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ADAPTIVE RESPONSE TO ALKYLATION STRESS IN *SACCIIAROMYCES CEREVISIAE*

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ABSTRACT

Nitrosoguanidine (MNNG), methylmethane sulphonate (MMS) and ethyl methane sulphonate (EMS) mediated adaptive repair was studied in *Sac-cdtaromyces cerevisiae* XV185-14C. MNNG adapted cells showed improved survival at certain MNNG doses, compared to the nonadapted. Analogous results were observed with EMS and MMS. However, the EMS mediated adaptation was more pronounced than that of methylating agents. Cross adaptation studies revealed the existence of his phenomenon when MNNG adapted cells were treated with lethal doses of MMS and vice versa. However, there was no cross a daptation when EMS adapted cells were treated with lethal doses of MNNG. SDS-PAGE studies with crude protein extracts showed an elevated Ada enzyme profile only in induced wild type cells.

Introduction

The 'adaptive response' is *RecA-lex*A independent, inducible yet error-proof phenomenon resulting from the activation *of ada* regulon, which is responsible for the prevention of mutations and killing by alkylating agents (Samson and Cairns, 1977). This phenomenon has provided an insight into repair mechanism of DNA by a unique enzymatic response to alkylation damages and in turn some concepts relating to carcinogenesis and anticarcinogenesis (Lindahl et al., 1988). Killing and mutagenic adaptation are partly under different genetic controls. Whereas, killing adaptation is correlated with the induction of DNA glycosylases; the mutagenic adaptation is ascribed to the induction of transferases (Evenson and Seeberg, 1982; Vaughan and Sedgwick, 1991). Recent biochemical and genetic approaches including recombinant DNA techniques have provided neo-insights into the molecular mechanisms of DNA repair in terms of the tertiary structures of proteins and DNA (Morikawa, 1993).

S. cerevisiae (an ideal eukaryotic model) provoked our interest because adaptive response has been negatively reported earlier (Friedberg et al., 1988). The present work

includes: the survival pattern of the non-adapted/adapted *S. cerevisiae* cells against challenge doses of methylating (MNNG, *MMS)* and ethylating (EMS) agents; cross adaptation studies i.e. adaptation by either of the alkylating agents and their resistance behaviour against the unrelated alkylating agents (not used for adaptation) and SDS-PAGE of acetone precipitated crude extracts of ultrasonicated preparations *of* adapted and nonadapted wild type andngs MR0192 mutant cells of S. *cerevisiae.*

Experimental

S. cerevisiae XV 185-14C was requested from Dr. Anwar Nasim of NRC, Ottawa, Canada. The culture was maintained on Sabouraud's dextrose agar (SDA). Cells were grown in the SD broth which was also used during adaptation and for determination of the dose response MNNG (Aldrich, USA) and MMS, EMS (Sigma) were diluted in citrate phosphate buffer (pH 6) (Plummer, 1978). Fresh stock solutions were prepared every time. Method of Ather et al. (1984) with necessary modification was followed for the viable counts of nonadapted and adapted cells. Cross adaptation studies were per-formed as per Rasool et al. (1992). Methods (for SIDS-PAGE) of Flames and Rick-wood (1990) were followed for the Ada protein profiles in parent and the ngs mutant strains of *S. cerevisiae.*

