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PHOTOREACTIVATION IN *BACILLUS THURINGIENSIS* STRAINS WITH DIFFERENT SENSITIVITY TO UV RADIATION

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The effect of photoreactivation in four *B. thuringiensis* ssp. *kurstaki* strains with different sensitivity to UV radiation was investigated. The results obtained show that photoreactivation is more efficient in the strains more sensitive to UV radiation. The reason(s) for the decreased effect of photoreactivation in UV resistant mutants are not known. One of the possible causes is the reduced quantity of pyrimidine dimers (the substrate for photolyase) in the strains in which dark repair mechanisms are more efficient. The cause can also be some unknown interaction between photoreactivation and other repair mechanisms. UV-induced mutagenesis in four strains was not detected.

Key words: *Bacillus thuringiensis,* ssp. *kurstaki,* photoreactivation, UV-sensitivity, mutagenesis.
Ključne reči: *Bacillus thuringiensis,* ssp. *kurstaki,* fotoreaktivacija, osetljivost na UV, mutageneza.

INTRODUCTION

Bacillus thuringiensis is a gram-positive soil bacterium which, during sporulation, forms parasporal crystalline inclusions that have insecticidal properties. Thanks to this ability, the preparations of *B. thuringiensis* are being used as insecticides for more than 20 years.

Genetic research of this bacterium, is mostly designated for industrial production of bioinsecticides i.e. mapping and sequencing the genes for the crystal proteins and cloning these genes into different subspecies of *Bacillus thuringiensis* as well as other species of microorganisms; discovering the processes of transfer of genetic material in these bacteria, etc.

Mechanisms of DNA repair in *B. thuringiensis* have not been intensively studied. However, for the analysis and manipulation of genetic material, it is of great importance to distinguish the DNA repair capacity of the bacterial strains. Auffray and Boutibonnnes (1987a) have proved the existance of photoreactivation (PHR) in *B. thuringiensis*, and they have also assumed the presence of excision mechanism of DNA repair in these bacteria.

In this work we have investigated the effect of photoreactivation in four *B. thuringiensis* ssp. *kurstaki* with different sensitivity to UV radiation. The results obtained show that photoreactivation is more efficient in the strains more sensitive to UV radiation.

MATERIALS AND METHODS

Bacillus thuringiensis ssp. *kurstaki* strains, used in this work, are listed in Tab. 1.

Tab. 1. Bacterial strains.

strain	name	source
L20001	HD-1	D. Karamata
L20002	CryB	D. Karamata
A-1		this work*
EY-3		this work*

* the strains obtained as UV resistant mutants of HD-1

Bacteria were grown in LB medium at 30°C. Exponentially growing cells (approximately 5×10^7 cells/ml, OD_{610} /1:4/=0,1) were centrifuged and resuspended in the same volume of 10 mM $MgSO_4$. 8 ml of cell suspension were UV irradiated with constant mixing, in a glass Petri-dish (diameter 90 mm) using germicidal lamp having a maximum output at 254 nm. Dose rates were measured with the Latarjet dosimeter. 10 ml of 10^{-2} dilution of bacterial suspension, irradiated with each UV dose, were

equally divided into two test tubes, one of which was protected from the light with a protective covering. Such pairs of test tubes were put in vessels with water, temperature 20°C, and illuminated with a light bulb (150 W) at the distance of 35 cm. After different periods of illumination, the number of bacteria in the samples was determined by plating on LA plates. The colonies were counted after 16 hours of incubation. The effect of photoreactivation was determined by comparing the number of bacteria in the non-protected test tubes where photoreactivation was allowed (+PHR) and the appropriate protected test tubes where photoreactivation was prevented (-PHR).

RESULTS

The differences in UV-survival of the tested strains, shown in Fig. 1A,B, are most probably the result of their different capacities for DNA repair. The strain CryB, which is a plasmid-less derivative of HD-1 was more resistant to UV light as shown by ten-fold increase of UV doses for the same survival (Fig. 1B). The absence of plasmids could increase UV survival in one of the following ways:

