Removal of UV induced pyrimidine dimers from a transcriptionally active MATα-gene of *Saccharomyces cerevisiae*

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ABSTRACT

The ability to remove UV-induced pyrimidine dimers from the transcriptionally active MATα gene was examined in nine radiation-sensitive (rad) mutants of the yeast Saccharomyces cerevisiae using the scission activity of T4 endonuclease V at pyrimidine dimer sites. The rad2 and rad3 mutants, belonging to RAD3 epistasis group, were found to be defective in their ability to remove UV-induced dimers, while rad7 mutant, belonging to the same group, showed a slightly low rate of removal of T4 endo V sensitive sites (ESS) after 4 and 5 hr. In RAD52 epistasis group, the removal of ESS in rad24 progressed to 50% after 4 hr and then leveled off to 5% after 5 hr. While, both rad50 and rad52 mutants proceeded gradually to remove the ESS until reached 55 and 50% after 5 hr, respectively. In RAD6 epistasis group, rad6 and rad8 mutants revealed high removal of ESS after 3 hr with removal of 75.5 and 90%, respectively. In rad18 strain, the removal of ESS was fluctuated with different repair rates and the highest removal of ESS of rad18 was obtained after 5 hr, which reached 87%. The main purpose of the study is to examine DNA repair at the level of a specific gene in yeast and to analyze the role of such a gene involved in the removal of damage from DNA. The study produced a rapid method, which can be used as a standard for the detection of damages due to numerous environmental pollutants.

Kew words: *Saccharomyces cerevisiae, MATα-gene, rad mutants, UV-induced pyrimidine dimers and T4 endo V sensitive sites (ESS).*

INTRODUCTION

Excision repair is an important repair process in virtually all organisms, principally two different mechanisms are recognized; base excision and nucleotide excision. In prokaryotes these mechanisms are understood in great detail at the molecular level. An example of the nucleotide excision pathway in *E. coli* is the UvrABC gene products, which are involved in recognition of the lesion in the DNA, and incision of the damaged strand at both sites of the strand (van Houten, 1990). In the case of base excision, the damaged base is removed by a damage specific glycosylase. Well known base excision repair mechanisms, which are active on UV induced pyrimidine dimers, are the phage T4 denV system (Dodson and Lloyd, 1989) and the *Micrococcus luteus* endonucleases system (Grafstrom *et al.*, 1982). In eukaryotes, the DNA repair processes are far less understood at the molecular level, whereas a larger number of loci is involved in the excision repair process compared to prokaryotes (Friedberg, 1988). In *Saccharomyces cerevisiae*, approximately 30 UV-repair genes (*rad* mutant loci) have been assigned to three epistasis groups RAD3, RAD6 and RAD52 (Prakash *et al.*, 1993). Analysis of *rad* mutants has indicated that loci in the RAD3 epistasis group are involved in

nucleotide excision repair, whereas those in the RAD6 epistasis group are required for induced mutagenesis, i.e., error-free and error-prone repair. Loci in RAD52 epistasis group are involved in post-replication repair. The ability to remove UV-induced pyrimidine dimers from the nuclear DNA of yeast was examined in many radiation-sensitive (*rad*) mutants of the yeast *Saccharomyces cerevisiae*. For example, *rad7* and *rad14* (Prakash and Prakash, 1979), 11 different *rad* mutants representing six RAD loci; *rad1, rad2, rad3, rad4, rad14 and rad18* (Reynolds and Friedberg, 1981), *rad9, rad16* and *rad24* (Terleth *et al.*, 1990), *rad6* and *rad18* (Cassier-Chauvat and Fabre, 1991), and *rad16* (Bang *et al.*, 1992).

Early DNA studies had been performed with methods based upon the assumption that repair along the genome is homogenous. Since this is obviously not the case, methods were needed to allow the study of repair in defined sequences of the genome. A method was developed, which utilizes the scission activity of T4 endo V at pyrimidine dimer sites. With this method, the removal of those sites recognized by T4 endo V can be determined (Bohr and Okumoto, 1988).

The main purpose of the present study is to examine DNA repair at the level of specific nucleotides in selected yeast genes and to analyze the roles of particular genes involved in the removal of damage from DNA. In general, the study produces a rapid method, which can be used as standard for the detection of damages due to numerous environmental pollutants. This focuses on *Saccharomyces cerevisiae*, which possesses the same excision repair processes as man, yet provides a simpler model system for dissecting relationships between the repair mechanisms.