Results and Discussion

S. cerevisiae has showed an increased resistance after step-wise adaptation (upto 1 μgmL^{-1}) to the killing effects of certain specific challenge doses of MNNG. Doses > 23 μgmL^{-1} (during challenge) did not induce resistance compared to doses between 0.5-25 μgmL^{-1}. It may be presumed that the adaptive repair enzymes are synthesized to a certain limit and are able to handle the damages only upto an extent beyond which the lethal doses are free to exert the lethality. It means that beyond a limit, the cells undergo 'cumulative damage infliction'. Thus, higher doses of MNNG may be perceived as a shock to the cells (Fig. 1), Dose dependent effect has also been observed in H4 rat hepatoma cells (Laval and Laval, 1984). Findings on the existence of adaptive repair in *S. cerevisiae* were further elaborated through similar experiments with a previously isolated ngs mutant MR0192 (Fig.2; Rasool and Mirza, 1994) which lacks in the induction of inducible adaptive repair; hence undergoes cumulative damage infliction (during adaptation and challenge). It appears that inducible adaptive repair in yeast (as in other organisms) functions under the control of *ada* regulon. Figures 3 and 4 indicate the MMS and EMS mediated adaptive repair in *S. cerevisiae* cells respectively. However, a better survival was observed at higher doses of EMS compared to MMS doses. In this context, EMS induced adaptive repair in *S. cerevisiae* has a close analogy with a classical adaptive response in prokaryotes (Rasool and Ali, 1991). R seems that ethylating agents like EMS induce more repair enzymes than the methylating agents in yeast cells. Cross

adaptive repair studies (Fig.5-7) have revealed that pretreatment of cells with sublethal MNNG doses conferred a marked resistance to inactivation by subsequent exposure to lethal MMS doses. However, this resistance was observed only at lower (<80 μlmL^{-1}) doses. Similar cross adaptation pattern was also reported in *E. coli* (Jeggo, 1979). MMS and MNNG (both being the methylating agents) have almost same sites of action in DNA (Hurst and Nasim, 1984). MNNG and MMS (as one would expect) have infact, shown the cross adaptation. An analogous cross adaptation has also been observed by MNNG and MMS adapted cells of *S. cerevisiae* to EMS challenge. Enzymes known to act on methyl adducts can co-remove the ethyl adducts in *E. coli.* Thus, an *E. coli* DNA glycosylases that catalyzed the release of 7 meG from alkylated DNA also liberated 7 ethylG (Laval et al., 1981). Similar pattern was observed in EMS adapted MMS challenged cells of *S. cerevisiae.* However, the extent of survival was lower compared to MMS adapted EMS challenged cells. It may be concluded that MMS inflicts more lethal lesions compared to EMS during challenge. However, the phenomenon of cross adaptation was not observed in EMS adapted MNNG challenged cells. Inspite of a few common sites of action for both methylating and ethylating agents, the EMS is also reactive at N6 and N7 of adenine; O2 of adenine and N4 of cytosine (Singer and Grunberger, 1983). With the increase in the number of adducts, cross adaptation is not observed probably because of lack of site specificity between the induced damages and available enzymes. Results of the SDSPAGE (Fig. 8) of ultrasonicated crude extracts (for Ada proteins) of parent and the ngs mutant MR0192 of *S. cerevisiae* showed an elevated profile of proteins in adapted parent cells only. Earlier Chao and Tillman (1982) in *E. coli* and Rasool et al. (1990)

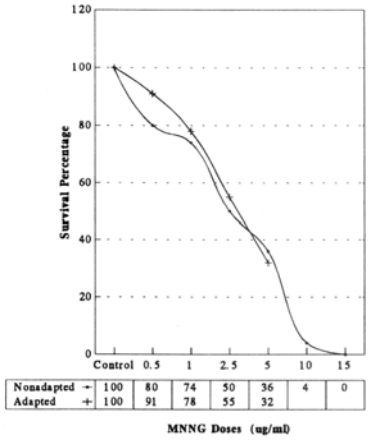


Fig. 1: MNNG mediated survival response of adapted and nonadapted cells of *S. cerevisiae* XV185-14C.

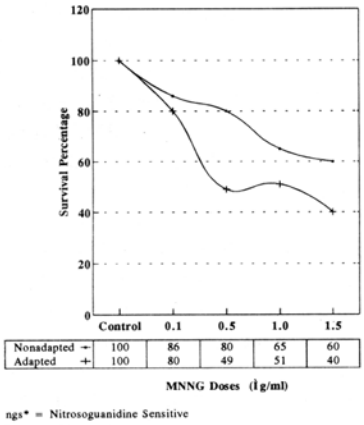


Fig. 2: MNNG mediated survival response of adapted and nonadapted cells of *ngs** mutant MR0192 *S. cerevisiae* XV185-14C.

