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A gene responsible for Ozone sensitivity (ozrB) in chromosome of *Escherichia coli* B. MQ 1844

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E. coli B. MQ 1844 균주의 오존감수성 유전자(ozrB)의 특성

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ABSTRACT: An ozone-sensitive mutant of *Escherichia coli* strain B, MQ 1844 is described. Its properties, including high sensitivity to ozone and radiation, inducible filamentation, extensive DNA degradation and impaired DNA synthesis following ozonation, are attributable to a mutation in *ozrB*, a gene which is cotransducible with *malB*. Based on differences in phenotypic expression as well as on the particular location of this gene on the bacterial chromosome, *ozrB* appears as distinct from the other ozone-or radiation-sensitivity genes previously described.

KEY WORDS □ *E. coli*-DNA repair-Mapping-Ozone

Mutants either more resistant or sensitive to ozone than wild-type (OZ^r or OZ^s) are obtained following treatment of *Escherichia coli* with different chemical or physical agents. Recent experiments from this laboratory have shown that the sensitivity of this organism to ozone is under the control of at least two genes which map between *argEH* and *metA* on the bacterial chromosome (Côtes and Chung 1979; Poliquin *et al.* 1982). The *ozrA* mutation was obtained following treatment of strain B251 with nitrosoguanidine and was shown to confer sensitivity to ozone, ultraviolet light and X-rays, as well as to induce extensive DNA degradation following ozonation (Hamelin 1975; Poliquin 1979). A similar treatment of the wildtype strain yielded *ozrC*, a mutation which is also closely linked to *argEH* but determines only the sensitivity of the cells to ozone (Côtes and Chung 1979).

Several ozone-sensitive mutants were also isolated following exposure of wild-type strain B Hill to ozone but preliminary mapping data suggested that at least one of these OZ^s strains, MQ1844, was carrying a mutation in a different gene (*ozrB*). The present study has thus been undertaken to determine if this gene (subsequently designated *ozrB*) was part of the *ozrA-pozrC* gene cluster previously described (Côtes and Chung 1979). In addition, we wished to determine the radio-sensitivity of the *ozrB* mutant as well as the magnitude of the effects of ozone upon cell survival, DNA degradation and DNA synthesis kinetics in this strain. Wild-type and X-ray-sensitive mutants of *E. coli* were also used for comparison.

MATERIALS AND METHODS

Bacterial strains

The following closely related strains of *E. coli* B Hill were used: MQ1844 (*ozrB*) was isolated after

treatment of the parental strain with 50 u*l*/*l* ozone for 30 min (Poliquin *et al.* 1982); Bs-2 (*exrA*) and PAM452 (*exrB*) were obtained from J. Donch. (Greenberg *et al.* 1974); MQ910 (*lexA*) was derived from *E. coli* K-12 strain AB2494 by Pl-mediated transduction (Chung *et al.* 1975); ozone- and radiation-resistant strain AB1911 was kindly supplied by B.J. Bachmann. The old *exrA* and *exrB* gene symbols have been maintained to provide easy access to the literature cited in the text.

Media

Cells were grown at 37°C in M9 medium (Emmerson and Howard-Flanders 1965) supplemented with the necessary growth factors to the following concentrations; thymine, 50 ug/m*l*; DL-amino acids, 40 ug/m*l*; L-amino acids, 20 ug/m*l*. Viable counts and survival curves were done on this medium solidified with 1.5% Bacto agar (Difco). For transduction experiments, Davis minimal agar (Difco) plates containing the appropriate amino acids and 0.5% glucose or maltose were used.

Transduction

Phage P1*kc*-mediated transduction were done according to Donch and Greenberg (1968). Colonies having appeared after 3 days of incubation at 37°C on minimal agar without arginine or methionine, and those on minimal agar lacking any other carbon sources than maltose, were respectively scored as Arg$^+$, Met$^+$ and Mal$^+$ transductants. Ozone-recombinant strains were rapidly detected as described earlier (Hamelin and Chung 1976).

Sensitivity to UV and ozone

Survival curves were obtained as described elsewhere (Chung and Greenberg 1968; Hamelin and Chung 1974). Log-phase cells (5×10^8/m*l*) were used for both the UV and the ozone treatments.