MATERIALS AND METHODS

Yeast strains and culture medium

The genotypes of the yeast strains used in this study are listed in Table (1). Cells were grown in complete medium (YEPD), which consisted of 1% yeast extract, 2% peptone and 2% dextrose according to Yang and Friedberg (1984).

Table (1): Yeast strains used and their genotypes.

Strains*		Genotypes
RAD3 epistasis group		
- X16-18C	*rad2*	ade 2-1
- LP2649-1A	*rad3*	leu 2-3, 112, ura 3-52, can 1
- LP2741-1B	*rad7*	ade 1, his 3-1, leu 2-3,112, trp 1, ura 3-52
RAD6 epistasis group		
- X10-2A	*rad6*	ade 2-1, his 3-200, lys 2-801, ura 3
- WW-YH5	*rad8*	ade 2, his 3, lys 2-801, 101, ura 3-52
- LP2729-4B	*rad18*	his 3, leu 2-3,112, trp 1-289, ura 3-52
RAD52 epistasis group		
- XK5255-1A	rad24	his 3, leu 2-3,112, lys 2, trp 1-289, ura 3-52
- SX131-5A	*rad50*	leu 2-3,1 12, ura 3-52
- STX507-9D	*rad52*	ade 2-1, his 7, leu 2, lys 7, trp 1, ura 3

* All strains were kind gifts from Dr. R. Waters (Univ. College of Swansea, Wales, England).

UV irradiation

Yeast cells were grown in YEPD medium at 28 °C until exponential phase. Cells were then irradiated with 70 J/m^2/sec UV light and subsequently collected by centrifugation, resuspended in YEPD and incubated for various time periods until 5 hr in the dark at 28 °C. DNA was isolated from

these cells to determine the repair efficiency by the removal of ESS.

T4 endo V isolation

The enzyme T4 endoV was isolated from *E. coli* cells carrying a plasmid containing the *den V* gene that can be induced by isopropyl-β-D thiogalactopyranoside (IPTG) (a kind gift of Dr. R. A. Brouwer, Leiden Univ., Molecular Genetics Lab., The Netherlands). The enzyme was purified according to Nakabeppu *et al.* (1982). The T4 endoV introduced single-strand nicks next to the dimer in the DNA samples as presented in Fig. (1). On a denaturing gel, nicked strands disappear in a smear, while the intact strands are still visible as a band, which are used to measure the repair efficiency.

DNA extraction and purification

DNA extraction and purification was carried out according to Campbell and Duffus (1988). Genomic DNA was cut with the restriction endonuclease HaeII, which generates a 3.6 Kb MAT α fragment and a 4 Kb HMLα fragment. DNA samples were then digested with the restriction endonucleases *Eco*RI and *Hind*III according to the manufacturer instructions (Boehringer Mannheim) to generate the transcriptionally active MATα gene. After UV irradiation, DNA samples were divided in two equal parts; one of which was incubated with T4 endoV and both were loaded on denaturing agarose gel according to Bohr *et al.* (1985). After electrophoresis, the DNA was transferred to Southern blot and hybridized with an internal Yα-specific probe (a kind gift of Dr. R. A. Brouwer). The gels were scanned using Gel Doc 2000 Bio-Rad system and analyzed with the Quantity One software package supplied by the manufacturer.

The absorption unit measurements [AU] were used to calculate the percentage of DNA that is sensitive to T4 endoV (nonrepaired DNA) according to Terleth *et al.* (1989) and Terleth *et al.* (1990) by the following equation: Percentage of DNA sensitive to T4 endo V= [1-AU (fragment + T4 endo V) / AU (fragment - T4 endo V)] x 100.

