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RESEARCH

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Possible repair of UV-damaged *Spodoptera littoralis* nucleopolyhedrovirus DNA via generation of mutant carrying photolyase gene with DNA repair activity

Wael Elmenofy[1,3*], Lamis Gomaa[1], Nourhan Albeltagy[1], Norhan Yasser[1], Alshaimaa Mahmoud[1] and Engy Osman[2]

Abstract

Background Baculoviruses can be inactivated by ultraviolet (UV) light, which limits their application as an efficient biopesticide. In an attempt to enhance virus resistance to UV inactivation, a photolyase gene, isolated from *Spodoptera litura* granulovirus (SpliGV), was expressed in a photolyase deficient *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) and the infectivity of recombinant virus expressing *phr* gene was determined after exposure to UV irradiation.

Results Expression of the photolyase resulted in a fivefold resistance of virus occlusion bodies of the recombinant virus (vSpli-phr) compared to the wild type SpliNPV upon exposure to UV irradiation as measured by LC_{50} value when fed on *S. littoralis* neonate. Furthermore, the recombinant virus expressing *phr* showed a significant decrease in the time required for lethality than the control virus with about 22 h, as measured by ST_{50} value. Hence, expression of photolyase via vSpli-phr relatively may rescue the viral DNA damage caused by UV irradiation.

Conclusions This study provided the evidence that the generated recombinant virus encoding a functioning photolyase, which may have implications for the development of a new generation of baculoviruses with enhanced insect pest management capabilities.

Keywords *Spodoptera littoralis* nucleopolyhedrovirus, *Spodoptera litura* granulovirus, Ultraviolet (UV) irradiation, Biological control, Photolyase

Background

Baculoviruses form a large group of double-stranded DNA that primarily infects phytophagous insects. These viruses are frequently utilized as vectors for the mass production of foreign proteins and as biocontrol agents for insect pests (Inceoglu et al. 2006). Different reports have demonstrated that UV radiation quickly inactivates baculoviruses occlusion bodies (OBs) on plant leaves upon spreading of virus OBs on the infested fields (Sun et al. 2004). Due to the high costs associated with adding UV-protective compounds to baculovirus formulations, this UV-sensitivity constitutes a significant barrier to the widespread adoption of baculoviruses as biocontrol agents (Baskaran et al. 1997). Sunlight (i.e., UV radiation) is a major factor in the inactivation of baculoviruses in the field (van Oers et al. 2008). Cyclobutane pyrimidine

*Correspondence:
Wael Elmenofy
wael.elmenofy@ageri.sci.eg; welmenofy@kfu.edu.sa
[1] Agricultural Genetic Engineering Research Institute, Agricultural Research Center, Giza 12619, Egypt
[2] Faculty of Agriculture, Cairo University, 9 Gamaa St., Giza 12619, Egypt
[3] Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia

dimers (CPDs) and (6-4) photoproducts ((6-4)PPs) are two forms of DNA lesions that are introduced when organisms are exposed to UV radiation with a wavelength between 250 and 320 nm (Weber 2005).

CPD photolyases are enzymes that repair CPDs by photoreactivating them in the presence of visible light. Photolyases can be categorized into two groups based on the divergence of their amino acid sequences (Kimura et al. 2004). Cryptochromes, (6-4)PP-photolyases from plants and animals (ClassII), and CPD-photolyases from bacteria and other microbial eukaryotes make up the extremely diverse (Class I). As reported in case of plants (Kimura et al. 2004), and fowlpox virus (Srinivasan and Tripathy 2005), CPD-photolyases are crucial for protecting organisms and certain viruses from UV radiation. Both viruses and parasites have been found to have class II photolyases; these include a number of entomo-pox viruses (Bennett et al. 2003), and a microsporidian intracellular insect parasite (Slamovits and Keeling 2004).