DNA degradation

For degradation experiments, the bacterial DNA was labelled by incubation of the cells in M9 medium containing 10 uCi of ^{3}H-thymidine (specific activity: 40-60 Ci/mM) per m*l*, purchased from New England Nuclear. Grown cells were washed twice, resuspended in the same volume of fresh medium without label, and incubated with aeration at 37°C for another 1.5 h. After washing one more, the cells were exposed to 50 u*l*/*l* ozone. The procedure for measuring ozone-induced DNA degradation was that of Strike and Emmerson (1974). Control experiments were carried out as above, except that clean air was used instead of ozone.

DNA synthesis kinetics

For DNA synthesis studies, unlabelled cells were preincubated, ozonated, then postincubated in M9 medium containing 20 uCi ^{3}H-thymidine and 100 ug deoxyadenosine per m*l*. At 30-min intervals, 100 u*l* samples were pipetted onto filter paper disks and assayed for trichloroacetic acid insoluble radioactivity by liquid scintillation counting. Cells exposed to clean air were used as control.

RESULTS

Mapping data

P1 grown on MQ1844 (*malB* OZ^s) was first used to transduce the Arg$^+$ character to AB1911 (*argEH metA* OZ^r), and 1.5% of the transductants analysed were found to be as sensitive to ozone as the donor strain (Table 1). An equivalent number of Arg$^+$, Mal$^-$ transductants was also obtained. Both the MQ1844 gene for ozone sensitivity (*ozrB*) and the *malB* gene for maltose fermentation are therefore located at a relatively large distance from the *argEH* gene.

When the Met$^+$ character was selected, about 3% of the transductants growing on minimal agar lacking methionine were OZ^s and Mal$^+$. Accord-

Table 1. *Transduction from MQ 1844 to AB1911**

Selected Markers	Number of Transductants Analysed	Cotransduction Frequency (%)			
		arg$^+$	*metA*$^+$	*malB*	*ozrB*
arg$^+$	133	—	27	1.5	1.5
metA$^+$	135	24	—	3	3

* MQ1844: *arg*$^+$, *metA*$^+$, *malB*, OZ^s(*ozrB*$^-$)
* AB1911: *arg*, *metA*, *malB*$^+$, OZ^r(*ozrB*)

ing to these data, *ozrB* was more closely linked to *malB* than *argEH* and thus represents a new gene for ozone sensitivity in *E. coli*. In agreement with this conclusion, other experiments (Poliquin *et al.* 1982) have shown that *ozrB* is cotransduced with *metA* and *malB* at a frequency of about 4% and 25%, respectively, which places the locus on the right side of *malB* at about 91 min on the conventional map of *E. coli* (Bachmann and Low 1980).

Survival curves

Radiosensitive mutants of *E. coli* deficient in the repair of X-ray-induced single-strand breaks in DNA are abnormally sensitive to ozone (Hamelin and Chung 1974; Hamelin *et al.* 1977); and many of the genes controlling radiation sensitivity have been located in the *metA–malB* region of the bacterial chromosome (Chung and Greenberg 1968; Chung *et al.* 1975). Pl-mediated transduction was thus used to bring some of these mutations from a different origin into the same genetic

