Journal of Invertebrate Pathology 137 (2016) 58–61

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

Brevibacillus laterosporus inside the insect body: Beneficial resident or pathogenic outsider?

Maria Giovanna Marche [a,b], Maria Elena Mura [b], Luca Ruiu [a,b,*]

[a] Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, University of Sassari, via E. De Nicola, 07100 Sassari, Italy
[b] Bioecopest, Tramariglio, Alghero, SS, Italy

ARTICLE INFO

Article history:
Received 31 March 2016
Revised 11 May 2016
Accepted 12 May 2016
Available online 12 May 2016

Keywords:
Entomopathogen
Microbiota
Detection
House fly
Honeybee
Integrated pest management

ABSTRACT

Brevibacillus laterosporus is an entomopathogenic bacterium showing varying degrees of virulence against diverse insect pests. Conversely, it is regarded as a beneficial component of the intestinal flora in different animals and in some insect species including the honeybee. *B. laterosporus* was detected through a species-specific PCR assay in the body of different insects, including *Apis mellifera* and *Bombus terrestris*. A strain isolated from a honeybee worker was pathogenic to the house fly *Musca domestica*, thus supporting the development of either mutualistic or pathogenic interactions of this bacterium with diverse insect species, as the result of a coevolutionary process.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Brevibacillus laterosporus Laubach is an ubiquitous spore-forming bacterium belonging to the *Brevibacillus brevis* phylogenetic cluster in the family Paenibacillaceae (Shida et al., 1996). Its spores are easily recognizable under phase microscopy because of the typical canoe-shaped parasporal body (CSPB) attached to one side (Hannay, 1957). Several strains of this species show significant pathogenicity against mollusks, nematodes, and insects in different orders, including Lepidoptera, Coleoptera, and Diptera (Ruiu, 2013). In line with other entomopathogens, different strains have varying degrees of virulence, and the insecticidal action appears to be a toxin-mediated process taking place in the gut of susceptible hosts (Ruiu et al., 2012). Conversely, *B. laterosporus* is regarded as a beneficial component of the intestinal flora influencing health in mammals (Hong et al., 2005) and birds (Porubcan, 2003), which is probably related to its antimicrobial properties and antagonism against hostile microbes. In the case of *Apis mellifera* L. (Hymenoptera: Apidae), *B. laterosporus* was initially considered to be a secondary invader in honeybees affected by European foulbrood (White, 1912). More recently, its inhibition potential against the etiological agent of American foulbrood of honeybees, *Paenibacillus larvae* White, was reported (Alippi and Reynaldi, 2006), and a functional role in maintaining and improving honeybee health was deduced (Hamdi and Daffonchio, 2011). Besides, *B. laterosporus* has only sporadically been isolated from the body of other insects, and its biological and evolutionary relationship with this arthropod class, represented by 31 orders and over 1,200,000 species, still need to be investigated.

The purpose of this study was to gather basic information about the distribution of this bacterial species within insect microbiota. Additional experiments were conducted to study the dynamics of *B. laterosporus* across different honeybee stages and to verify if strains isolated from honeybees can be pathogenic to insects that are normally susceptible to this bacterial species.

2. Materials and methods

2.1. Insect sampling and B. laterosporus detection

All insect specimens were field-collected in Sardinia (Italy) during spring 2015 and, after being identified, were subjected to total DNA extraction. Insect species were selected so as to include a wide variety of orders and families. Immature honeybee stages were collected from apiaries in the same areas. Individual or pooled samples were preliminarily surface-sterilized with sodium hypochlorite (0.2%) and homogenized in phosphate-buffered saline (PBS 1X) using sterile plastic pestles. The homogenate was filtered

* Corresponding author at: Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, University of Sassari, via E. De Nicola, 07100 Sassari, Italy.
E-mail address: lucaruiu@uniss.it (L. Ruiu).

http://dx.doi.org/10.1016/j.jip.2016.05.002
0022-2011/© 2016 Elsevier Inc. All rights reserved.

through sterile gauze to remove chitinous debris, and the remaining suspension was centrifuged at 15,000*g* for 15 min at 4 °C. The supernatant was discarded and the pellet was used for DNA extraction using Wizard Genomic DNA Purification Kit (Promega®, Madison, USA) in compliance with the manufacturer's instructions.

PCR reactions with primer pair *BlFind* (Bioecopest, Italy), designed to specifically detect *B. laterosporus* (Ruiu et al., 2016), were performed in a volume of 25 µl per sample containing 1× reaction buffer; 1.5 mM $MgCl^{2+}$; approximately 100 ng DNA; 0.4 µM of each primer; 0.2 mM of each dNTPs; and 0.75 U GoTaq® DNA Polymerase (Promega®, Madison, USA). PCRs were performed using a Veriti™ Thermal Cycler (Applied Biosystems) with the following thermal profile: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 57 °C for 45 s, and 72 °C for 45 s, followed by final extension at 72 °C for 10 min. PCR products were routinely analyzed by 1% (wt/vol) agarose gel electrophoresis using SYBR® Safe DNA stain (Life Technologies Europe BV, Bleiswijk, The Netherlands). The amplicons were observed in a UV light transilluminator. Samples with a clear DNA band of the expected size (709 bp) were considered positive. *B. laterosporus* reference strain ATCC 9141 was used as a positive control.