The genomes of the baculoviruses Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV), Trichoplusia ni (Tn) SNPV, and most recently *Spodoptera litura* granulovirus (SpliGV) (GenBank DQ288858) have also been identified to include putative *phr* genes. The ChchNPV genome was shown to have two ORFs that resemble class II CPD-photolyases (van Oers et al. 2004). The amino acid sequences of these ORFs, Cc-phr1 (ORF68) and Cc-phr2 (ORF72), showed 48% similarity.

Recently, a photolyase gene (*phr*) homolog from *Spodoptera littoralis* granulovirus (SpliGV-EG1) was fully characterized. Obtained results suggested that the SpliGV photolyase may have an activity toward SpliGV UV-resistance and this activity is crucial for enhancing the persistence of SpliGV OBs in the field under UV light inactivation stress (Elmenofy et al. 2022).

In the present study, a class II CPD-photolyase of *S. littoralis* granulovirus (SpliGV-EG1, an Egyptian isolate) was analyzed in rescue experiment in a photolyase deficient *S. littoralis* nucleopolyhedrovirus. The, SpliGV photolyase was transposed into SpliNPV genome, purified and further characterized with respect to biological activity.

Methods

Insect and viruses

The cotton leaf worm *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae) used for *S. littoralis* NPV & *S. littoralis* GV propagation and bioassay experiments were derived from the insect rearing facility of the Agricultural Genetic Engineering Research Institute, Agricultural Research Center (ARC), Giza, Egypt. For the rearing of *S. littoralis* larvae with different instars, insects egg clusters were kept in paper towels for few days subsequently transferred to an empty glass bottle for egg hatching. Next day, the new hatched larvae (neonates) were reared on semi-artificial diet until reached 4th instar (7–10 days) in plastic cups. The 4th instar *S. littoralis* larvae were used subsequently for virus propagation.

PCR amplification of photolyase gene using SpliGV genomic DNA

One set of photolyase gene (*phr*)-specific primers was designed based on the published sequence of *S. litura* GV (SpltGV) isolate sequence (Acc. No. OM256472.1) and used to amplify *phr* full length gene using SpliGV virus genomic DNA as a template. The *phr* gene was amplified by High-fidelity PCR, using the primer set SpliGV phr-F (5'-***CTGCAG***ATGGATTCCACGTTCGCGCAACT-3') and SpliGV phr -R (5′-**AGGCCT** TTATTTTCTGTA TTGGTTGATAT-3') with *Pst*I and *Stu*I on the 5′-end of the forward and reverse primer, respectively, (bold). High-fidelity PCR reaction was performed by using high-fidelity reaction mixture as follow: 1X Phusion® HF Buffer, 0.2 mM dNTPs, 25 pmol of each forward and reverse primers, 500 ng SpliGV DNA and 2U Phusion® DNA Polymerase (ThermoFisher, USA). The reaction was completed up to 50 µl using ddH2O. The PCR was performed under the following conditions: 98 °C for 30 s, followed by 30 amplification cycles of 98 °C for 10 s, 54 °C for 30 s, and an elongation stage of 72 °C for 1 min, followed by final extension at 72 °C for 10 min. Obtained PCR fragment was separated on 1% TAE agarose gel and stained with ethidium bromide (0.1 µg/ml).

Transferring of the photolyase gene through site-specific transposition into SpliNPV genome and selection of mutants

The PCR-amplified SpliGV photolyase gene was cloned into the CloneJET PCR Cloning vector and subsequently into pFB-GFP (ie-2)-hr5-(ie-1) vector under ie-1 promoter using *Pst*I and *Stu*I sites. Transformants carrying photolyase gene was selected out of 10 screened colonies. For the transposition of photolyase gene (*phr*) into Splibac, one nanogram of the pFB-GFP(ie-2)-hr5-(ie-1)-phr plasmid DNA was transformed into competent DH10β cells, which contains Splibac and the helper plasmid contains transposase gene, to facilitate transposition of the recombinant cassette into the bacmid mini-*att*Tn7 site within 4 h shaking at 37 °C. Five serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) of the obtained culture were prepared and spread on LB-Agar plates contains (15 µg Kanamycin, 7 µg Gentamycin and 10 µg Tetracycline). To facilitate blue/white colonies screening, X-Gal (100 mg/ml) and IPTG (40 µg/ml) were added to each plate before spreading of the culture. All plates were incubated for 48 h at 37 °C.