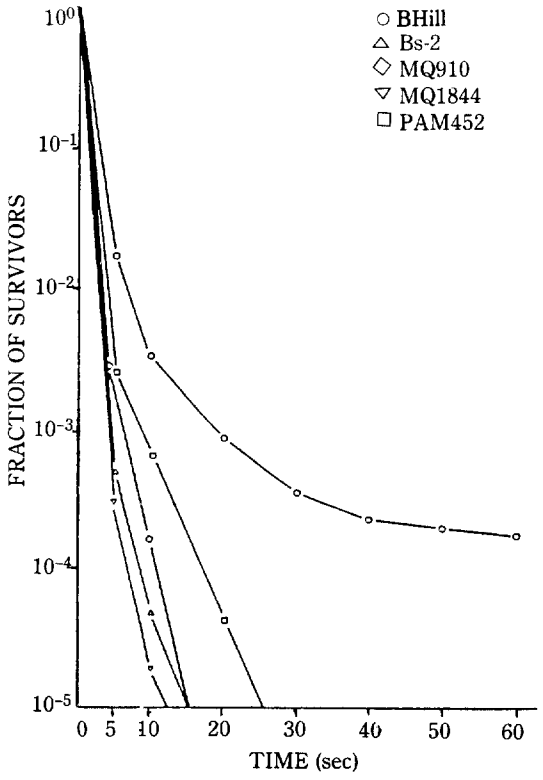

Fig. 1. *Survival of E. coli strains BHill, Bs-2 (exrA), MQ910 (lexA), MQ1844 (ozrB) and PAM452 (exrB) to ultraviolet light (1.54 Jm^{-2}sec^{-1}).*

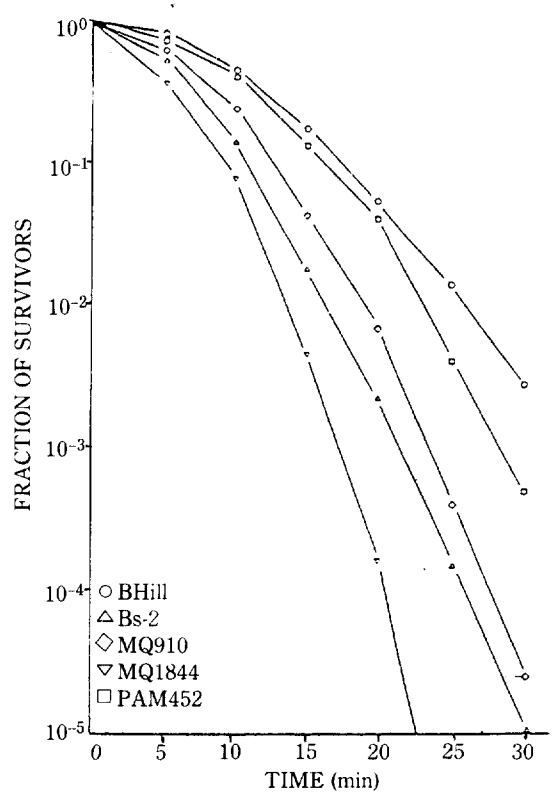

Fig. 2. *Survival of strains after exposure to 50 ul/l ozone for 0-30 min in growth medium.*

background and these derivatives of parental strain B Hill were compared to the *ozrB* (MQ1844) strain as to their sensitivity to ultraviolet (UV) light and ozone.

The *lexA* (MQ910) and *exrA* (Bs-2) strains are extremely UV-and X-ray-sensitive while the *exrB* (PAM452) strain shows an intermediate radio-sensitivity when compared to the parental (B Hill) strain (Donch and Greenberg 1968; Greenberg *et al.* 1974; Fig. 1). In agreement with the radiation data, PAM452 (*exrB*) was found to be slightly more sensitive to ozone than B Hill but not as sensitive as Bs-2 (*exrA*) and MQ910 (*lexA*) (Fig. 2). The most significant cell-killing effects of UV light and ozone were observed, however, with the MQ1844 (*ozrB*) mutant strain.

DNA degradation

Survival of *E. coli* cells and DNA degradation following exposure to ionizing radiation are closely correlated with the repair of DNA single-strand breaks (Youngs and Bernstein 1973; Myers 1975;

Hamelin *et al.* 1976; Hamelin 1984). When ozonated cells were held for 3 h in M9 medium at 37°C, extensive DNA degradation occured and the relative rate of this DNA degradation was both faster and more extensive in the *lexA* (MQ910) and *exrA* (Bs-2) strains than in the *exrB* (PAM452) and the parental strains (Fig. 3). Ozone may thus affect the DNA in a similar fashion to ionizing radiation, notably by introduction breaks into the molecule; and unrepaired single-strand breaks in the polynucleotide chains are probably responsible for the increased cell-killing and DNA degradation observed in these phenotypically different mutants following ozonation. In accordance with the ozone survival data shown in Fig. 2, mutant strain MQ1844 (*ozrB*) was found to degrade more or its DNA than any of the other strains studied.

