NOTES

Improving DNA damage repair ability of *E. coli* to UV- radiosensitivity by DNA-mediated gene transfer of low energy ion beam

SONG Daojun[1] and YU Xun[2]

1. *Center of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei* 230031, *China*;
2. *College of Life Science, University of Science and Technology of China, Hefei* 230026, *China*

Abstract Using calcium chloride method of transfer gene as control, a new technique of transferring gene by low energy ion beam has been applied to the study of improving DNA damage repair ability of *E. coli* to UV-radiosensitivity. The genome DNA pieces of *Deinococcus radiodurans*, as "foreign" genetic materials, were introduced into the UV-radiosensitive strains of *E. coli* by implantation of 20 keV Ar^+ at doses ranging from 1×10^{15} to 2×10^{15} ions/cm^2. Results show that the transfected strains present higher UV-radioresistance than that of un-transfected ones and start ones. The survival rate of transfected strains and their unscheduled DNA synthesis (UDS) ability is increased, indicating that the transfer gene is a success.

Keywords: **ion implantation, *Deinococcus radiodurans*, transfer gene, survival rate, unscheduled DNA synthesis.**

SINCE the 1980s, a series of achievements have been acquired in industrial and agricultural fields of mutation breeding using ion beam bioengineering[1—3]. In the meantime, breakthroughs have also been made in the research of gene transfer by low energy ion beams. It is reported that this new method of gene transfer has already been successfully applied to crops, such as rice and corn[4—6]. But reports on the application of this biotechnology to microorganisms have not been found. On the other hand, *Deinococcus radiodurans*, one species of bacteria with extreme resistance to ionizing radiation (such as UV and Gama-ray) and a variety of chemical mutagens[7], was found to possess a highly-efficient DNA damage repair system, which was regarded as responsible for such resistance[8]. However, little research has been reported on the change of radio-resistance of other acceptive cells after the delivery of the genome DNA of *Deinococcus radiodurans*.

In this note, using calcium chloride method of gene transfer as control, the genome DNA pieces of *Deinococcus radiodurans* were introduced into mutagen-induced UV-radiosensitive strains of *E. coli* with the technique of gene transfer assisted by low energy ion beam. The change of *E. coli*'s resistance to UV-radiation and the ability of DNA damage repair after such delivery have been studied.

1 Materials and methods

(ⅰ) Bacterial strains and growth conditions. *Deinococcus radiodurans* AS1.633 were obtained from the Institute of Microbiology, the Chinese Academy of Sciences. *E. coli* B were obtained from Dr. Li Hong at the Center of Ion Beam Bioengineering of the Institute of Plasma Physics, the Chinese Academy of Sciences. *D. radiodurans* strains were grown at 32℃ in TGY broth (10 g tryptone, 1 g glu-

cose, 5 g yeast extract, 1 000 mL distilled water) with shaking at 200 r/min or TGY agar (1.5% agar). *E. coli* strains were grown at 37℃ in Luria-Bertani broth (10 g tryptone, 5 g yeast extract, 10 g NaCl, 1 000 mL distilled water) with shaking at 200 r/min or LB agar (1.5% agar).

(ii) DMS, EI mutagenization and the screening of UV-sensitive *E. coli*. DMS and EI mutagenization were made by the method described in ref. [9]. Exponentially growing cultures of *E. coli* B were treated with the DMS and EI solution of different concentrations (DMS was dissolved in phosphate buffer with 0.1 mol/L and pH 7.0; EI was dissolved in aseptic water) for 120 min at 37℃ with shaking at 160 r/min, then the mutagenized cells were harvested and washed twice with LB broth, and the samples were spread on LB plates to give about 80—100 colonies per plate after incubation at 37℃ for 2 d. The mutagenized population was screened for UV-sensitivity by patching individual colonies onto LB plates and exposing the plates to 200 J/m^2 of UV-radiation.

(iii) Chromosomal DNA isolation. The chromosomal DNA isolation of *D. radiodurans* was made following the method in ref. [10]. The procedure yielded approximately 400 mg of chromosomal DNA per 500 mL culture. Analyzed by pulsed-field gel electrophoresis, this preparation routinely generated 10—50 kb DNA fragments.