To verify successful transposition, eight clear white colonies were PCR analyzed using specific primer pair for *phr* gene. The recombinant bacmid DNA was isolated from 2 ml of overnight culture of one single positive white colony following the manufacture instruction (Thermofisher). One positive colony was selected and cultured in 50 ml LB medium with appropriate activity overnight at 37 °C, and in the next day, midi-preparation of DNA was carried out to be subsequently used for *S. littoralis* 4th instar larvae transfection in order to examine the infection capacity of the recombinant virus. Obtained mutant virus harboring photolyase gene was injected into 4th instar *S. littoralis* larvae hemocoel using cellfectin complex for virus assembly and OBs production.

Transfection of mutant virus into *S. littoralis* larvae

Larvae were disinfected with 0.4 Hyamine (Sigma-Aldrich, USA) before injection, and they were subsequently given a 3 min anesthetic with diethyl ether vapor in a closed Petri dish. Using a 10 µl syringe, an aliquot of 1 µl of the mixture was injected into the hemocoel of 4th instar *S. littoralis* larvae. Each treatment contains fifteen 4th instar larvae. Following injection, larvae were reared on fresh semi-artificial medium and left until larval death. After 7 days, *S. littoralis* cadavers were subjected for OB purification and the purified OBs were used to inoculate more *S. littoralis* larvae. Larvae cadavers died from viral infection were kept at − 20 °C until OBs purification, which were subsequently used to calculate the LC_{50} and ST_{50} values.

Exposure of the mutant virus OBs to UV-irradiation

The repair activity of the mutant virus carrying *phr* gene was determined after exposure to UV-Irradiation, using low timing (90 s) via determination of LC_{50} toward neonates of *S. littoralis* larvae. For this, purified mutant vSpli-phr OBs were exposed to UV-Irradiation by putting them in a 6-well plate in front of UV-Lamb for 90 s using Philips TUV 15W G5 T8 germicidal bulbs at 254 nm. Then, the treated virus OBs were collected and subjected for LC_{50} determination.

Determining the LC_{50} & ST_{50} of the mutant virus before and after exposure to UV-irradiation

Spodoptera littoralis first instar larvae were infected with a concentration range causing 5–95% mortality in a 7 day post-infection. For each virus concentration, 35–40 larvae were infected and each bioassay was three times independently repeated. The final virus concentrations performed were 10^3, 5×10^3, 10^4, 5×10^4, 10^5 and 5×10^5 OBs/ml. Bioassays were performed in autoclavable 50-well plates containing 45 ml of artificial diet (valdi-Sender 1974), mixed with 5 ml of OBs suspension of different concentrations per plate. For control plates, the diet was mixed with 5 ml H_2O instead of OBs suspension. Bioassay plates containing diet were kept open overnight to allow evaporation of excess humidity. On the following day, larvae per virus concentration (i.e., treatment) were individually placed in each well of a bioassay tray. After adding of neonates on all plates, all plates were closed tightly and were kept at 26 °C with a 16/8-h light/dark photoperiod. Larvae that died within the first 24 h of the assay were assumed to have died from handling and were not included in the scoring. Mortality was scored on day 7 post-infection (p.i.). On the same context, the ST_{50} value was calculated by infection of 5 day's old *S. littoralis* larvae using LC_{80} value. Mortality was monitored every 12 h until larval death or pupation.

Statistical analysis

The EPA Probit analysis program (Version 1.5) was used for calculation of LC_{50} for SpliNPV as well as the mutant carrying *phr* gene before and after exposure to UV-Irradiation for 90 s (Robertson and Preisler 1992). Furthermore, the ST50 value was determined using the Kaplan–Meier estimator analysis method (Kaplan–Meier 1958).