DNA synthesis kinetics

To assess the possible contribution of unrepaired single-strand breaks toward the sensitivity of *E. coli* to ozone, DNA synthesis was

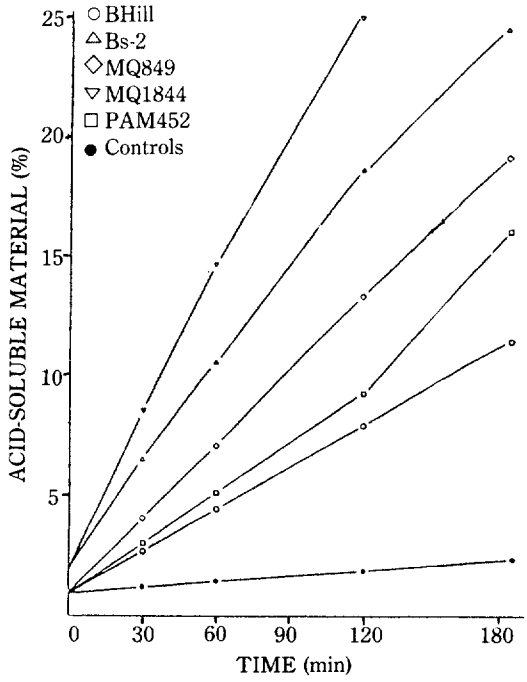

Fig. 3. *Acid-soluble material appearing during incubation after a 30 min ozonation.*
The control cultures exposed to clean air are represented by a closed symbol. Similar results were obtained for the five controls.

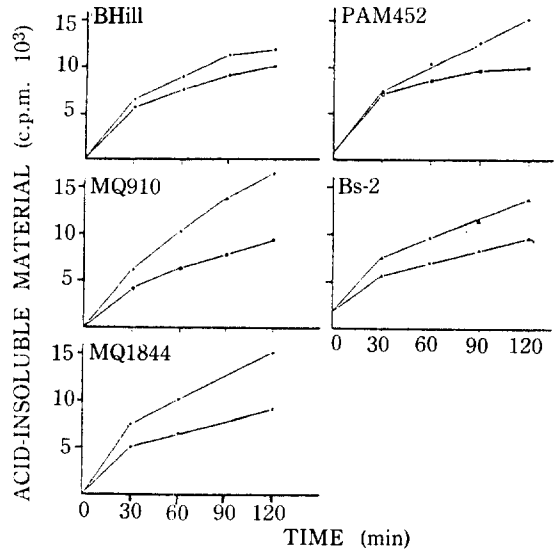

Fig. 4. *DNA synthesis kinetics after ozonation.*
The corresponding unozonated controls incubated in the same conditions are represented by a closed symbol.

measured in the five isogenic strains under the same conditions used for the cell survival and DNA degradation experiments (Fig. 4). After a 2 h incubation period at 37°C, incorporation of ^{3}H-thymidine in the B Hill, PAM452 (*exrB*), MQ910 (*lexA*), Bs-2 (*exrA*) and MQ1844 (*ozrB*) cultures that had been exposed to ozone was only 85, 66, 57, 70, and 60% of that measured in the control cultures. On the basis of these data, it thus appears that the lesions produced in *E. coli* DNA by ozone interfere, as well as radiation products, with the synthesis of DNA; and that the local delays in DNA synthesis resulting from these lesions are longer in strains deficient in DNA single-strand break repair than in wild-type. Differences in the overall DNA synthesis kinetics were observed between the *ozrB* and the X-ray-sensitive mutants which more of less correlate with the cell killing and DNA degradation data.

DISCUSSION

Repeated isolations of ozone-sensitive mutants of *E. coli* B after treatment with nitrosoguanidine (Hamelin 1975; Côtes and Chung 1979) suggested

that the susceptibility of this organism to ozone might be under the control of specific genes. In agreement with this, strains highly sensitive to ozone were found to carry a mutation in genes (*ozrA* and *ozrC*) located between *argEH* and *metA* on the bacterial chromosome (Hamelin 1975; Côtes and Chung 1979). With the discovery of *ozrB* on the right side of *malB*, it thus seems that at least three genes are involved in the resistance of *E. coli* to ozone.

