Journal of Stored Products Research 53 (2013) 61–66

Contents lists available at SciVerse ScienceDirect

Journal of Stored Products Research

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

Potential of *Photorhabdus temperata* K122 bioinsecticide in protecting wheat flour against *Ephestia kuehniella*

Wafa Jallouli [a,*], Lobna Abdelkefi-Mesrati [a], Slim Tounsi [a], Samir Jaoua [a,b], Nabil Zouari [a,b]

[a] Biopesticides Team (LPAP), Centre of Biotechnology of Sfax, Sfax University, P.O. Box 1177, 3018 Sfax, Tunisia
[b] Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar

ARTICLE INFO

Article history:
Accepted 1 March 2013

Keywords:
Photorhabdus temperata K122
Ephestia kuehniella
Toxicity
Histopathological effect

ABSTRACT

The present study reports investigations on the insecticidal activity of the entomopathogenic bacterium *Photorhabdus temperata* K122 against the Mediterranean flour moth *Ephestia kuehniella*. Cultured in the optimized medium, *P. temperata* K122 cells aged 32 h exhibited 51% growth inhibition at a concentration of 9×10^8 cells/ml. However, culture must be prolonged up to 48 h incubation in the proteose peptone medium to reach only 28.6% inhibition. At the same concentration, no adult emergence was observed in the case of larvae feeding on wheat flour treated with the whole culture of *P. temperata* K122 after physical lysis. Interestingly, *P. temperata* K122 cells in the viable but non culturable (VBNC) state retained the same toxicity level as the culturable cells. At a high concentration of 12×10^8 cells/ml, 100% mortality of E. kuehniella larvae could be reached. Insect mortality is due to toxaemia as confirmed by the absence of Variants small colony (Vsm) or *P. temperata* colonies in *E. kuehniella* tissue. The investigation of the histopathological effect of *P. temperata* toxins on the gut of infected *E. kuehniella* larvae showed destruction of the gut epithelium, appearance of large cavities and cellular disintegration.

1. Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller is a serious pest of stored food products, especially whole and milled grains. *Ephestia kuehniella* attacks also all sorts of grains, dried fruits, cocoa, nuts and almonds (Jacob and Cox, 1977). Larvae cause direct damage by feeding and they also reduce product quality by their presence and by the production of frass and webbing (Johnson et al., 1997). Moreover, *E. kuehniella* larvae grow completely and form pupae within the same products they infest. Its close association with human foods makes it a prime target for biological control methods other than chemical pesticides. Endotoxins from *Bacillus thuringiensis* Berliner are the most used biopesticides (Schnepf et al., 1998). But, tolerance of the flour moth *E. kuehniella* can be induced by pre-exposure to a low concentration of *B. thuringiensis* formulation. Tolerance correlates with an elevated immune response and can be transmitted to offspring (Rahman et al., 2004). Therefore, we have focused our research on the use of the entomopathogenic bacterium *Photorhabdus temperata* K122 belonging to the *Enterobacteriacae* family (Wang and Dowds, 1993). The strain K122 of *P. temperata* was isolated from the nematode *Heterorhabditis downesii* K122 (Stock et al., 2002). When the infective dauer juvenile (IJ) nematode larvae carrying symbiotic *Photorhabdus* cells enter the insect haemocoel, the cells are directly released into the open blood system of the insect larvae. The released *Photorhabdus* cells propagate and kill the insect host (ffrench-Constant et al., 2003) by insecticidal toxins such as the toxin complexes (Tcs) (Waterfield et al., 2001), the "makes caterpillars floppy" (Mcf) toxins (Daborn et al., 2002), the "*Photorhabdus* insect-related" (Pir) toxins (Waterfield et al., 2005) or/and the "*Photorhabdus* virulence cassettes" (PVCs) (Yang et al., 2006).

Photorhabdus temperata K122 was also shown to exhibit toxicity independently from nematode larvae against the lepidopteran olive tree pest *Prays oleae* (L.) (Tounsi et al., 2006) and the sugarcane stalk borer *Diatraea saccharalis* (F.) (Carneiro et al., 2008). Moreover, *P. temperata* K122 was shown to be a suitable control agent when ingested by *E. kuehniella* larvae (Jallouli et al., 2008). Indeed, oral toxicity, assessed as the growth inhibition of *E. kuehniella* larvae fed with *P. temperata* K122 at 4×10^8 cells/ml, was 27%, when cultured in the optimized medium (OM) (Jallouli et al., 2008). Several studies have been already carried out to improve toxicity of *P. temperata* K122 by avoiding Vsm polymorphism (Jallouli et al., 2008) and addition of sodium chloride at 5 g/l (Jallouli et al., 2011). Improvement could be achieved also by control of the dissolved oxygen concentration during the fermentation process using the OM or the complex medium.

* Corresponding author. Tel./fax: +216 74 874 446.
E-mail address: jallouliwafa2@yahoo.fr (W. Jallouli).

0022-474X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jspr.2013.03.001

Indeed, toxicity reached 64.2% when two-stage oxygen supply was applied for *P. temperata* bioinsecticide production in the complex medium (Jallouli et al., 2012). However, there are no reports in the literature on the toxicity or histopathological effects of *P. temperata* K122 on *E. kuehniella* larvae. In the present study, we were interested to investigate the toxicity of *P. temperata* K122 cells, particularly when entered in the viable but non culturable (VBNC) state, the mortality elicited and the histopathological effect on the gut of infected *E. kuehniella* larvae.

2. Materials and methods

2.1. Strains

This work was carried out with *Photorhabdus temperata* strain K122, *Photorhabdus luminescens* sp. Q167/2 (Ehlers and Niemann, 1998) offered by courtesy of Dr. Mark Blight (CNRS, GIF sur Yvette, France) and *Escherichia coli* strain Top 10 (Amersham, France).