Results

Generation of vSpli-phr mutant carrying copy of SpliGV photolyase gene

As shown in Fig. 1a, the PCR products by the specific amplification of *phr* gene using SpliGV DNA as a template were placed in agarose gel from left-to-right orientation as well as the negative control closed to the control lane. The PCR amplicon showed clear and dense band corresponding to the expected size of 1.4 kbp, which was cloned into pFB-GFP(ie-2)-hr5-(ie-1) vector under ie-1 promoter via *Stu*I/*Pst*I digestion generating the recombinant vector pFB-ie2-GFP-ie1-phr of ~ 8.8 kb (Fig. 1b). Subsequently, the transposition of *phr* gene into *att*-T7 transposition site of Splibac, previously generated using SpliNPV genomic DNA, was performed (data not shown). A schematic outline for the transposition strategy is presented in Fig. 1c. The generated recombinant vector denoted pFB-GFP(ie-2)-hr5-(ie-1)-phr was transformed into DH10BAC competent cells, that carrying Splibac and helper plasmid (carrying transposase gene), to allow transposition of *phr* gene cassette into *att*-T7 transposition site located in Splibac.

To verify successful transposition of *phr* cassette into Splibac, eight clear white colonies were obtained and analyzed by PCR using the specific primer pair for *phr* gene. The recombinant Spli-bac DNA carrying *phr* gene named vSpli-phr was isolated from overnight culture generated using single positive white colony. The vSpli-phr