(iv) Genetic transformation procedure. Transformation by calcium chloride ($CaCl_2$) solution was carried out according to the method in ref. [11]. The final $CaCl_2$ was 30 mmol/L, 20 μg of chromosomal DNA was added to 1 mL of LB containing 2×10^7 UV-sensitivity cells. Transformation by DNA-mediated low energy ion beam implantation was performed as follows: the mutagenetic cells of UV-sensitivity were harvested and washed twice with aseptic water, then diluted and plated on glass plates (2 cm × 3 cm). The samples, put into vacuum target chamber (10^{-5} Pa), were implanted by 20 keV Ar^+ and the pulse implantation technique was used with a pulse time of 5 s and interval time of 30 s. The implantation dose was $(1 \times 10^{15}—2 \times 10^{15})/cm^2$ and the fluency of each pulse to the sample was 10^{14} ions (D_0). The sample in vacuum chamber without ion implantation was used as ck1, and the sample in aseptic air was used as ck2. Transformed cells by the above method were soaked in LB broth containing DNA fragments of *D. radiodurans* for a 2-h incubation at 37℃. The isolation of bacteria transformed to UV resistance was described above.

(v) The survival measurement. The survival rate was determined according to the method described in reference [12].

(vi) The UDS DNA determination. The UDS DNA determination was made according to the method in ref. [13], the concentration of ^{3}H-TdR was 10 μCi/mL, the cpm counters were measured by the method of Song[14].

2 Results and analysis

(i) Sensitivity of *E. coli* B to DMS and EI treatment. It can be seen from fig. 1 that the survival rate of *E. coli* B declines almost linearly with the increase of DMS concentration. When the concentration of DMS reached 12%, the survival rate drops nearly to zero. In the meantime, the survival rate of *E. coli* B appears as a small shoulder-type curve first with the treatment of EI. Only after the concentration of EI exceeds 1/1 000, the survival rate decreases greatly. These facts indicate that *E. coli* B has higher sensitivity towards DMS treatment; as to EI, it only shows its relatively-high sensitivity in the concentration range of 1/1 000—1/250. Certainly high sensitivity does not necessarily represent high mutational efficiency. In this study we used DMS (0.5%) and EI (1/1 000), both in their generally-accepted optimal mutagenetic concentration on bacteria, to treat almost the same amount of *E. coliB* cells, respectively. Through the same screening method, we obtained 3 strains of *E. coli* B from each of the two treatments (named *E. coli* BDsu1, *E. coli* BDsu2, *E. coli* BDsu3 and *E. coli* BEsu1, *E. coli* BEsu2, *E. coli* BEsu3), which are all sensitive to UV-radiation with a radiation dose of 200 J/m^2. The mutagenesis frequency of the two groups are 6.3% and 7.8%, respectively.

(ii) Stability of mutational strains and possibility of back mutation. The 6 mutational strains, which had been obtained with two mutagens, were cultured continuously for a month under no UV radia-

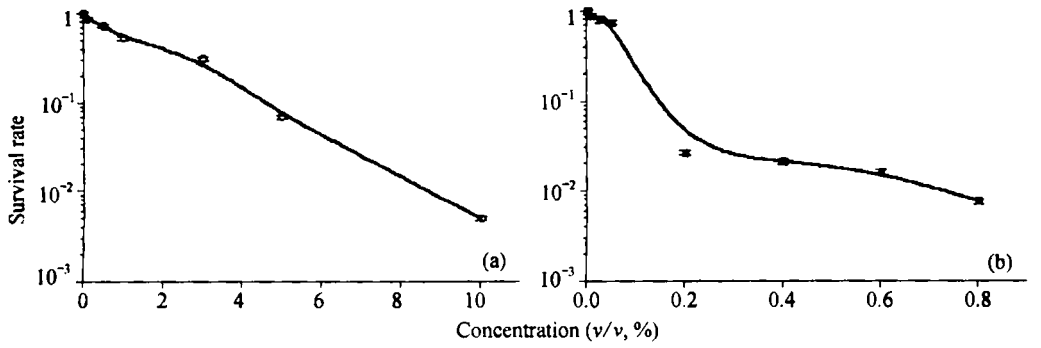

Fig. 1. Survival curves of *E. coli* B treated with DMS(a) and EI(b).

tion. Consequently, their sensitivity towards UV radiation did not change. This indicates that the 6 strains were the result of cell's gene mutation caused by two alkylating mutagens. These mutant genes, like the wild-type ones, possessed relatively stable structures. The stability of such UV-radiosensitive strains not only means the stability of mutant genes in these strains, but also means that there was no back mutation (from mutant-type to wild-type) happening during one month's passage culture. Of course the stability of mutant genes and the lack of back mutation are only relative conceptions. Just like wild-type genes, mutant genes also have the possibility of spontaneous mutation; such spontaneous mutation also contains back mutation.