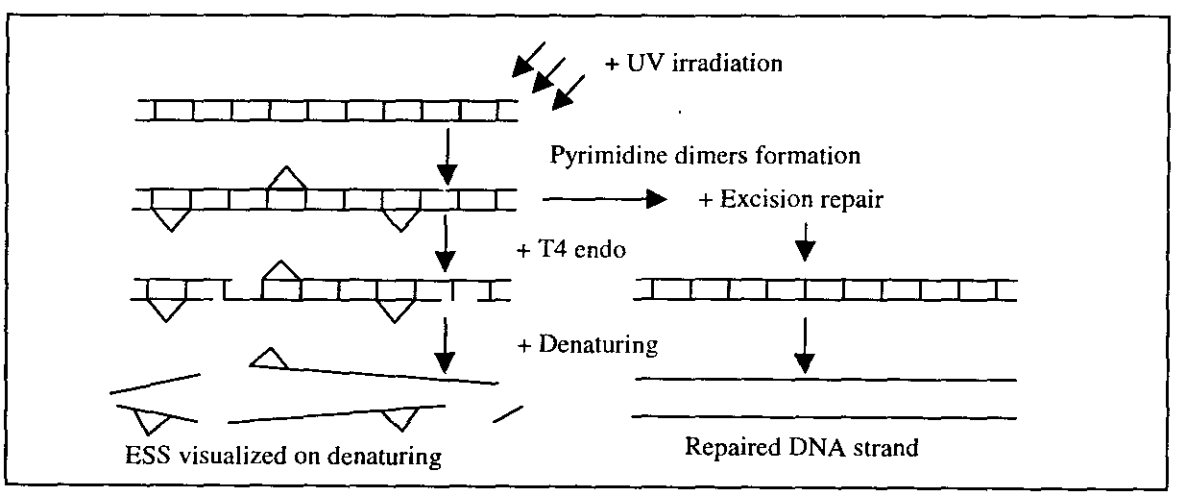

Fig. (1): Schematic representation showing the T4 endonuclease V assay. The UV induced DNA dimers forming T4 endo V sensitive sites (ESS) at the strand containing dimers that were recognized by T4 endo V. Cleavage of these fragments was obtained, while DNA derived from repaired DNA will be intact.

RESULTS AND DISCUSSION

Nine different *rad* mutants belonging to the three epistasis groups RAD3, RAD6 and RAD52 were irradiated with UV light and examined for their DNA repair efficiency at the level of the transcriptionally active MATα locus, which involved in the removal of T4 endo V sensitive sites (ESS) from DNA.

Differential repair with normal ESS removal in some *rad* mutants

In RAD52 epistasis group, three different *rad* strains; *rad24*, *rad50* and *rad52* were used. In *rad24* mutant strain, the removal of ESS from the active MATα locus progressed to 50% after 4 hr and then leveled off to 5% after 5 hr (Fig. 3). However, in a previous study, it reached 50% after 1.5 hr (Terleth *et al.*, 1990). The *rad24* functions both in excision repair (RAD3 epistasis group) and post-replication repair (RAD52 epistasis group) as reported by Eckardt-Schupp *et al.* (1987). They stated that the time lag before the start of removal of ESS is prolonged in *rad24* strain, which agrees with the obtained results in Fig. (2). Both *rad50* and *rad52* mutant strains proceeded gradually to the removal of ESS from MATα locus until reached 55 and 50% after 5 hr, respectively. These data are in agreement with Cox and Parry (1968).

Differential repair with high ESS removal in some *rad* mutants

In RAD6 epistasis group, three different *rad* strains; *rad6*, *rad8* and *rad18* were tested for the removal of ESS after UV-irradiation (Figs. 2 and 3). The three different *rad* strains have a differential repair and the removal of ESS occurred at the same rate, whereas *rad6* and *rad8* mutant strains revealed high removal of ESS after 3 hr with 75.5 and 90%, respectively. After 4 hr, the removal of ESS in the two strains was reduced to 60% and then raised up to 67.5 and 88.1%, respectively. In *rad18* strain, the removal of ESS fluctuated with different repair rates; 30, 2 and 25% after 1, 3 and 4 hr, respectively. The highest removal of ESS of *rad18* was obtained after 5 hr, which reached 87% as presented in Figs. (2 and 3). It has been reported that *rad6* and *rad18* mutant strains are both highly sensitive to the lethal effects of UV-induced mutagenesis and their genes were shown to be involved in the filling of gaps after UV irradiation (Prakash, 1981), but their mutagenic responses have been reported to differ where the induction of mutations in *rad6* cells is almost completely depressed (Morrison *et al.*, 1988), while the *rad18* mutations were shown to reduce UV-induced reversions of some mutation but not of other mutated sites (Cassier-Chauvat and Fabre, 1991).