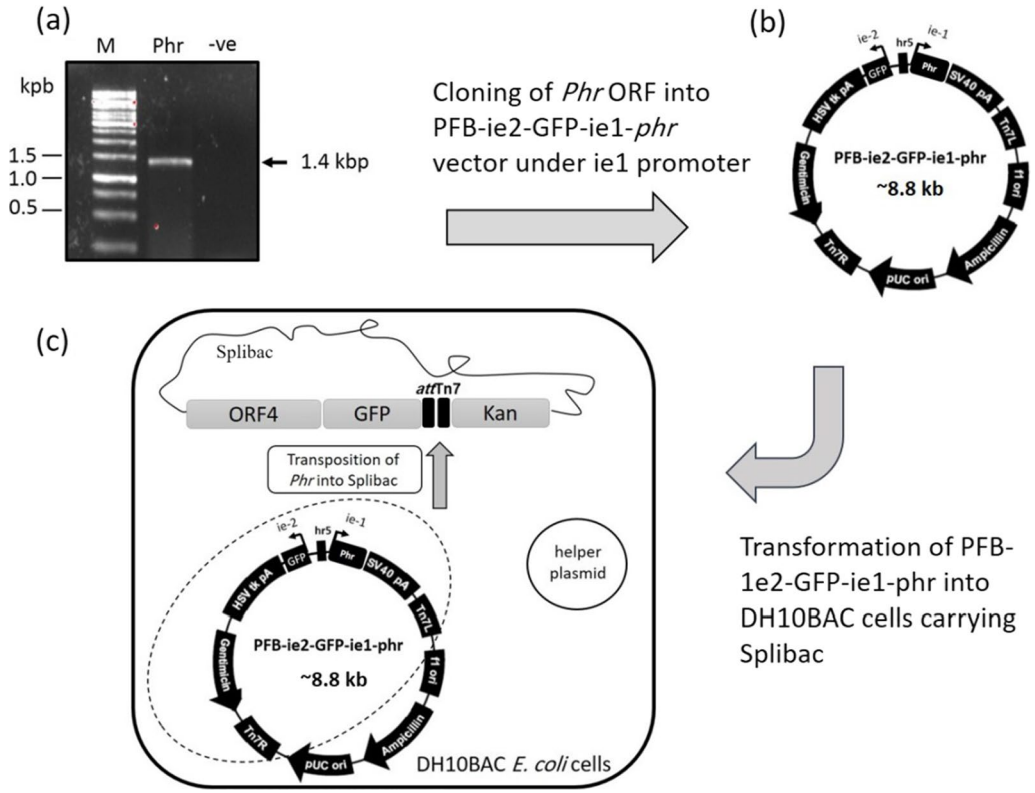

Fig. 1 Schematic representation shows construction of the recombinant vSpli-phr harboring the *phr* gene ORF. **a** A total of 1% agarose gel shows the amplified photolyase gene ORF (*phr*) of 1.4 kpb. Lane (—ve): PCR negative control. M: 1 kb ladder. **b** Plasmid map shows the *phr* gene that was cloned downstream from the ie-1 promoter of the modified pFastBac-Dual vector generating the hybrid plasmid PFB-ie2-GFP-ie1-phr of about 8.8 kb. **c** Transposition of the *phr* cassette into the *att*Tn7 transposition site of the Splibac in the presence of the helper plasmid. Dotted line circle shows the *phr* cassette

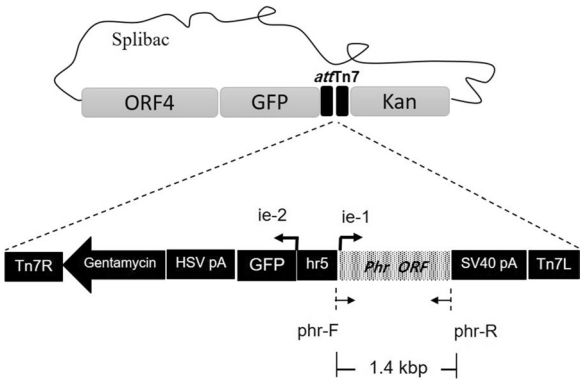

Fig. 2 Generation and evaluation of the recombinant virus Spli-phr carrying the photolyase gene in its genome. The drawing shows the location of the phr ORF under the control of ie1 promoter. Arrows shows the location of primers used for the verification of the selected clones to give a fragment of 1.4 kbp corresponding to the *phr* gene ORF in some clones

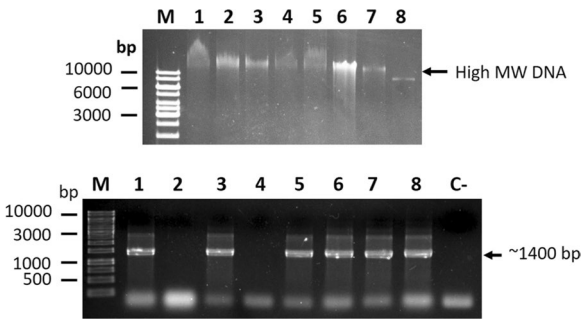

Fig. 3 A stained agarose gels show the verification of different selected colonies upon transformation of pFB-ie2-GFP-ie1-phr plasmid into DH10β cells carrying Splibac. The upper agarose gel shows High Molecular Weight (HMW) genomic DNA isolated from 8 screened colonies. The lower agarose gel shows amplification of *phr* gene ORF of eight selected colonies using phr-F and phr-R primers. M: 1 kbp DNA ladder. Arrow shows a clear band of 1.4 kbp corresponding to the *phr* gene ORF

construct and location of primer pairs used for verification of the recombinant virus is represented in Fig. 2. As shown in Fig. 3 (upper gel), high molecular weight DNA

was isolated from eight putative colonies and subjected for PCR analysis using *Phr* specific primer pair for verification of the isolated clones. A successful detection of mutants by PCR using *phr* gene specific primers is shown in Fig. 3 (lower gel). Different colonies gave the expected PCR fragments of ~ 1400 bp corresponding to *phr* gene suggested the successful transposition of *phr* gene into Splibac generating the recombinant virus vSpli-phr. Enough amount of the mutant vSpli-phr DNA was prepared and used for subsequent *S. littoralis* virus transfection for virus assembly and OBs propagation.

