6.40±3.17	4.83±2.85	–	6.82±2.69
% High	22.16±7.51	17.57±6.31	22.04±9.86	20.69±7.62	24.96±9.44	23.47±8.60	34.32±11.20	20.38±8.07

Assay 2. Mean values of intensities (million spores) and standard deviation for general average intensity and intensities for each percentage range (day 10 p.i.)

S2 surfactin 2 produced by C4, *B1* bacteriocin of *E. avium* DSMZ17511 (pH5.5, 7), *B2* bacteriocin of *E. faecium* CRL1385 (pH4.5, 5.5, 7), *CTRL* Control groups of CFS and S2

[a] Indicates significant differences from control group when compared to intensity values (Mann–Whitney $p<0.001$)

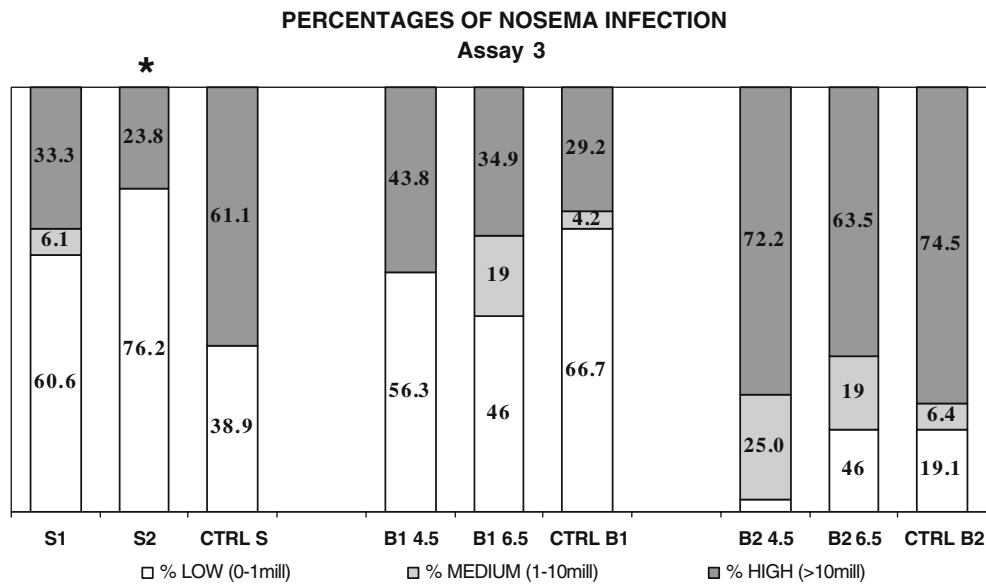## PERCENTAGES OF NOSEMA INFECTION
Assay 3

Fig. 2 Assay 3 ($n=90$ bees/treatment). Percentage of individuals at each level of infection on the arbitrary scale for each treatment (day 20 p.i.).%LOW, %MEDIUM, %HIGH: percentage of individuals with low ($0–1\times10^6$), medium ($1–10\times10^6$), or high ($>10\times10^6$) intensity of spores in midgut. *Bars* indicate percentages above 3%. *S1* surfactin 1 produced by Mori2, *S2* surfactin 2 produced by C4, *B1* bacteriocin of *E. avium* DSMZ17511 (pH4.5, 6.5), *B2* bacteriocin of *E. faecium* CRL1385 (pH4.5, 6.5), *CTRL* control groups of CFS (B1 and B2) and surfactins (S). *Asterisk* indicates significant differences between spore intensities when compared to control group (Mann–Whitney $p=0.038$)

In vitro studies have proven the powerful cytotoxicity effect of surfactin on Vero cells (kidney epithelial monkey cells) after 1-h exposure (From et al. 2007), as well as the disruption of the viral lipid membrane and partially of the capsid observed by electron microscopy after a few minutes (Vollenbroich et al. 1997). Indeed, further in vitro and in vivo experiments should be conducted so as to elucidate the optimum exposure time affecting spores viability and to determine the way in which surfactin interacts with the vegetative phase of the parasite's cycle. The possible enterotoxicity exerted on the bee midgut cells should also be addressed.