Absence of removal of ESS in some *rad* mutants

The incision-deficient *rad* mutants were defective in the removal of ESS. Therefore, no removal of ESS occurred in both *rad2* and *rad3* mutants in the MATα gene. They were extremely UV sensitive as presented in Fig. (2), as well as in the band profiles of the RAD3 epistasis group (Fig. 3). However, *rad7* mutant that belonged to RAD3 epistasis group showed a slightly low rate of removal of ESS after 4 and 5 hr. It has been proposed by Perozzi and Prakash (1986) that the *rad7* mutant is proficient for repairing a certain fraction of the DNA upon UV irradiation.

These results are in agreements with previous studies, which reported that several mutants from the RAD3 epistasis group, such as *rad2* and *rad3*, have been shown to be completely incision-deficient, whereas *rad7* and others possess residual incision activity

(Wilcox and Prakash, 1981; Scott and Waters, 1997). However, no mutants have been found in *S. cerevisiae* that are specifically deficient in the repair of active DNA as found in human (e.g., Cockayne s syndrome cells) (Terleth *et al.*, 1991; Venema *et al.*, 1991).

On the other hand, *S. cerevisiae* genome displays many features common to the higher eukaryotes, rodent cells and xeroderma pigmentosum (XP-C) cells and only repairs a low percentage of lesions (10%-20%) of the genome overall after UV, while paradoxically displaying a high resistance to UV in comparison with human cells which repair ~70% of the genome overall (Bohr *et al.*, 1986; and Kantor and Elking, 1988). The presence of dimer blocks transcription effectively (Protic-Sabljic and Kraemer, 1985) and, therefore, efficient repair of active genes in rodent cells in combination with tolerance mechanisms to allow bypass of replication across the lesions (Meneghini *et al.*, 1981), would enhance the survival of these cells. Furthermore, it has been stated that dimers do not have to be removed from the whole genome for cells to survive (Cleaver, 1989).

In general, the repair of active genes in yeast, possesses the same excision repair processes as human, will have important applications for the process of induced mutagenesis. One of the consequences of repairing the transcriptionally active gene is to prevent the accumulation of DNA adducts in non-active regions of the chromosome. Mutagenic bypass of these lesions will, therefore, not lead to accumulation of mutations in non-active regions of the DNA, and such mutations will prevent the activation of genes (e.g., oncogene activation) (Terleth *et al.*, 1991) or alternatively become important when the cell due to differentiation or environmental changes needs to express a different subset of genes (Reed *et al.*, 1996).