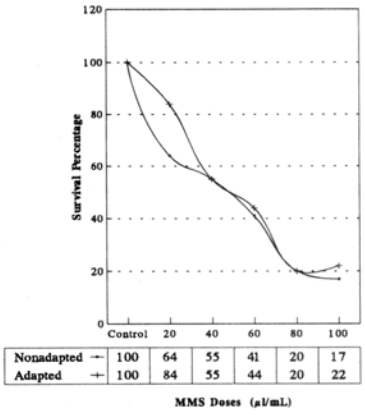


Fig. 3: MMS mediated survival response of adapted and nonadapted cells of *S. cerevisiae.*

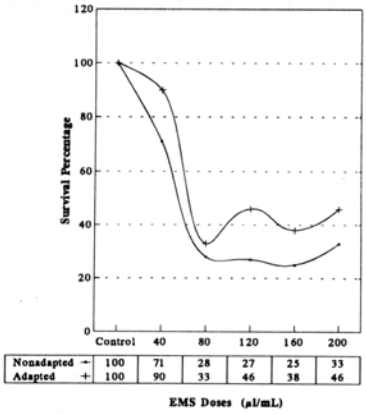


Fig. 4: EMS mediated survival response of adapted and nonadapted cells of *S. cerevisiae.*

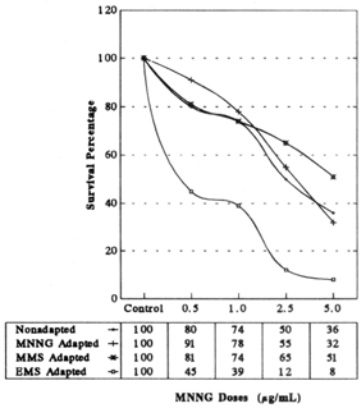
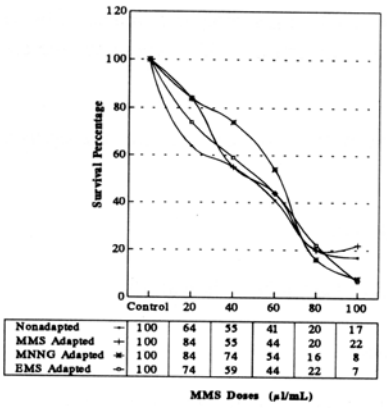
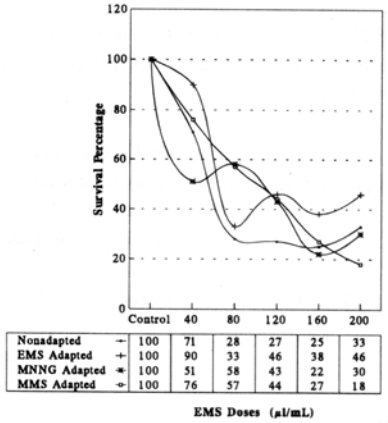


Fig. 5: MNNG, MMS and EMS mediated survival response of adapted cells of *S. cerevisiae* XV185-14C with MNNG.



Nonadapted	→	100	64	55	41	20	17
MMS Adapted	+	100	84	55	44	20	22
MNNG Adapted	*	100	84	74	54	16	8
EMS Adapted	○	100	74	59	44	22	7

Fig. 6: MMS, MNNG and EMS mediated survival response of adapted cells of *S. cerevisiae* XV185-14C with MMS.



Nonadapted	→	100	71	28	27	25	33
EMS Adapted	+	100	90	33	46	38	46
MNNG Adapted	*	100	51	58	43	22	30
MMS Adapted	○	100	76	57	44	27	18

Fig. 7: EMS, MNNG and MMS mediated survival response of adapted cells of *S. cerevisiae* XV185-14C with EMS.