(Ⅲ) Comparison of radiosensitivity of UV-sensitive mutational *E. coli* strains before and after the delivery of genome DNA. Donor DNA extracted from *Deinococcus radiodurans* was introduced separately into 6 radiosensitive strains of *E. coli* B obtained. After repeated screening, $CaCl_2$ method was used to get one strain *E. coli* r/BEsu1 which had resistance to UV-radiation. While 2 more UV-radioresistant strains (*E. coli* r/BEsu2 and *E. coli* r/BDsu1) were obtained using the method of gene delivery assisted by low energy ion beam. Changes of their sensitivity to UV-radiation are shown in fig. 2. The 2 controls received identical treatments except that they were neither induced by ion beam nor by $CaCl_2$, and no UV-radioresistant strains of *E. coli* were obtained. Hence we may conclude that in order to deliver genes successfully, it is necessary for acceptive cells to be treated with extrinsic physical or chemical methods before the transfer.

The results of the experiment show that those strains into which the DNA of *Deinococcus radiodurans* were transferred have a higher resistance to UV-radiation not only than controls, but also than the original *E. coli* B strains of this study. This character is displayed through the increase of 2 parameters which describe the extent of radiosensitivity: D_0 (the radiation dope when the survival rate equals zero) and D_{37} (the radiation dope when the survival rate equals 37%). It proves that *Deinococcus radiodurans*' donor DNA, which was transferred into acceptive cells and which was related to radio-resistance, has brought about its expression in UV-sensitive *E. coli* strains, causing the considerable increase of their resistance to UV-radiation.

(Ⅳ) Comparison of the ability of DNA damage repair of UV-sensitive *E. coli* strains before and after the delivery of genome DNA. The main action of UV-radiation on DNA in organic cells is the formation of pyrimidine dimer. If the the organism lacks the ability to repair such DNA damage, or the repair system itself has been hurt, the organism will behave as UV-radiosensitive. The resistance to UV-radiation for *Deinococcus radiodurans* is much higher than that of ordinary microorganism; not because its DNA receives less damage when under UV-radiation, but that it has much better ability to repair DNA damage than usual microorganism. In this study we treated *E. coli* B with 2 mutagens to get 6 UV-radiosensitive mutational strains, which indicated that the treatment of mutagens had damaged its DNA repair system. After the delivery of genome DNA pieces of *Deinococcus radiodurans* using two different methods, we obtained 3 UV-resistant strains (*E. coli* r/BEsu1, *E. coli* r/BDsu1 and *E. coli* r/BEsu2) with a lot of screening. Unscheduled DNA synthesis (UDS) measurement was made after UV-radiation, and a consid-