For relative quantification of *B. laterosporus* in honeybee, DNA from three biological replicates of insect pools (n = 10) was used in Quantitative Real-Time PCR (qPCR) reactions carried out employing *Power* SYBR® Green PCR Master Mix (Life Technologies) and primer pair *BlQuant* (Bioecopest, Italy) (Ruiu et al., 2016) in an Applied Biosystems 7900HT Fast Real-Time PCR System according to manufacturer's instructions, with the following cycle conditions: denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 60 °C for 1 min. Each sample was run in technical triplicates, after the primers efficiency had preliminarily been tested by standard curve and dissociation curve analyses (Pfaffl, 2009). β-actin was used after being validated as a suitable honeybee internal control gene for PCR normalization, in line with the results of preliminary tests involving also other candidate reference genes (Ryabov et al., 2014). The relative abundance of *B. laterosporus* population was calculated in agreement with Livak and Schmittgen (2001), and fold variations were analyzed by one-way ANOVA, followed by an LSD test for post-hoc comparison of means.

2.2. Bacterial isolation and bioassays

B. laterosporus was isolated from *A. mellifera* by streaking on LB agar plates serially diluted suspensions obtained homogenizing surface-sterilized *A. mellifera* workers as previously described. After incubation for 48–72 h at 30 °C and pure colony isolation, *B. laterosporus* was preliminarily identified under phase microscopy by detecting the typical canoe-shaped parasporal body attached to one side of the endospore. Morphological observations were confirmed by 16S rRNA gene amplification and sequencing according to Shida et al. (1996).

To assay the pathogenic properties of *B. laterosporus* isolated from the honeybee, bacteria were cultured on LB broth at 30 °C for 72 h up to sporulation (Ruiu et al., 2007), and spore suspensions were administered by ingestion to adult house flies provided by the Entomology Section of the University of Sassari (Italy). For this purpose, four replicated groups of 10 flies were fed a liquid diet (30% saccharose solution) incorporating 10^9 *B. laterosporus* spores/ml, administered by capillary tubes (7.5 µl/fly/day) as described in Ruiu et al. (2006). Fly mortality was assessed daily for 5 days in comparison with untreated control. Bioassays were repeated three times with different batches of flies and the entomopathogenic *B. laterosporus* strain NCIMB 41419 (=UNISS 18) was used as a positive control (Ruiu et al., 2012).

All statistical analyses were performed with SAS software (version 9.1) with significance level set at $\alpha = 0.05$ (SAS Institute, 2004). Mortality data were compared across treatments using one-way ANOVA, followed by an LSD test to separate means in each sampling interval.

3. Results and discussion

Insect samples belonging to 30 different species and 10 orders were subjected to *B. laterosporus* detection by species-specific PCR assay (Table 1). In addition to the honeybee, the bacterium was significantly detected in some females of the following species: *Icerya purchasi* Maskell (Homoptera: Margarodidae), *Calliphora vicina* Rob.-Des. (Diptera: Calliphoridae), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), *Andrena nigroaenea* Kirby (Hymenoptera: Andrenidae), and *Bombus terrestris* L. (Hymenoptera: Apidae). Whilst the relationship between *B. laterosporus* and these species still need to be clarified, our results corroborates previous studies where this bacterium was successfully isolated as a microbial pathogen from the red palm weevil *R. ferrugineus* (Salama et al., 2004) and as a component of resident gut microflora from the bumblebee *B. terrestris* (Přidal et al., 1997). Although the presence of this microorganism in the body of some insect species may just be exceptional, in the case of the honeybee, *B. laterosporus* was consistently found in association with different stages (larvae, pupae, emerging workers, foragers), disclosing a significantly higher relative abundance in foragers ($F_{3,26} = 10.30$, $P < 0.001$), as determined by qPCR analyses (Fig. 1). Our results confirm the endogenous presence of *B. laterosporus* during the whole honeybee life, which is in agreement with its envisioned role in contributing to the maintenance of a good gut microbiota (Hamdi et al., 2011) and to health improvement (Hamdi and Daffonchio, 2011). Because *B. laterosporus* was not found in honeybee eggs, our work does not provide evidence to support a possible trans-ovarian mechanism of transmission (Bright and Bulgheresi, 2010). While we cannot exclude that the titer of this bacterium in egg samples was below the PCR-detection threshold, this finding is in line with previous studies reporting a lower bacterial diversity and load in immature stages compared to adults (Martinson et al., 2012). On the other hand, there are different mechanisms associated with honeybee behavior in the colony (i.e., nursing, trophallaxis), through which honeybees could assume this bacterial species during their very first days of life. Besides, a higher *B. laterosporus* load in foragers might be a consequence of metabolic and physiological changes favouring gut microflora development or just the result of an increased environmental uptake (Powell et al., 2014).

Spore suspensions of a *B. laterosporus* strain isolated from a honeybee worker were toxic by ingestion to *Musca domestica* adults ($F_{2,33} = 751.58$, $P < 0.0001$) in comparison to untreated control, achieving 75.0 ± 2.9% mortality (mean ± SE) after 5 days, which was however lower than average mortality level (99.2 ± 0.8%) in groups of flies treated with the entomopathogenic strain NCIMB 41419 (Ruiu et al., 2007). This finding is in agreement with a well documented pathogenicity of *B. laterosporus* against the house fly, and with an expected strain-specific virulence (Oliveira et al., 2004).

Beyond highlighting another strain toxic to the house fly, this study reveals that a *B. laterosporus* strain that is regarded as a symbiont with probable beneficial effects on the honeybee, may significantly act as a pathogen against *M. domestica*. It is remarkable that *B. laterosporus* was never detected in house fly samples, while it was regularly found in the more advanced eusocial species, *B. terrestris* and *A. mellifera*. It can be inferred that during a coevolutionary process, this spore-forming bacterium may have developed specific virulence factors against certain insects

Download English Version:

https://daneshyari.com/en/article/4557557

Download Persian Version:

https://daneshyari.com/article/4557557

Daneshyari.com