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SPONTANEOUS MUTAGENESIS IN WILD TYPE AND RADIATION DEFECTIVE MUTANTS OF CYANOBACTERIUM *ANACYSTIS NIDULANS*

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Abstract

Comparative studies on the spontaneous mutation rate on ery^r marker of wild type cyanobacterium *Anacystis nidulans* and its UV sensitive (and UV resistant) mutants were carried out in order to determine the effect of repair processes affecting mutations. Some of the *UVS* and *XRS* mutations decreased the rate of spontaneous mutations while other similar mutations did not affect the level of spontaneous mutagenesis compared with the increased level of UV induced mutations. The process of spontaneous mutagenesis in cyanobacterium *A. nidulans* is controlled by complicated genetical systems that are responsible for radiation sensitivity of the cells. Moreover, the rate of spontaneous and induced mutagenesis was correlated in majority of the strains tested.

Introduction

The origin and mechanism of spontaneous mutations is not clearly understood. As a result of extensive work with bacteria and phages, a number of mechanisms for spontaneous mutagenesis have been proposed. The mechanism of spontaneous mutagenesis may be traced to errors either in the metabolism of DNA synthesis processes (Liberfarb & Bryson, 1970), or in DNA replication apparatus (Drake, 1973) or in the functioning of DNA repair enzymes (Okazaki *et al.*, 1971).

While considerable amount of genetical work has been done on bacterial, phage and fungal objects, very little is known about obligately phototropic cyanobacteria that are characterized by high level of radioresistance and genetical stability. The present work deals with the comparative study of spontaneous mutagenesis in wild type *Anacystis nidulans* and its radiation defective mutants in order to determine the effect of different mutations affecting the repair processes on the rate of spontaneous mutagenesis.

Material and Methods

Culture of *Anacystis nidulans* (strain No. 602) obtained from the Institute of Biology, Leningrad State University, USSR, and its *UVS* and *UVR* mutants isolated and characterized in the department of Genetics, Moscow State University, USSR (Shestakov, 1974) were used.

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Cyanobacteria were grown in modified medium C (Staletov *et al.*, 1965) at 36°C ± 1° under 2500 lux of white light from fluorescent lamps. Solid medium for colony counts contained 1.5% agar. Replica plate technique was used for characterization of the mutants. For viability, 3-day old exponentially grown cultures were serially diluted. Viability based on the number of colonies was determined after 10–11 days of incubation. Modified method of antibiotic concentration gradient (Shestakov & Mitronova, 1971) was used for determining the frequency of ery^r mutants. After 72 hr of incubation, erythromycin @ 5 μg/ml was added in each Petri dish. The ery^r colonies were scored in the lysis zone after 12 to 16 days of the addition of antibiotic. The ery^r colonies were checked for antibiotic resistance by growing each clone in liquid medium containing varying concentrations of antibiotic. Colonies that were resistant to more than 1 μg/ml of antibiotic were considered stable mutants. In a different set of experiments, the minimum inhibitory concentration (MIC) of erythromycin was calculated and found to be 0.05 μg/ml for all the strains of *A. nidulans*. Concentration of spontaneous ery^r mutants was calculated out of 10^8 viable cells. Result is based on the average of 3 repeated experiments. Standard deviation was calculated according to the formulae of Urbakh (1963).

Results

Table 1 shows the frequency of spontaneous ery^r mutants in the wild type cells and radioresistant mutant UR 17; while Tables 2 and 3 present the results of *UVS* and *XRS/lex* mutants respectively. Whereas *hcr* mutants An 7, An 12 and *XRS* mutant Fr 13 do not differ much on the frequency of spontaneous ery^r mutants from the wild type cells of *A. nidulans*, however, radioresistant mutant UR 17 and *XRS* mutants An 52, Br 54 and An 59 differ from the wild type cells and are characterized by significant decreased frequency of spontaneous mutations.

Rec mutant Fr 15 is the morphological revertant of filamentous *XRS/rec* mutant Fa 25 that is resistant to streptomycin (Mitronova *et al.*, 1973). Therefore, the frequency of spontaneous mutations of Fr 15 was calculated on str^r marker. Mutant Fr 15 showed

Table 1. Frequency of spontaneous ery^r mutants in the populations of wild type and *UVR* mutant cells of *Anacystis nidulans*.

Strain	Number of plates checked	Number of ery^r colonies	Frequency of ery^r mutants per 10^8 cells (M ± m)
Wild type	45	103	4.7 ± 1.7
UR 17	36	8	0.7 ± 0.2

Table 2. Frequency of spontaneous ery^r mutants in the populations of *UVS* mutants of *Anacystis nidulans*.

Strain	Nature of mutations*	Number of plates checked	Number of ery^r colonies	Frequency of ery^r mutants per 10^8 cells (M ± m)
An 7	*hcr*	30	25	2.6 ± 0.9
An 12	*hcr*	26	17	3.9 ± 1.34
Mt 1	*phr*	36	20	1.1 ± 0.3

*As per Shestakov, S.V. (1974).

a reliably decreased frequency of Str^r mutants (2.10^{-9}) compared with the wild type cells (3.10^{-8}) on str^r marker.