Assembly of the mutant vSpli-phr OBs

In order to evaluate the replication efficiency of vSpli-phr, and to produce viral OBs, the purified genomic DNA of the mutant vSpli-phr was injected into the hemocoel of 10 4th instar *S. littoralis* larvae as a liposome complex. As shown in Fig. 4, injection using vSpli-phr in a lipofectin complex resulted in virus infection symptoms after 5–7 days post-injection. Occlusion bodies were purified from cadavers larvae and used for inoculation of 4th instar larvae to test their *per os* infectivity and to prepare more OBs. The results showed that *S. littoralis* larvae died of viral infection symptoms after larvae re-inoculation confirming that OBs isolated from *S. littoralis* larvae injected with vSpli-phr were perorally infectious.

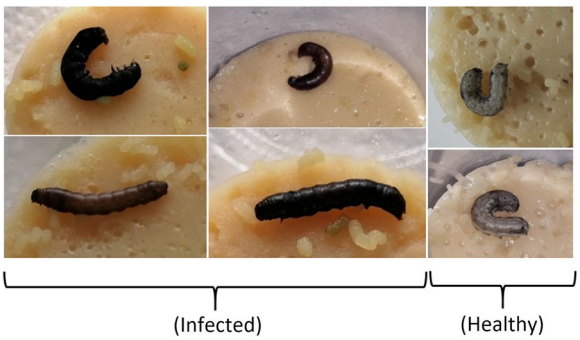

Fig. 4 Photographs showing differences between infected *Spodoptera littoralis* 4th instar larvae (to the left) and healthy larvae (to the right), upon transfection of the recombinant virus vSpli-phr DNA into larval hemocoel by injection. After 5–7 days of larval infection, the infected *S. littoralis* larvae became visibly swollen with fluids as typical symptoms of baculovirus infection

Median lethal concentration (LC_{50})

The virulence of the mutant virus (vSpli-phr) as well as the wild type SpliNPV was determined, using median lethal concentration (LC_{50}) and median survival time (ST_{50}) value. As shown in Table 1, the calculated LC_{50} was 1.3×10^4 (OBs/ml) for SpliNPV before exposure to UV-Irradiation and 1.6×10^5 (OBs/ml) after exposure to UV-Irradiation for 90 s. These results suggested that the SpliNPV OBs were dramatically affected by exposure to UV-Irradiation with about 11.96-fold lower activity. Since the wild-type SpliNPV virus lacks the photolyase repair activity, decreasing of OBs activity due to exposure to UV-Irradiation was expected.

However, the generated mutant virus vSpli-phr that harboring a copy of *phr* gene in its genome showed resistance against UV-Irradiation. As shown in Table 1, the results showed that the calculated LC_{50} value was 22,908.5 (OB/ml) for the mutant virus harboring the *phr* gene after exposure to UV-irradiation. This results suggested that the insertion of the *phr* gene into SpliNPV genome relatively improved the resistance to UV-irradiation with about fivefold.

Median survival time (ST_{50})

The median survival time (ST_{50}) was determined in order to examine the speed of kill of the generated mutant Spli-phr that harboring a copy of *phr* gene in its genome after exposure to UV-Irradiation compared with the wild type SpliNPV. The calculated ST_{50} values were 120 h for SpliNPV before exposure to UV-irradiation and 144 h for SpliNPV after exposure to UV-Irradiation. On the other hand, the mutant Spli-phr showed an ST_{50} value of 120 h after exposure to UV-Irradiation (Table 2). These results suggested that, after exposure to UV-Irradiation, the recombinant Spli-phr needs less time (120 h) to reach 50% mortality of the treated insects compared to the wild type SpliNPV, which needed more time (144 h) to reach 50% mortality of *S. littoralis* larvae. Hence, this confirms that the photolyase protein, expressed by the phr gene in SpliNPV-phr mutant, may improve the resistance of

Table 1 Median lethal concentration (LC_50) of *Spodoptera littoralis* neonates infected with OBs derived from SpliNPV or vSpli-phr viruses after 7 days p.i

Virus	Nr	LC_{50} (CI)	Slope (SE)	χ^2
SpliNPV before exposure to UV	631	13,312.4 (8589. 3–19,775.9)	0.699 (0.058)	7.885
SpliNPV after exposure to UV	559	159,272.4 (110,342–211,924.8)	3.65 (0.632)	9.488
SpliNPV-phr after exposure to UV	536	22,908.5 (648.6–63,304.9)	1.9 (0.566)	9.488