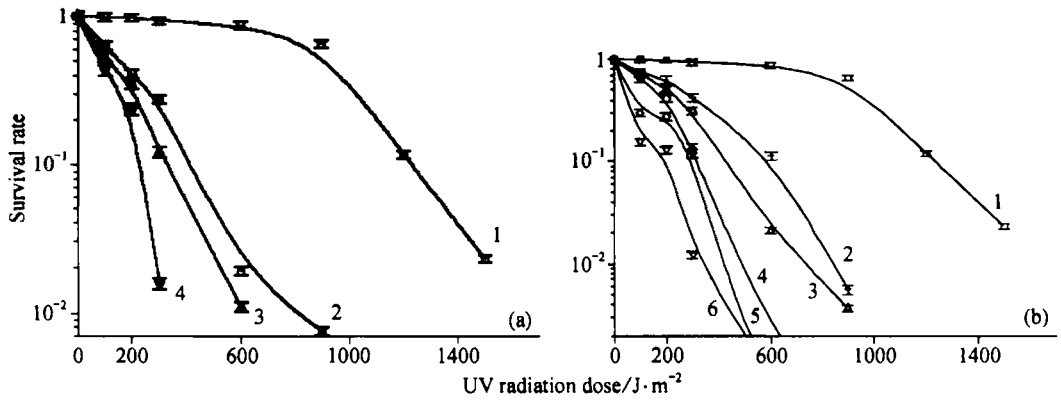

Fig. 2. Survival curve of *D. radiodurans* and *E. coli* by UV radiation. (a) *E. coli* r/BEsu1 were transfected by $CaCl_2$ treatment. 1, *D. radiodurans*; 2, *E. coli* r/BEsu1; 3, *E. coli* B; 4, *E. coli* BEsu1; (b) *E. coli* r/BEsu2 and *E. coli* r/BDsu1 were transfected by ion implantation. 1, *D. radiodurans*; 2, *E. coli* r/BDsu1; 3, *E. coli* r/BEsu2; 4, *E. coli* B; 5, *E. coli* BDsu1; 6, *E. coli* BEsu2.

erable increase in their UDS ability has been found. This further proves that not only such genome DNA pieces coming from *Deinococcus radiodurans* were delivered into those *E. coli* B strains, but that among these DNA pieces genes which were related to the character of radio-resistance have accomplished their expression.

Table 1 Comparison of UDS of *D. radiodurans*, *E. coli* B and transfected strains

Strains	Cells number ($\times 10^7$)	^{3}H-TdR/count·min^{-1}	Normal UDS (%)
D. radiodurans AS1.633	2.1	98 947	1 741.1
E. coli			
E. coli B	2.3	5 683	100
E. coli BEsu1	2.56	936	16.5
E. coli BEsu2	2.45	1 083	19.1
E. coli BDsu2	2.31	1 013	17.8
E. coli r/BEsu1	2.35	3 638	64.02
E. coli r/BEsu2	2.47	6 417	112.9
E. coli r/BDsu2	2.38	6 149	108.2

3 Discussion

Mechanism of 2 mutagens in the role of organic mutagenesis. Besides UV and ionizing radiation, chemical mutagens are also widely used in studies to mutagenize organisms. In our research we applied 2 types of mutagens, DMS and EI, which were both alkylating agents and had good mutagenetic action on organic cells. The reaction of these mutagens on organic cells mainly focuses on the alkylation of base (especially on the base of G) and phosphate group, provoking the breakage of carbohydrate-phosphate bond, and trending to error-DNA repair in heredity, the transformation mutation of AT→CG, the transition mutation of AT→TA and of CG→GC, etc.[15]. More research is needed to explain which type of damage towards *E. coli* B DNA is caused by the 2 mutagens in this experiment.

Comparison of 2 methods of DNA-mediated gene transfer. In this study 2 different methods, the method of gene transfer by low energy ion beams and the method of calcium chloride, were applied successfully to introducing the genome DNA pieces of *Deinococcus radiodurans* into the UV-sensitive strains of *E. coli* B. Through the test of resistance to UV-radiation and the determination of ability in DNA damage repair, we conclude that the gene pieces which were delivered into acceptive strains have made their expression.

One method which has been used widely from an early time is to induce organic cells into compe-

tence with calcium chloride solution of appropriate concentration to process gene transfer. But because different cells have different living conditions and because $CaCl_2$ solutions with different concentrations have different induction abilities, which add difficulty to the control of optimal conversion condition, this method has been limited in the increase of its conversion rate, though it has relatively simple operations. Using this method, the absolute conversion rate obtained in our experiment was 0.32%.

The method of gene transfer assisted by low energy ion beams is a new way to deliver gene making use of the principle of the etching action of ion beam implantation on cells[3,4]. Microholes or pores can be formed from the cell's surface to its inner part with the etching of ion beams. Donor DNA pieces are able to make their way into acceptive cells successfully through these passageways. Our research indicates that this method assisted by ion beams is not only fit for plant cells, but also practicable for microbial cells. Its absolute conversion rate is 2.4%, which is higher than that of the method using $CaCl_2$ solution. Though this new method of gene transfer is a fairly good one, it no doubt has some flaws that need improvements. For example, various types of ion beams with different amounts of energy will bring different etching extents to manifold acceptive cells. We should try to find out which kind of ion beams, and how large the implantation dose and energy we are to use to obtain the best effect for acceptive cells.