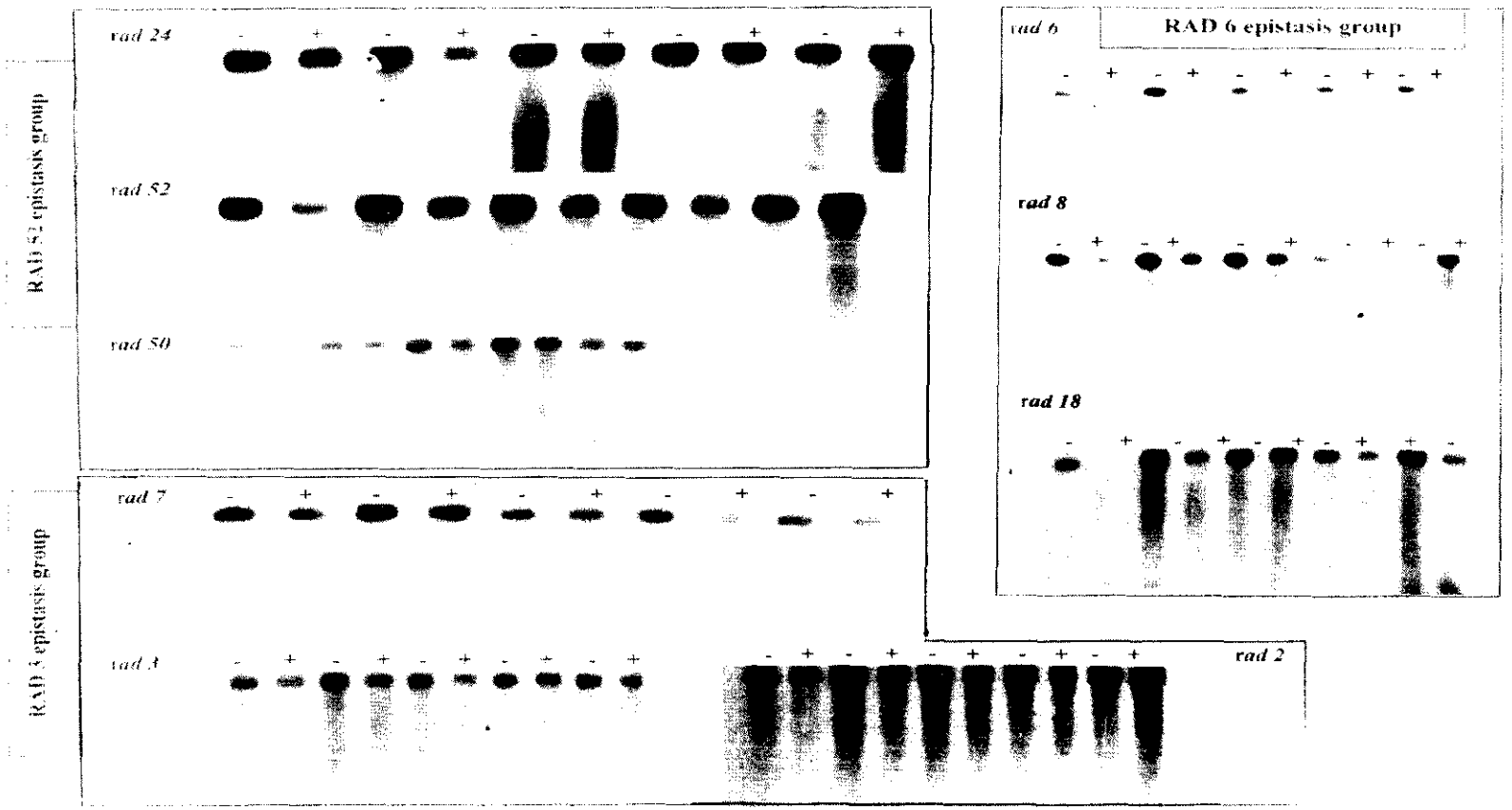

Fig. (2): The removal of ESS from MATα gene of S. cerevisiae among nine radiation-sensitive (rad) mutants belonged to the three epistasis groups.