When the activity from two surfactins produced by two different *Bacillus* strains was compared, surfactin S2 was the only one exhibiting statistically significant antiparasitic

Table 3 Average values of daily intake intensity and mortality in assay 3

Average	Surfactins			Bacteriocins					
	S1	S2	CTRL S	B1 4.5	B1 6.5	CTRL B1	B2 4.5	B2 6.5	CTRL B2
GRAL.	11.5±13.3	12.96±12[a]	12.07±9.90	14.28± 17.26	10.32± 14.14	7.98± 13.68	22.80± 15.3	21.35± 16.94	23.13± 15.41
Low %	0.013±0.1	0.007±0.01	0.005±0.005	0.07±0.19	0.19±0.30	0.12±0.22	0.17± 0.001	–	0.11±0.12
Medium %	6±1.06	–	–	–	2.61±2.41	3.27±2.79	4.13±2.92	6.82±2.69	3.77±3.31
High %	26.03±9.4	26.63±7.6	19.45±7.13	32.54±8.79	27.88±9.44	26.62± 12.1	30.13± 11.1	20.38±8.07	30.71±9.4
Mortality									
% Days 10 & 17 p.i.	19.6 & 27.2 A	11.3 & 19.4 B	21.8 & 41.9 C	8.6 & 12.9	2.8 & 12.5	9.8 & 18	7 & 29.6	10.3 & 26.9	6 & 26.2
Daily intake p.i. (μL/bee)	30.43 AB	24.1 B	37.56 A	23.22 A	26.09 B	21.1 A	24.85 A	29.83 B	21.22 A

Assay 3. Mean values of intensities (million spores) and standard deviation for each percentage range and general average for treatments (day 17 p.i.). Daily intake of solutions and average values of cumulative mortality are indicated (10 and 17 days p.i.). Different capital letters indicate significant differences (Tukey's test; mortality $p<0.001$; daily intake $p<0.05$). Consumption values were obtained from post infection period

S1 surfactin 1 produced by Mori2, *S2* surfactin 2 produced by C4, *B1* bacteriocin of *E. avium* DSMZ17511 (pH4.5, 6.5), *B2* bacteriocin of *E. faecium* CRL1385 (pH4.5, 6.5), *CTRL* control groups of CFS (B1 and B2) and surfactins (S)

[a] Indicates significant differences with respect to control group (Mann–Whitney $p=0.038$)

activity, yet both metabolites (S1 and S2) extended the infected bees survival. This could be ascribed to a major reduction of the parasitic load, although other mechanisms able to prolong survival should not be discarded.

The antibacterial activity against *L. monocytogenes* provided by S2 was lower than that obtained with surfactin S1, the lipopeptide produced by *B. subtilis* Mori 2. Hence, in this particular case, the greater antibacterial effect could be correlated with the antiparasitic effect. Conversely, even when the bacteriocins tested yielded the highest AU values of bactericidal effect, no antiparasitic action was detected on *N. ceranae*. Despite the lack of anti-microsporidial activity, the results obtained are attractive, as it is the first time that bacteriocins potential has been tested on a target other than bacteria, microorganisms generally affected by such compounds (Jack et al. 1995).

Syrup acidification has been proposed as an effective practice against nosemosis development (Mottoul 1996); however, field and laboratory studies have demonstrated that *Nosema* prevalence or development is not affected by acidified feed (Vaillant 1989; Forsgren and Fries 2005). This work shows that bacteriocins administered on acidified medium have no effect on spore viability after contact with metabolites as well as on Nosema development after ad libitum consumption. The lactic acid concentration derived from the strains employed (32 mM) is not very high compared to that of other members of the family, such as *Lactobacillus* genera, which may produce concentrations close to 175 or 220 mM (Audisio et al. 2010). Therefore, further experiments using higher lactic acid concentrations, or administrating lactic acid exclusively, could offer different results.

Spores refrigerated at 8°C loose nearly 50% viability (Fries and Forsgren 2009) and degrade more quickly when stored at 4°C (Fenoy et al. 2009). Then it is reasonable that spores in assay 3, stored for 24 h at 4°C (treatments with bacteriocin produced by *E. avium*) yield less infectivity than those stored for 2 h.

This research shows that an alternative treatment for the nosemosis caused by *N. ceranae*, based on bacterial metabolite activity is feasible. Surfactin postulates as a molecule capable of reducing parasitosis development, acting either by direct exposure to spores or incorporated to the luminal medium of bee midgut. This characteristic, added to the non toxic effect on bees, renders its inclusion as an antiparasitic drug viable. During the experiment, two concentrations of surfactin were used: 50:50 in syrup 66% *w/v* (assays 1 and 3) or at maximum concentration (assays 1 and 2). Further analyses should be conducted so that minimum inhibitory concentrations are also addressed. Likewise, it would prove interesting to thoroughly analyze the effects of metabolites on bee physiology, such as the mechanisms of antiparasitic action against *N. ceranae* and other microsporidian parasites.

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