Discussion

The results on comparative study of spontaneous mutations on ery^r (str^r) marker suggest that some of the mutations causing changes in radiosensitivity of cells of *A. nidulans* do affect the frequency of spontaneous mutations. In mutants An 52, An 59, Br 54, An 7, An 12, Fr 15 and UR 17 there exists a correlation between the changes in spontaneous and UV induced mutagenesis (Rasool, 1976). Perhaps some of the white light used for the cultivation of cyanobacteria induced lesions are shown in the form of spontaneous mutations (Zhevner & Shestakov, 1972). Lethal and mutagenic effects of visible light on bacteria have been reported by Webb & Malina (1967). Thus, a portion of spontaneous mutations do infact constitute a part of induced mutations with an interrelationship between spontaneous and UV induced mutations in cyanobacteria. If a

Table 3. Frequency of spontaneous ery^r mutants in the populations of *XRS/lex* mutant cells of *Anacystis nidulans*.

Strain	Nature of mutations*	Number of plates	Number of ery^r colonies	Frequency of ery^r mutants per 10^8 cells (M ± m)
An 52	*recB (C)*	60	18	0.9 ± 0.72
Br 54	*recB (C)*	48	18	0.8 ± 0.36
Fr 13	*XRS*	29	30	2.8 ± 0.86
An 59	*lex*	60	13	0.6 ± 0.56

* As per Shestakov, S.V. (1974).

portion of UV induced mutations is formed as a result of the impairment in the functioning of replication apparatus that is responsible for spontaneous mutability (Fersht, 1979), it may be expressed in the form of a simultaneous change in the rate of induced and spontaneous mutagenesis. Amla & Saxena (1983) have also shown the existence of relationship between DNA replication and repair of UV induced lesions for mutations.

Spontaneous mutation rate of *XRS* mutant FR 13 on ery^r marker is about $3X10^8$. This result does not correspond to the rate of spontaneous mutations in other *XRS* mutants of An 59 which showed a low rate of spontaneous mutation. Probably, the increased spontaneous mutability in *XRS* mutant Fr 13 pertains to the 'mutator effect'. The mutator effect of the genes has been reported in phages and bacteria (Hershfield & Nossal, 1973; Sekiguchi *et al.*, 1983).

Radioresistant mutant UR 17 has also shown a decreased rate of ery^r marker spontaneous mutation rate. Davies *et al.*, (1973) reported a radioresistant strain D2IR6008 of *Salmonella typhimurium* that is able to repair the gamma induced damages more rapidly than the wild type. Hence the low generation of spontaneous mutations in UR 17 may be explained in the light of the existence of powerful error proof repair mechanisms.

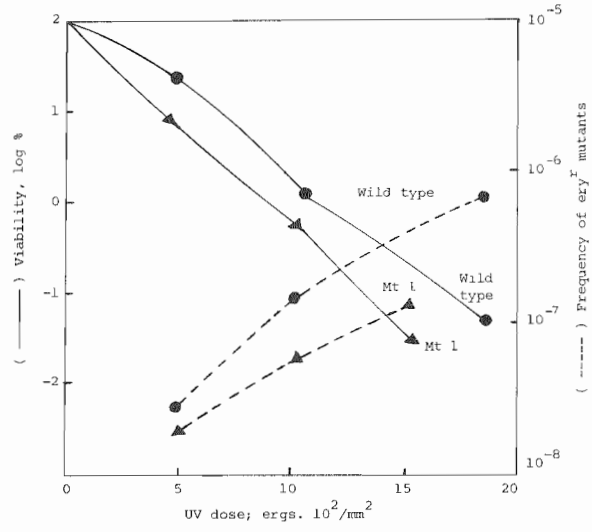

Fig. 1. Comparative lethal and mutagenic action of UV irradiation on wild type and *phr* Mt 1 mutant cells of *A. nidulans*. Exponentially grown (4 ml quantity) were irradiated with varying doses of UV and inoculated on solid medium. Viability and ery^r mutation rate were determined as given in the text. While *phr* Mt 1 mutant is more sensitive compared with the wild type; its UV induced mutability is appreciably decreased.

Low spontaneous mutation rate of *phr* mutant Mt 1 is explained in the light of its low UV induced mutation generating ability (Fig. 1). Zhevner & Shestakov (1972) reported a similar mutant M 6 of *Synechocystis aquatilis* which simultaneously possesses a decreased photoreactivating ability and compared with the wild type a decreased spontaneous and UV induced mutability. It is of interest that a number of UV induced mutations in a *phr* mutant of *E. coli* B were found to be photoreversible. This observation lead to suggest that there may be two enzymes involved in photoreactivation; one which breaks pyrimidine dimers and the other deals with some mutagenic photoproduct that is not a dimer (Smith & Hanawalt, 1969). Low spontaneous mutability of Mt 1 could also be attributed to the 'antimutator gene effect' which has also been found and studied in ϕT4 (Bessman *et al.*, 1974).

Appreciably low spontaneous mutability trend has been observed in *rec* mutants of *A. nidulans*. Similar results have been observed in other bacterial strains that have been shown to have increased sensitivity to mutagenic factors and decreased recombination ability. Such *rec* mutations have been identified in *E. coli* (Kondo, 1973) in *Bacillus subtilis* (Prozorov & Barabanshikov, 1976) and in *Proteus mirabilis* (Bohme, 1967).

The process of spontaneous mutagenesis in cyanobacterium *A. nidulans* is controlled by complicated genetical systems that are responsible for radiation sensitivity of the cells. The rate of spontaneous and UV induced mutability in these bacteria is correlated as also observed in yeast (Hannan & Nasim, 1985).

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