Nr Number of tested larvae. The LC_{50} of probit analysis and the slopes of the probit lines are given. CI 95% confidence interval; *SE* Standard error. LC_{50} value and confidence limits are given in OBs/ml. All Wald χ^2 tests (DF = 1) were significant at $P < 0.0001$ according to Robertson and Preisler (1992) [29]

Table 2 Kaplan–Meier estimation of ST50 for *Spodoptera littoralis* larvae infected with OBs derived from SpliNPV or vSpli-phr in a single infection

Virus	No	LC_{85} (OBs/ml)	ST_{50} (h)	CI (Lower–Upper)
SpliNPV before exposure to UV	110	79,604.492	120	115–125
SpliNPV after exposure to UV	108	306,009.844	144	140–150
SpliNPV-phr after exposure to UV	112	404,504.250	120	115–125

Spli-phr to UV-Irradiation than the wild type SpliNPV under the same condition.

Discussion

Due to their high virulence, host specificity, environmental friendliness, and safety for animals other than the target, baculoviruses are used as biocontrol agents of insect pests. The DNA damage is assumed to be the main mechanism by which UV light inactivates baculoviruses. In the present study, the expression of photolyase protein via integration of *phr* coding sequence into SpliNPV that would rescue the viral DNA damaged by UV irradiation was hypothesized.

A recombinant SpliNPV virus with DNA repair activity via incorporation and expression of a *phr* gene encoding photolyase enzyme, which was isolated from SpliGV genome, in order to determine the possible improvement of SpliNPV resistance toward UV-Irradiation, was constructed. The SpliNPV-phr bacmid via site-specific transposition between SpliNPV bacmid and pFB-GFP(ie-2)-hr5-(ie-1)-phr vector that harboring photolyase coding sequence under the control of the early ie-1 promoter was successfully constructed. The generated SpliNPV-phr bacmid was transfected into *S. littoralis* larvae hemocoel for virus assembly and OBs production. Obtained recombinant vSpli-phr can be propagated in *E. coli* as a BAC, and it is still infectious to *S. littoralis* larvae. Obtained results, using virus occlusion bodies (OBs), support our hypothesis. The obtained viral OBs expressing photolyase protein were approximately fivefold more resistant to inactivation by UV irradiation than wild type SpliNPV as measured using LC_{50} value. These results indicated that the presence of PHR has stimulated the repair of the damage resulted from the direct exposure to UV irradiation. Even if a fivefold improvement was not that much. These results suggested that our concept had a chance of being successful. However, the question here, how this fivefold reduction in UV inactivation would affect a natural infection in the field? But if the recombinant virus harboring the *phr* gene had an advantage fivefold to resist the damage caused by UV-Irradiation than wild type virus, this would be a considerable improvement.

Direct effects of UV radiation on biopolymers were linked to the virus's loss of potency (Ignoffo and Garcia 1994). A DNA break or the constructions of pyrimidine dimers inside the DNA chain, as well as additional photoproducts such pyrimidine photohydrates, thymine glycol, and DNA-DNA and DNA–protein binding agents, were caused by UV radiation (Girard et al. 2011). The induction of reactive oxygen form (hydroperoxide, hydroxyl radical, and singlet oxygen), which oxidized nucleotides and destroyed the DNA chain, may also by induced indirectly via UV radiation (Douki et al. 1999).

Different trials have been performed in order to improve virus resistance to UV radiation. A recombinant AcMNPV that expresses cv-PDG protein, an algal virus pyrimidine dimer specific glycosylase that is involved in the initial stages of UV damaged DNA repair, was constructed (Petrik et al. 2003). The A50L gene, encoding cv-PDG protein, was expressed by the mutant AcMNPV (vHSA50L) under the control of D. melanogaster's hsp70 promoter. While budded virus (BVs) of the mutant vHSA50L were three times more resistant to UV radiation than BVs of the wild-type AcMNPV, the assembled OBs of the mutant vHSA50L did not show resistance to UV radiation compared to the wild type AcMNPV.