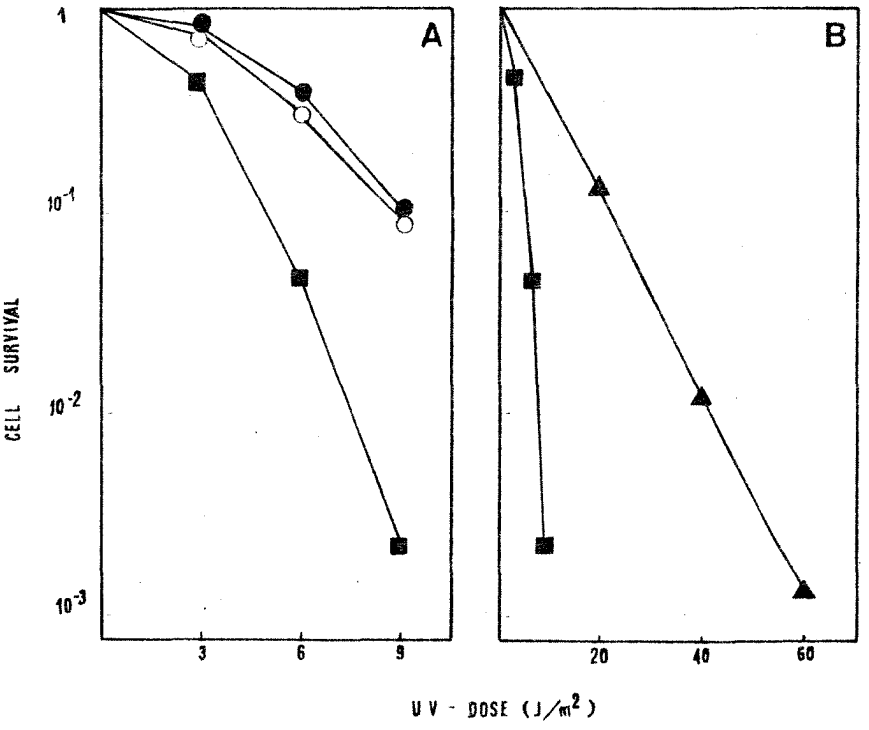

Fig. 1. – Survival of strains HD-1 (■), A-1 (O), CryB (▲) and EY-3 (●) after UV radiation. Each value represents the mean of four independent experiments.

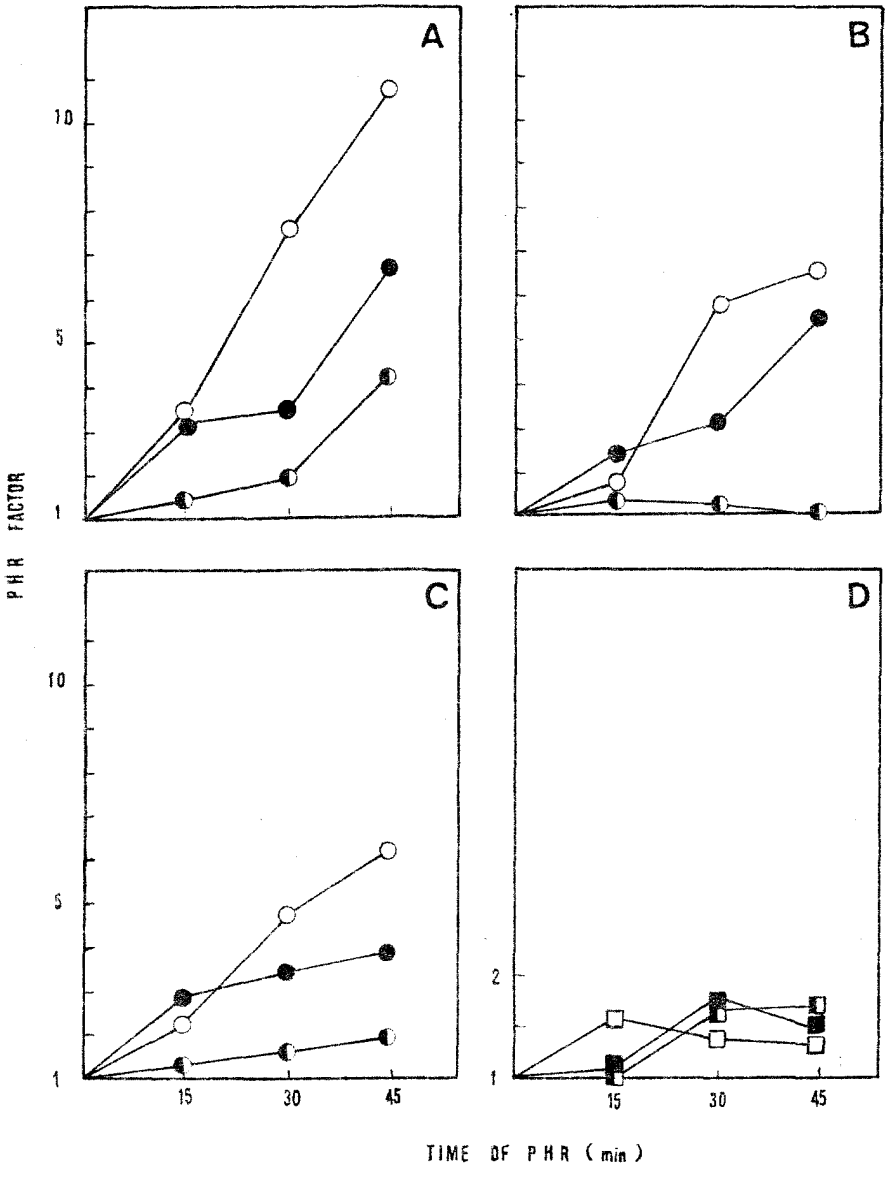

Fig. 2. – Photoreactivation in strains HD-1 (A), A-1 (B), EY-3 (C) and CryB (D). The cells were irradiated by UV: 3 J/m^2 (◐) 6 J/m^2 (●), 9 J/m^2 (O), 20 J/m^2 (□), 40 J/m^2 (■) and 60 J/m^2 (◧). Photoreactivation factor was calculated by dividing UV survival of illuminated cells with UV survival of non-illuminated cells. Each value represents the mean of 3-5 independent experiments.

