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Isolation and characterization of nucleotide excision repair deficient mutants of the entomopathogenic fungus, *Beauveria bassiana*

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Abstract

To better understand DNA repair in the entomopathogenic fungus *Beauveria bassiana*, three ultraviolet (UV) light sensitive mutants were isolated and characterized to be deficient in nucleotide excision repair (NER). The UV sensitive mutants were scored by comparison to survival of the parental isolate, GK2016, after 36 J/m^2 UV-C irradiation. At this dose, conidial survival of GK2016 was 98% and the mutants LC75, LC194, and LC85 had survival values of 63%, 45%, and 31%, respectively. An immunological method which measured the removal of pyrimidine-(6-4)-pyrimidone photoproducts during repair confirmed the decreased ability of LC75, LC194, and LC85 to remove these UV-induced dimers by NER. The mutants were also found to be deficient in NER at swollen/ germinating conidia and blastospore life cycle stages. The germination of the moderately UV sensitive mutant, LC75, was similar to that of the parental isolate, GK2016, after UV irradiation and incubation to enhance NER. The more sensitive mutants, LC194 and LC85 were 2.1- or 2.7-fold, respectively, less likely to germinate after UV irradiation based on their ability to carry out NER. These NER deficient mutants, the first to be derived from *B. bassiana*, reveal the importance of NER in spore survival post-UV irradiation.

Keywords: *Beauveria bassiana*; Entomopathogenic fungi; Ultraviolet radiation; Thymine dimers; Nucleotide excision repair; UV sensitive mutants

1. Introduction

The entomopathogenic fungus, *Beauveria bassiana* is of commercial importance as an alternative to chemical insecticides in an agroecosystem (Khachatourians et al., 2002). The widespread use of entomopathogenic fungi (EPF) is somewhat limited because they are too dependent on environmental factors (Fargues, 2003). Of main concern is ultraviolet (UV) radiation from the sun, which was found to "inactivate" spores of EPF in early studies (Ignoffo et al., 1977). In addition to spore inactivation, UV radiation will also cause lowered insect mortality and spores to exhibit delayed germination (Morley-Davies et al., 1995; Hu et al., 1996; Varela and Morales, 1996; Inglis et al., 1997). For *Metarhizium* spp., post-UV delay in germination was shown to be a result of DNA repair (Alves et al., 1998; Braga et al., 2001a,b,c). To mitigate, the focus for improving survival of EPF in the field has been the use of sunscreens in the final formulation (Inglis et al., 1995; Fargues et al., 1996) and integrated pest management (IPM) practices (Fargues, 2003).

In a previous paper we have shown that by using current methods of analyses quantification of UV-C irradiation induced cyclobutane pyrimidine dimers (CPDs) and their removal in EPF is possible (Chelico et al., 2005). This approach is more direct than estimations that rely on the loss of viability. We have also shown that mechanisms of nucleotide excision repair (NER) and photoreactivation (Phr) are operative in *B. bassiana*, *Beauveria brongniartii*, *Beauveria nivea*, *Metarhizium anisopliae*, *Paecilomyces farinosus*, and *Verticillium lecanii* (Chelico et al., 2006).

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The NER repair pathway is a versatile mechanism of DNA damage repair that can repair UV photoproducts such as CPDs and is required to repair pyrimidine-(6-4)-pyrimidone photoproducts ((6-4)PPs) in organisms that to do not have photoproduct-specific repair systems (Friedberg et al., 1995). In addition, NER can repair bulky DNA base adducts from chemical mutagens or other damage that distorts the DNA helix structure (Friedberg et al., 1995). Phr is a repair mechanism carried out by a photolyase that monomerizes UV-induced pyrimidine–pyrimidine dimers in DNA (Wang, 1976). Classical Phr is known as that which is specific to repair of CPDs (Kelner, 1949) and is the definition that will be used here.

We currently report the results of investigations of DNA repair in *B. bassiana* with UV sensitive mutants, which characterize survival under conditions of deficient NER. This approach can be used to study *B. bassiana* DNA repair and to decipher the role of DNA repair pathways in the survival of this fungus. Comparison of the NER mutants' physiological response to the *B. bassiana* parental isolate, GK2016, after treatment with UV enabled loss of function characterization of DNA repair. By establishing a model of DNA repair in *B. bassiana* a more rational research approach to improving *B. bassiana* spore survival after field application should be possible.