Although the median lethal concentrations (LC_{50}) of vHSA50L and AcMNPV in concentration-mortality bioassays in neonatal *T. ni* were non-significantly different, as well as the median survival time (ST_{50}), the mutant vHSA50L showed more virulence against neonates of *Spodoptera frugiperda* by 15-fold (7.1×10^5 vs 1.15×10^7 OBs/ml). Moreover, the mutant vHSA50L carrying the A50L coding sequence showed reduction in ST_{50} with about 41% (73.6 vs 124.4 h) than the wild type AcMNPV. Hence, the cv-PDG protein enhances BVs resistance to UV radiation but not virus assembled OBs.

In agreement with obtained results, an active DNA photolyase encoded by a baculovirus from the insect *Chrysodeixis chalcites* was verified (van Oers et al. 2008). Two putative classes II CPD-DNA photolyases Cc-phr1 and Cc-phr2 showed to be encoded in the genome of Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV). *Escherichia* coli cells that lacking photolyase displayed photo-reactivation activity when Cc-phr2 was expressed but not with Cc-phr1, demonstrating that only Cc-phr2 encoded an active photolyase. In the present study, the analysis performed clearly demonstrates

that SpliGV *phr* gene encodes an active photolyase, which was able to complement a photolyase deficiency in SpliNPV and had in vivo photolyase activity. Under light conditions, UV-treated vSpli-phr expressing photolyase showed a high virulence against the 1st instar of *S. littoralis* larvae compared to UV-treated wild type SpliNPV that lack a copy of photolyase gene. Moreover, the ST_{50} value of *S. littoralis* infected with LC_{90} of the UV-treated vSpliNPV-phr was lower (120 h) compared to wt SpliNPV-infected larvae under the same condition, which was 144 h. These results demonstrated that the vSpli-phr encoding the photolyase could to rescue the virus activity. In the same context, improvement in the speed of DNA repaired and/or the amount of time needed for repair should be considered. If this strategy was to be practical in pest management program, further studies are required in order to determine the best strategies that can be used for the efficient repair of DNA damage upon virus OBs exposure to UV radiation via natural sunlight for a long time.

Conclusions

In order to examine the possible repair of UV-damaged baculovirus DNA, a recombinant SpliNPV harboring a copy of photolyase gene was generated and its infectivity was determined after exposure to UV irradiation. Upon exposure to UV-Irradiation, the recombinant virus (vSpli-phr) showed a fivefold increase in UV irradiation resistance than the wild type SpliNPV under the same conditions as measured by LC_{50} value. In addition, the ST_{50} value of the recombinant virus expressing *phr* revealed a considerable reduction in the time needed for *S. littoralis* speed of mortality of about 22 h than to larvae treated with the wild-type SpliNPV. Hence, UV-induced viral DNA damage could potentially be repaired by the production of photolyase via the recombinant vSpli-phr. The results of the present study confirmed that a functional photolyase was expressed by the generated recombinant virus, which may have the potential for the development of new generation of baculoviruses with DNA-repair activity.

Abbreviations

SpliNPV	*Spodoptera littoralis* nucleopolyhedrovirus
Obs	Occlusion bodies
CPDs	Cyclobutane pyrimidine dimers
LT50	Median lethal time
LC50	Median lethal concentration
h	Hours
Min	Minute
hpi	Hours post-infection
p.i	Post-infection

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Author contributions

WE developed original approach; LG and EO contributed to improving and confirming the technique; LG, NA and AM contributed to performing experiments; WE, NY and LG contributed to analyzing data; WE and EO contributed to writing the manuscript LG and AM contributed to molecular genetic studies; WE and EO contributed to participating in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed in this work are available in the published manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

This study does not contain any individual person's data.

Competing interests

The authors declare not to have any competing interests regarding the publication of this work.

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