1) by reducing the total amount of DNA in the cell, which decreases the number of UV-induced lesions, which could otherwise compete for the repair enzymes;
2) by the absence of some plasmid encoded negative regulator(s) of DNA repair genes.

Increased survival of UV-treated cells after illumination with visible light, compared to the appropriate non-illuminated samples, was noticed in all tested strains. The results obtained for the strains HD-1, EY-3 and A-1 (Fig. 2A,B,C) which were irradiated with the same UV doses (3, 6 and 9 J/m^2), show that photoreactivation is more efficient when the mentioned strains are irradiated with higher doses. Moreover, the longer time of exposure to visible light increases the effect of photoreactivation (Fig. 2 A,B,C). After the same UV doses, photoreactivation is less efficient in strains EY-3 and A-1 which are more UV resistant compared to HD-1 (Fig. 2). Photoreactivation in CryB strain (Fig. 2D) was less pronounced.

From the results obtained (Figs. 1,2) it seems that the effect of photoreactivation is directly opposite to the UV-resistance of the investigated strains. This conclusion is in agreement with the finding that the effect of photoreactivation is increased in UVS-mutant (YA 200) compared to the wild type strain *B. thuringiensis* ssp. *thuringiensis* S1$_2$ (Auffray and Boutibonnes, 1987a).

DISCUSSION

The reason(s) for the decreased effect of photoreactivation in UV resistant mutants are not known. One of the possible causes is the reduced quantity of pyrimidine dimers (the substrate for photolyase) in the strains in which dark repair mechanisms are more efficient. The cause can also be some unknown interaction between photoreactivation and other repair mechanisms. In *E. coli* SSB protein has the ability to stimulate photolyase in the absence of RecA protein, and photolyase stimulates UvrABC excision nuclease (Lerš et al., 1989). It is possible that such and/or different interactions exist in *Bacillus* species.

The difficulties in discussing our data arise from the fact that little is known about the mechanisms of DNA repair in *Bacillus thuringiensis* and from the lack of well-defined mutants in DNA repair. Extensively studied repair mechanisms in *E. coli* (Walker, 1984, 1985) facilitate the discovery of similar repair mechanisms in *Bacillus*.

It has been demonstrated that *Bacillus thuringiensis* possesses inducible error-free systems (Auffray and Boutibonnes, 1987b). In addition, this bacterium responds to DNA-damaging agents by eliciting several inducible SOB phenomena (SOS in *E. coli*) such as cell filamentation (Boutibonnes et al., 1984), prophage induction (Auffray and Boutibonnes, 1985) and W-reactivation and W-mutagenesis (Auffray and Boutibonnes, 1987b). However, we could not detect UV-induced mutagenesis in any of our strains of *B. thuringiensis* ssp. *kurstaki*.

The most extensive study of inducible SOB system in *Bacillus subtilis* (Love and Yasbin, 1986; Yasbin et al., 1988) indicates very complex regulation of RecE

protein (homologous to RecA in *E. coli*) wich has a possible role in mutagenic DNA repair.

The prospective research in this field should be directed toward identification of regulatory and structural genes involved in the processes of DNA repair and mutagenesis in *Bacillus thuringiensis,* by isolating and characterizing different repair deficient mutants. For this purpose, it is primordial to establish the conditions that increase mutagenesis in *Bacillus thuringiensis.*

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Rezime

VLADIMIR LAZAREVIĆ, SLAVIŠA STANKOVIĆ, DRAGA SIMIĆ

FOTOREAKTIVACIJA U *BACILLUS THURINGIENSIS* SOJEVIMA SA RAZLIČITOM OSETLJIVOŠĆU NA UV-ZRAČENJE

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U ovom radu je ispitivan efekat fotoreaktivacije na četiri soja *Bacillus thuringiensis* ssp. *kurstaki* sa različitom osetljivošću na UV-zračenje. Dobijeni rezultati pokazuju da je fotoreaktivacija efikasnija u sojevima koji su osetljivi na UV-zračenje. Nije razjašnjeno zašto dolazi do smanjenog efekta fotoreaktivacije u sojevima koji su rezistentni na UV-zračenje. Efikasni mehanizmi reparacije koji se odigravaju u odsustvu svetlosti i dovode do smanjenja dimera pirimidina (supstrata za fotoliazu) mogu biti uzrok ovog smanjenog efekta. Osim toga interakcije između različitih procesa reparacije mogu uticati na fotoreaktivaciju. U svim ispitivanim sojevima mutageneza indukovana UV-zračenjem nije detektovana.