2. Materials and methods

2.1. Fungal isolates and culture conditions

The parental isolate, *B. bassiana* GK2016 (BioInsecticide Research Laboratory, Department of Applied Microbiology and Food Science, College of Agriculture, University of Saskatchewan, Canada), and derived mutants were cultured on YPG agar (YPGA, 20 g/l glucose, 10 g/l peptone, 2 g/l yeast extract, and 15 g/l agar) at 27 °C for 7 days. Spores from a culture were harvested by flooding the plate with deionized distilled water (ddH_2O), dislodging of spores using a bent glass rod, and passage through a glass wool filter to remove mycelia. Spores were washed with ddH_2O and recovered by centrifugation at 4000*g* for 15 min at 25 °C.

2.2. Development and characterization of UV sensitive *B. bassiana* mutants

The mutagenesis of 20 ml of conidia resuspended in 0.02% (v/v) Tween 80 at 1×10^8/ml was accomplished by addition of methylmethane sulfonate (MMS) to a final concentration of 1% (v/v) and incubation with shaking for 90 min where survival represented 1–2% of the original population. The conidia were then plated onto YPGA. After 7 days at 27 °C, the resultant colonies were randomly selected and transferred to fresh YPGA to obtain isolated cultures. A pool of 200 mutants was generated and these mutants were screened for a UV sensitive phenotype using two selection levels (tests) which were qualitative (Level I) or quantitative (Level II).

In Level I, isolates were screened for UV sensitivity by spot inoculation on YPGA. A loop was used to transfer spores from each mutant isolate colony to a 1×1 cm area drawn as a grid on a YPGA plate. This inoculation was done in quadruplicate. Plates were exposed to UV-C light in the dark for 0, 120, 240, or 360 J/m^2 and growth was permitted for 3 d at 27 °C. Unless otherwise stated all UV-C exposures were conducted using the UVP Mineralight R52 G (Upland, CA) between 200 and 280 nm ($\lambda_{max} = 254$ nm) at an irradiance of 0.4 W/m^2 as determined by a Blak-Ray® Ultraviolet Intensity Meter model J225 (San Gabriel, CA). The UV sensitivity was ranked comparatively by assessing the quality of visible growth with that of the parental isolate, *B. bassiana* GK2016, for 3 days after UV exposure. This process was repeated three times independently. Mutants that showed a UV sensitive phenotype in three trials were moved into Level II screening. Mutants that did not show a consistent UV response were removed from the screening process.

The Level II screening was a quantitative measure of incorporation of ^{3}H-deoxythymidine triphosphate (^{3}H-dTTP) into blastospore DNA after UV-C irradiation. Since *B. bassiana* blastospores cannot take up ^{3}H-dTTP we used a permeabilization method, described in Chelico and Khachatourians (2003), to enable incorporation of ^{3}H-dTTP into blastospore DNA after a 36 J/m^2 dose of UV-C irradiation (Section 2.3). The ^{3}H-dTTP incorporation was measured with a scintillation counter as in Chelico and Khachatourians (2003). The ability to incorporate ^{3}H-dTTP was used as a measure of NER proficiency and mutants with reduced incorporation of ^{3}H-dTTP were defined as UV sensitive.

The stability of the mutants was assessed by the spontaneous reversion frequency. The spontaneous reversion frequency was determined by diluting and spread plating 100 μl of a conidial suspension of a mutant isolate at 6×10^7/ml onto YPGA, exposure to 360 J/m^2 UV-C and incubation at 27 °C for 5 days. The putative revertants were replica plated onto fresh YPGA and exposed to 360 J/m^2 UV dose then incubated at 27 °C for 5 days to confirm stability. Isolates demonstrating the loss of the UV sensitive phenotype after two successive trials were designated true revertants.

Mutagen induced reversion frequencies were used to further characterize the repair deficiency of the mutants. Induced reversion frequencies were calculated by exposing conidial suspensions of mutant isolates at a concentration of 1×10^7/ml to 12 J/m^2 UV dose (as described in Section 2.3). The conidia were then diluted, spread plated onto YPGA and incubated at 27 °C for 5 days. True revertants were determined by replica plating the resultant colonies and exposure to 360 J/m^2 UV dose. The wild-type control for reversion frequency data was measured by the mutation frequency of GK2016 under the same conditions to become more UV tolerant.