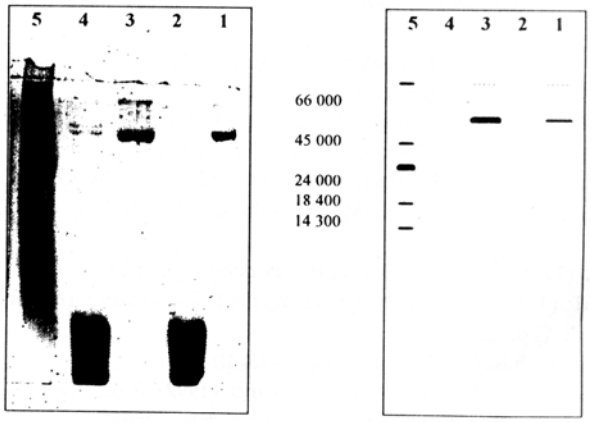


Fig. 8: Results of *SIDS-PAGE* of acetone precipitated crude extracts of ultrasonicated samples of parent strain and ngs mutant MR-0192.

From right to left:
1. Untreated parent strain of *Saccharonryces cerevisiae*
2. Untreated strain of ngs mutant MR-0192
3. Treated parent strain of *Saccharonryces cerevisiae*
4. Treated strain of ngs mutant MR-0192
5. Standard (SDS Molecular weight) markers

in *Ps. aeruginosa* also reported the analogous results. Sassanfar and Samson (1990) demonstrated the repair of methylated bases in S. *cerevisiide* DNA, only by *in vivo* experiments.

The present research findings are suggestive of the functioning of *the ada* operon regulated adaptive repair in *S. cerevisiae.*

Acknowledgment

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References

Ather A., Ahmed Z. and Riazuddin S. (1984). *Nucleic* Acids *Res.* 12: 2111.
Chao L. and Tillman D.M. (1982). *J. Bacteriol.* **151**: 764.
Evensen G. and Seeberg E. (1982). Natu*re* (London) **296**: 773.
Friedberg E.C., Cooper J, Couto L., Fleet It, Naumovaski L., Nicolet C.M., Robin-son G.M. and Weiss WA. (1988). In: *Laboratory Manual of Research Procedures.* Vo1.3 (eds.) Friedberg E.C. and Hanawalt P.C. (Makriel Dekker Inc., New York), p.l.
Flames B.D. and Rickwood D. (1990). Gel electrophoresis of proteins: A practical approach, 2nd edition (IRL Press Oxford), p.l.
Hurst A. and Nasim A. eds. (1984). *J. Bacteriol.* **139**: 783.
Laval F. and Laval J. (1984). *Proc. Natl. Acad. Sci.* USA **81**: 1062.
Laval J. Pierre J. and Laval F. (1981). *Proc. Natl. Acad. Sci.* USA **78**: 852.
Lindahi T., Sedgwick B., Sekiguchi M. and Nakabeppu Y. (1988). *Ann. Rev, Biochem.* **57**: 133.
Morikawa K. (1993). *Current opinion in structural* biology. **3**: 17.
Plummer D.T. (1978). *An Introduction to Practical Biochemistry*. (McGraw Hill Book Co. Ltd. UK) p.119.
Rasool S.A. and Ali R. (1991). *Pak J. Botany* **23**(2): 199.
Rasool S.A., Ghazala S.S., Iqbal A. and Ahmed B. (1992). *Proceedings of 12th Pakistan Congress of* Zoology; *Zoological Society of Pakistan.* **12**: 615.
Rasool S.A., Mirza A. and Khan MA. (1990). *Curr. Genet.* **17**: 417.
Rasool S.A. and Mirza A. (1994). *Pakistan Journal of Pharmacology.* **11**(2): 53.
Samson L. and Cairns J. *(1977). Nautre* (London) **267**: 281.
Sassanfar M. and Samson L. (1990). *J. Biol. Chem* **265**(1): 20.
Singer B. and Grunberger D. (1983). In: *Molecular Biology of Mutagenesis and Carcinogenesis* (Plenum Press, New York), p.45.
Vaughan P. and Sedgwick B. (1990). *J. Bacterial.* **173**(12): 3656.