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ERROR FREE REPAIR OF ALKYLATED DNA IN *STAPHYLOCOCCUS AUREUS*

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Abstract

The adaptive repair activity has been demonstrated in cells of *Staphylococcus aureus*. Reactivation of alkylated phage p68 has been shown to be dependent upon the host cell coded adaptive repair enzymes of the adapted *S. aureus* cells. *In vitro* biochemical studies have indicated the nitrosoguanidine (MNNG) adapted synthesis of alkyltransferase. However, DNA glycosylase activity has been insignificant.

Introduction

Error-proof adaptive repair involves both the enhanced survival and a decreased mutagenesis in cells that are pre-exposed to sublethal exposure with the same or related agents (Evensen & Seeberg, 1982; Lindahl, 1982). Killing and mutagenic/ carcinogenic adaptation are atleast partly under different genetic control viz., with the induction of DNA glycosylase and alkyltransferase (Hurst & Nasim, 1984). Universality of adaptive phenomenon still awaits confirmation (Rasool, 1987). The present studies were undertaken in order to point out a possible strategic change during the course of handling the alkylation mediated damages in Gram positive organisms like *Staphylococcus aureus*. Experiments were therefore carried out to study the survival pattern of the adapted and nonadapted *S. aureus* cells to MNNG-mediated stress; fate of lethally alkylated staph phages within the adapted cells and *in vitro* enzymatic assay for glycosylase and alkyltransferase in crude extracts of the pretreated *S. aureus* cells, using tritiated MNNG labelled *Micrococcus luteus* as a substrate.

Material and Methods

Culture of *Staphylococcus aureus* HER 1049 and its lytic phage p68 (Ackermann *et al.,* 1984) were obtained from Professor H.W. Ackermann of the University of Laval, Canada. MNNG (Fluka) was used as methylating agent. Fresh solutions were prepared each time in citrate phosphate buffer pH 6 (Plummer, 1978).

For viable counts of adapted and nonadapted cells, method of Ather *et al.,* (1984) with slight modification was followed. Method of Evensen & Seeberg (1982) was followed for studying the phage reactivation of lethally alkylated phages in adapted and nonadapted *S. aureus* cells based on plaque forming units. In *vitro* assays for DNA glycosylase and alkyltransferase activities in adapted and nonadapted cells

were carried out according to the methods of Samson & Cairns (1977) and Ather *et al.* (1984).

Results

The cell survival pattern of adapted and nonadapted cells of *S. aureus* showed a clear indication of the existence of adaptive repair activity in pretreated adapted cells (Table 1).

Phage reactivation studies based on plaque forming units (p.f.u.) of lethally treated phages in adapted and nonadapted cells have been depicted in Fig.1. The findings counterprove the results obtained in Table 1. The results of *in vitro* studies of alkyl-damage mediating alkyltransferase enzyme in adapted and nonadapted cells of *S. aureus* showed that only the induction of alkyltransferase activity was pronounced. This activity was monitored by measuring the released H^3 radioactivity (Counts per minute-c.p.m.) from the extracts of the nonadapted (540 c.p.m.) and adapted (1243 c.p.m.) cells.

Discussion

An increased resistance has been observed in *S. aureus* cells after stepwise adaptation (upto 1 $\mu g\ ml^{-1}$ with MNNG) to the lethal effects of the same alkylating agent, thereby indicating the existence of adaptive response in this microorganism. Ather *et al.,* (1984) have also observed similar results in *Micrococcus luteus*. Analogous results were reported by Hadden *et al.,* (1983) in *Bacillus subtilis*.

Bacteriophages serve as an ideal tools for studying DNA repair activities of the cells. Results of phage reactivation studies of alkylated phages in adapted and nonadapted cells confirm the occurence of inducible adaptive response in *S. aureus* cells. It is well understood that the adapted cells (with increased quantities of repair enzymes) are able to repair the lesions of lethally treated phages more efficiently than the nonadapted cells. Evensen & Seeberg (1982) also reported the alkylated *E. coli* phage reactivation in adapted cells only.

Adaptation involves a pleiotropic response and results in the induction of several enzymes (Evensen & Seeberg, 1982). O^6-methylguanine (O^6-meG) is specifically

Table 1. Cell survival pattern of adapted and nonadapted cells of *Staphylococcus aureus*.

MNNG challenge doses (μg/ml)	Cell survival percentage (%)	
	Nonadapted cells	Adapted Cells
0	100	100
5	80	86
10	77	82
15	74	77
20	67	74

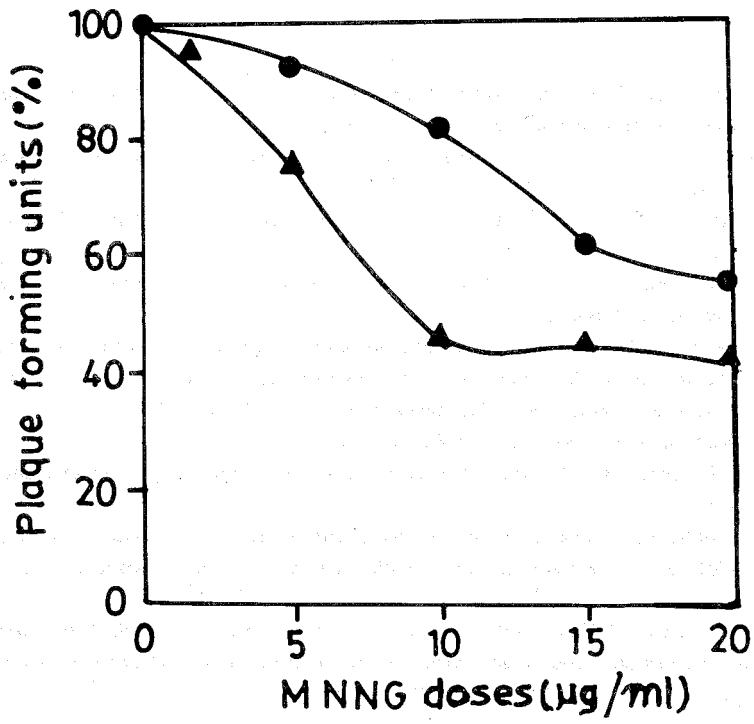

Fig.1. Phage reactivation pattern of lethally treated phages in adapted and non-adapted host cells based on p.f.u.

- Phage reactivation in adapted cells
- Phage reactivation in nonadapted cells

repaired by one of these inducible enzymes viz., alkyltransferase as also found in the present study. The extracts from the adapted cells of *S. aureus* removed the methyl group from the radioactively labelled alkylated DNA more efficiently than the nonadapted control cell extracts. Earlier, cell free extracts from MNNG-treated *E. coli* were found to remove O^6-meG from radioactively labelled alkylated DNA more efficiently than from the control extracts (Lindahl *et al.*, 1988). The glycosylase which is responsible for the repair of N^3-methyladenine (i.e., N^3-meA) activity has been insignificant in *S. aureus*, although, this enzyme has been reported to be induced during adaptive repair in *E. coli*. (Karran *et al.*, 1982). It is possible that lesions like N^3-meA are not introduced in sufficient quantities in *S. aureus* as a result of alkylation damage.

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Samson, L. and J. Cairas. 1977. A new pathway for DNA repair in *Escherichia coli. Nature*, 267: 281-282. involves a pleiotropic response and results in the induction of several enzymes (Evensen & Seeberg, 1982).

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