+ = Incubation with T4 endoV - = Incubation without T4 endoV

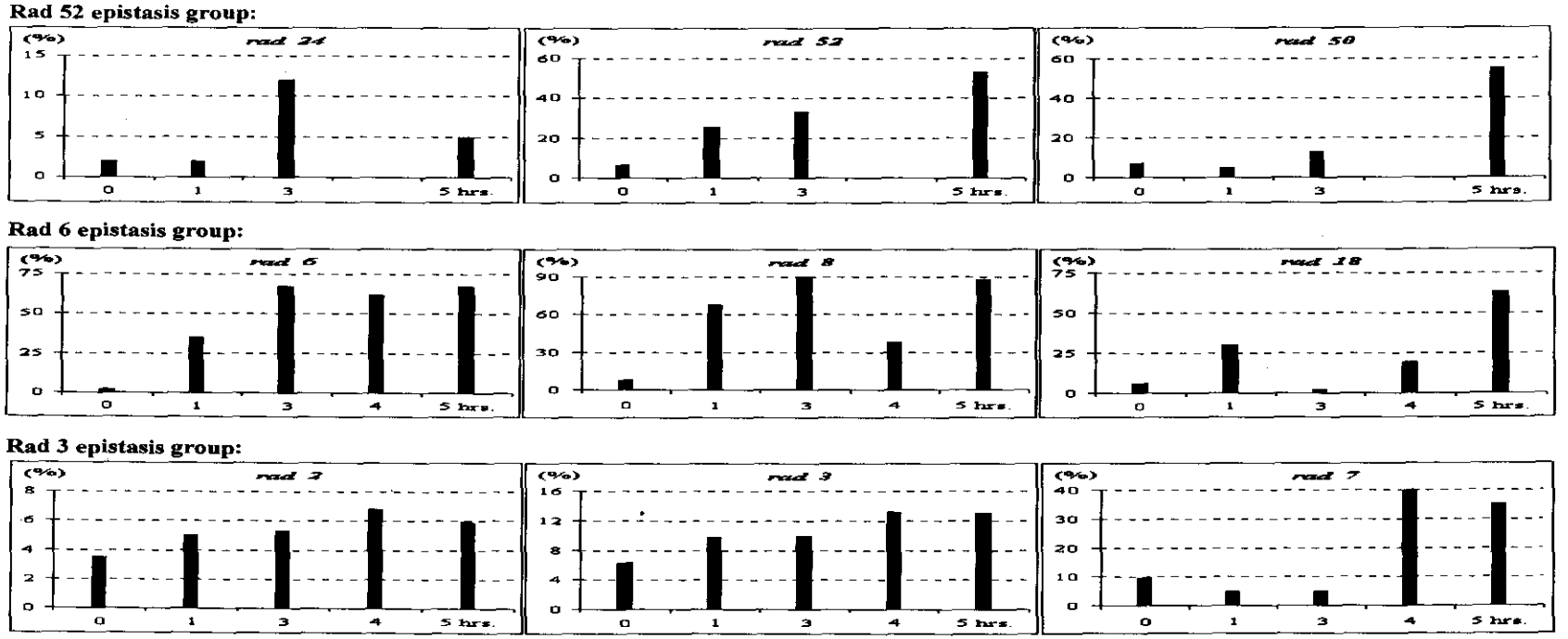

Fig. (3): The percentages removal of DNA resistant to T4 endonuclease V of rad mutants from the MAT gene at various post-irradiation times.

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الملخص العربي

إزالة ثنائيات البيريميدين الناتجة عن الأشعة الفوق بنفسجية من الجين النشط تناسخيا فى الخميرة الخباز

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تم اختبار القدرة على إزالة ثنائيات البيرميدين الناتجة من التعريض للاشعة فوق البنفسجية فى الجين النشط فى عمليـــة النســخ MATα gene وذلك فى تسعة من الطوافر الحساسة للإشعاع *rad* mutants فى خميرة الخباز وذلك باستعمال طريقة النشاط القاطع للمواقع الحساسة لثنائيات البيريميدين(ESS) بإنزيمات الفاج T4 الذى يحتوى على الجين V. ولقد أظهر كل من الطـــافرين *rad2* ، *rad3*التابعين للمجموعة RAD3 عدم القدرة على إزالة ثنائيات البيرميدين بينما أظهرت السلالة الطـــافرة *rad7* والتـــى تتبـــع نفـــس المجموعة معدلاً منخفضاً فى إزالة تلك الثنائيات بعد فترات تعريض للأشعة وصلت إلى ٤ و٥ ساعات. وفى المجموعة RAD52 فانـــه أمكن إزالة ٥٠% من ألثنائيات فى السلالة *rad24* بعد فترة ٤ ساعات من التعريض الإشعاعى، ثم انخفضت إلى ٥٥% بعد ٥ ســـاعات ، فى حين انه أمكن إزالة ٥٥ ، ٥٠% من ثنائيات البيرميدين فى الطفرتين *rad50* ، *rad52* بعد ٥ ساعات فى كل من السلالتين ،علـــى التوالى. وفى المجموعة RAD6 فانه أمكن إزالة قدر كبير من الثنائيات بعد فترة تعريض ٣ ساعات فقط ولقد وصلت النسبة إلـــى ٧٥,٥، ٩٠% فى الطافرين *rad6* ، *rad8* ، على التوالى. أما السلالة الطافرة *rad18* فقد أظهرت معدل إصلاح متباين عبر فترات التعريـــض وكان أعلى معدل لإزالة الثنائيات هو ٨٧% بعد ٥ ساعات.

والهدف الرئيسى من الدراسة هو اختبار القدرة على إصلاح التلف الذى يحدث فى DNA والناتج عن المعاملة بالأشعة الفـــوق بنفســـجية وذلك على مستوى جين متخصص فى الخميرة باستعمال طريقة سريعة ودقيقة يمكن استخدامها كمقياس عام لتحديد الأضرار التى تتعرض لها البشرية نتيجة الملوثات البيئية المتعددة.