Besides the formation of etching passageways into cells by ion beam implantation, which is one main action of ion-beam-assisted gene transfer, the positive charge carried by implanting ions has also undoubtedly played an important role in helping acceptive cells to absorb foreign DNA pieces that carry negative charge, making these DNA pieces enter acceptive cells more smoothly. In this study Ar^+ of 20 keV was implanted into *E. coli* cells, and it was indicated by preparation experiments that a radiation dose of 1×10^{15} ion/cm^2 would bring relatively good induction effect. As the structures of membrane and wall of different cells vary, the choice of type, energy and dose of implanting ions should correspondingly change. In spite of the fact that this method needs further improvement, it has many merits such as simple operation, no need to prepare competence cells and feasibility to be applied to both plasmid DNA and macromolecular genome DNA. We anticipate that this technology of gene transfer will grow into a comparatively ideal and efficient one.

Acknowledgement The authors are grateful to Dr. H. Li for providing generous gifts of *E. coli* used in the study and Prof. Z.L. Yu, Dr. F.L.Wu and Dr. H. Li for valuable comments on the manuscript, also to J. Hao and C.L. Yuan for their contribution to this work. This work was supported by the National Natural Science Foundation of China (Grant No. 19605005).

References

1 Yu, Z. L., Huo, Y. P., Review in low energy ion biology, *J. Anhui Agri. Univ.* (in Chinese), 1994, 21(3): 221.
2 *Jiang, Z. H., Peng, Z. H., Advances in the research on improved variety by ion implantation, J. Anhui Agri. Univ.* (in Chinese), 1994, 21(3): 295.
3 Yu, Z. L., Ion beam in life science——a new research field, *Physics* (in Chinese), 1997, 6: 333.
4 Yu, Z. L., Yang, J. B., Wu, Y. J. *et al.*, Transferring Gus gene into intact rice cells by low energy ion beam, *Nucl. Instru. Meth. B*80/81, 1993, 81: 1328.
5 Yang, J. B., Wu, L. J., Wu, J. D. *et al.*, Production of transgenetic rice plants via low energy ion beam-mediated DNA delivery into mature embryos, *Chinese Science Bulletin*, 1995, 40(5): 427.
6 Chen, B. J., Tian, Q. Y., Yao, Z. W. *et al.*, Studies on foreign gene transfer into cotton by ion beam, *J. Anhui Agri. Univ.* (in Chinese), 1993 (Supplement): 19.
7 Moseley, B. E. B., Radiation resistance and deoxyribonucleic acid base composition of Micrococcus radiodurans, *Nature*, 1964, 203: 1298.
8 Daly, M. J., Ouyang, L., Minton, K. W., Interplasmidic recombination following irradiation of the radioresistant bacterium *Deinococcus radiodurans*, *J. Bacteriol.*, 1994, 176(24): 7506.
9 Tempest, P. R., Boseley, B. E. B., Defective excision repair in a mutant of Micrococcus radiodurans hypermutable by some monofunctional alkylating agents, *Molec. Gen. Genet.*, 1980, 170: 191.
10 Mattimore, V., Udupa, K. S., Berne, G. *et al.*, Genetic characterization of forty ionizing radiation sensitive strains of Deinococcus radiodurans: linkage information from transformation, *J. Bacteriol.*, 1995, 177(18): 5232.
11 Udupa, K. S., O'cain, P. A., Mattimore, V. *et al.*, Novel ionizing radiation sensitive mutants of *Deinococcus radiodurans*, *J. Bacteriol.*, 1994, 176(24): 7439.
12 Driedger, A. A., James, A. P. Grayston, M. J. *et al.*, Cell survival and X-ray-induced DNA degradation in *Deinococcus*

radiodurans, *Radiat. Res.*, 1970,44: 835.
13 James, E., *DNA Repair*: *A Laboratory Manual of Research Procedures* (eds. Trieberg, E. C., Hanawalt P. C.), New York: Oxford University Press, 1981,1(Part B): 276.
14 Song, D. J., Yu, X., Shao, C. L. *et al.*, Studies on etching and damage action of low energy ion beam on microbial cells, *Acta Biochem. Biophys. Sinica* (in Chinese), 1998, 30(6): 570.
15 Zhu, G. J., Wang, Z. X., *An Application Technique Manual of Industrial Microorganism* (in Chinese), Beijing: Chinese Light Industry Press, 1997, 385.

(*Received March* 3, 1999)