2.2. Media

Three media were used: Lauria-Bertani (LB) medium (Sambrook et al., 1989) with a composition of (g/l): peptone, 10; yeast extract, 5; and NaCl, 5, 2% proteose peptone medium (Fluka 29185) and the OM composed of (g/l): Na_2HPO_4 $2H_2O$, 1.2; NH_4Cl, 1.07; KCl, 0.35; $C_6H_5O_7Na_3$ $2H_2O$, 0.5; Na_2SO_4, 0.28; $MgCl_2$, 0.12; $CaCl_2$, 0.05; NaCl, 5; $FeCl_3$ $6H_2O$, 0.0017; yeast extract, 10; and glucose monohydrate, 5 (Jallouli et al., 2008). Glucose stock solution (20%) was autoclaved separately and later added to the OM. The pH of the different media was adjusted to seven using HCl 1 N or NaOH 1 N before sterilization during 20 min at 121 °C.

2.3. Inocula preparation

The inocula were prepared as follows: A 48-h old *Photorhabdus* strain colony was dispersed in 3 ml of LB liquid medium and incubated overnight at 30 °C. For *E. coli* Top 10, a 24-h old colony was dispersed in 3 ml of LB liquid medium and incubated overnight at 37 °C. The culture broth was used to inoculate the culture medium at an initial optical density (OD) of 0.025 at 725 nm as previously optimized (Jallouli et al., 2008). Cultures were developed in 500 ml shake flasks containing 85 ml of the culture medium, incubated at 30 °C and 37 °C in a rotary shaker set at 200 rpm for *Photorhabdus* strains and *E. coli*, respectively. Shake flasks were incubated according to the culture condition for 72 h.

2.4. Biomass determination

Total cell count was determined by preparing dilutions of the sample and cells were counted microscopically using a Thoma counting microscope (ZUZI) at 100-fold magnification. Biomass was determined also by measuring the OD at 725 nm with a spectrophotometer (Biorad).

2.5. Bioassays

Bioassays were carried out using first-instar larvae of *E. kuehniella*. Ten larvae were weighed before they were transferred to a sterile Petri dish containing 1 g of wheat flour mixed with 800 µl of diluted sample at a final concentration of 9×10^8 cells/ml *P. temperata* K122 culture broth. After incubation at 26 °C for 7 days, the ten-larval weight was recorded. Oral toxicity was assessed as the growth inhibition of the fed larvae with *P. temperata* K122, compared to growth of similar larvae number fed with the non-toxic *P. luminescens* Q167/2. It was calculated as follows:

$$\text{Growth inhibition}(\%) = (((GQ167/2 - GK122)/GQ167/2)) \times 100$$

GQ167/2: (weight of the ten larvae fed with *P. luminescens* Q167/2 after 7 days) − (weight of the ten larvae fed with *P. luminescens* Q167/2 at $t = 0$).

GK122: (weight of the ten larvae fed with *P. temperata* K122 after 7 days) − (weight of the ten larvae fed with *P. temperata* K122 at $t = 0$).

The values presented in the results section are the average of the weight of 30 larvae collected from three replicates carried out with 10 larvae each.

2.6. Bacterial colonization

The number of recoverable bacteria within infected tissues of *E. kuehniella* larvae was determined after 7 days of incubation at 26 °C. Replicates of ten fed larvae with *P. temperata* K122 and *P. luminescens* Q167/2 were dissected after treatment. Each larva was surface sterilized with 70% ethanol and then bled to collect the internal organs. The gut and the fat body were homogenized in PBS using a hand-held Potter homogenizer. To determine the number of recoverable bacteria, serial dilutions of tissue homogenates were prepared with PBS and plated onto LB agar and LB agar supplemented with catalase at 2000 Units/plate to enhance the recovery of *P. temperata* K122 in the VBNC state (Jallouli et al., 2010).

2.7. Preparation and sectioning of insects tissues

After exposure to *P. temperata* K122 cells at a concentration of 12×10^8 cells/ml for 5 days, first-instar larvae of *E. kuehniella* were chilled on ice for 15 min. The guts were then excised and placed in 10% formaldehyde then dehydrated by increasing ethanol concentration, rinsed with 100% toluene, and embedded in paraffin wax. Sections (5 µm) were placed in carriers loaded with a mix of 1.5% egg albumin and 3% glycerol in distilled water. For histopathological localization of toxins effect, the sections already de-paraffinated by 100% toluene were stained with hematoxylineosin as reported by Ruiz et al. (2004).

2.8. Statistical analysis

All the results related to determination of bioassays and bacterial colonization were the average of three replicates of three separate experiments. They were statistically analyzed by SPSS software (version 100) using Duncan test performed after analysis of variance (ANOVA).

3. Results

3.1. Comparison of P. temperata K122 pathogenicity against E. kuehniella with P. luminescens Q167/2 and E. coli Top 10

Toxicity evaluation of *P. temperata* strain K122, P. luminescens strain Q167/2 and *E. coli* strain Top 10 was carried out by using the proteose peptone medium known to be a suitable medium for toxins production (Lining et al., 1999). By using similar cell counts of 9×10^8 cells/ml, it was shown that *P. temperata* cells exhibited a maximal growth inhibition of 28.6% after 48 h of incubation (Fig. 1). However, the strains Q167/2 and Top 10 were not toxic against *E. kuehniella* larvae even at high cell densities of 12×10^8 cells/ml. To confirm non toxicity of these strains, different culture fractions were prepared (supernatant, bacterial culture, washed bacterial cells and finally bacterial culture after physical lysis) and tested for