Strain MQ1844 (*ozrB*) was isolated following exposure of wild-type cells to ozone which confirms the usefulness of this strong oxidant in mutation research (Hamelin *et al.* 1981). However, OZ^s strains closely related to the *ozrB* strain have also been obtained with nitrosoguanidine (unpublished results). Induction of mutations in this gene is therefore not exclusive to ozone.

According to the transduction experiments, *ozrB* is independent from the *ozrA* and *ozrC* genes. In contrast to the latter gene which determine only the susceptibility of *E. coli* to ozone (Côtes and Chung 1979), *ozrA* and *ozrB* also control the response of the cells to radiation (Hamelin 1975; Poliquin 1979). However, *ozrA* strains filament spontaneously while this interference with normal cell division must be induced by UV or ozone in the *ozrB* strains (Poliquin 1979; Poliquin *et al.* 1982). Mapping data as well as differences in phenotypic expression bus indicate that *ozrB* is not an allele of *ozrA* or *ozrC*.

Several genes involved in resistance to radiation have been found in *E. coli* which are also cotransducible with *malB* (Chung *et al.* 1975; Greenberg *et al.* 1974). *exrA* strains are more sensitive to UV, X-rays and ozone than parental strain B (Greenberg *et al.* 1974; Hamelin and Chung 1974) but they do not form filaments even when irradiated (Donch *et al.* 1968). The gene *lex* isolated in a K-12 strain of *E. coli* confers phenotypic properties corresponding to those of *exrA* (Howard-Flanders and Boyce 1966; Mount *et al.* 1972; Hamelin and Chung 1974; Chung *et al.* 1975) but as mentioned above, *ozrB* does not suppress UV-induced filamentation and a strain carrying this mutation show a slightly higher sensitivity to radiation and ozone than the *exrA* and *lexA* strains. DNA degradation following ozonation is also more extensive in the *ozrB* strains than in the radiosensitive strains. Now *exrB* strains are moderately sensitive to UV and ozone, prevent excessive DNA degradation, and form filaments not only after irradiation but spontaneously (Greenberg *et al.* 1974). These phenotypic differences suggest that *ozrB* is not an allele of the radiation sensitivity genes; and this conclusion is supported by the fact that *ozrB* is transduced less frequently with *metA* or *malB* than *lexA*, *exrA* and *exrB* (Chung and Greenberg 1968; Chung *et al.* 1975; Greenberg *et al.* 1974).

However, no wild-type recombinants occurred in crosses between strains containing *ozrB* and *exrA* (unpublished results). Mutation of one cistron with different phenotypic expressions, depending on an altered gene product not yet identified, may explain this absence of complementation between the ozone-and the radiation-sensitive strains. Nevertheless, *ozrB* is a *malB*-linked gene as much involved in sensitivity to radiation and filament formation as *lexA*, *exrA* and *exrB*, genes found dominant over their wildtype alleles (Mount *et al.* 1972; Donch and Greenberg 1974). A similar dominance of *ozrB* over $ozrB^+$ would also make the demonstration of complementation among these closely related strains impossible. *ozrB* and *exrA* may also be mutants of different cistrons forming an operon concerned with DNA repair and synthesis, and which is involved in some may with cell division. Experiments now in progress in this laboratory should provid a genetic resolution to this problem.

적 요

오존 감수성 돌연변이 균주의 하나인 *E. coli* B. MQ1844를 실험재료로 오존가스 처리 후에 나타나는 DNA 사상체의 연결, DNA의 분해, 손상된 DNA의 합성 그리고 오존과 방사선의 고감수성 현상에 대하여 이 세균의 malB 유전자와 상호치환

이 가능한 유전자의 하나인 ozrB 유전자의 돌연변이로 연관하여 검토하였다. 세균 염색체상의 특수한 유전자 위치 뿐만 아니라 세균의 표현형질의 상이한 결과를 분석한 결과 ozrB 유전자는 기존 발표한 바 있는 오존 또는 방사선 감수성 유전자와는 별개의 것임을 확인하였다.

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