MUTANT RADIATION-RESISTANCE ALLELES FROM ESCHERICHIA COLI Gam^{r}444 : CLONING AND PRELIMINARY CHARACTERIZATION

V.N. Verbenko, V.L. Kalinin, L.V. Kuznetzova

The radiation-resistant mutant Gam^{r}444 selected in our laboratory by repeated cycles of γ-irradiation-growth [1] has at least two mutations involved in enhanced radioresistance. Cloning of these mutations is handicapped because direct selection of recombinant clones on selective media is imposible. Therefore it is necessary to use an enrichment of the transformed populations by γ-irradiation.

Plasmid pBC4042 carrying mini-Mu-transposon MudII4042 was chones as a vector for in vivo cloning. The wild-type recipient AB1157 (Mu_cts) was transduced to Cm^r by lysates of Gam^r444 (Mu_cts/pBC4042) donor and the populations of the transductants were enriched with the radiation-resistant clones by two alternative methods. The first one consisted in three concequtive cycles of γ-irradiation by increasing (up to 3 *kGy*) doses and regrowth of survived cells to select the radiation-resistant cell clones.

The second method was invented to select recombinant dominant plasmids capable to give the radiation-resistant transformants. Plasmid DNAs were isolated from primary Cm^r transductants and used for transformation of AB1157 (Mu_cts) recipient. Colonies of Cm^r transformants were resuspended into minimal medium, irradiated by a dose giving survival $S = 10^{-3}$ and plasmid DNA was reisolated from colonies of the survived cells. These cycles were repeated four times, the dose being gradually increased up to 3 *kGy*.

By the first method variants of the AB1157 (Mu_cts) were selected which gave positive response in a spot-test for γ-resistance (irradiation by 1,4 *kGy* on nutrient agar) and one of these variants (12 20) was studied. Its radioresistance level is nearly as high as for a mutant strain Gam^r445. However, the plasmid DNA isolated from this strain does not transfer Gam^r phenotype to Cm^r transfomants and the cells remained radiation-resistant even after spontaneous elimination of the plasmid. We can assume that a primary transductant from which the strain 12Δ20γ descended carried a recessive Gam^r mutation of the strain Gam^r444 on the recombinant plasmid but during repeated cycles of irradiation homozygotization took place and this mutation was introduced into a cell chromosome so that the strain 12Δ20γ acquired Gam^r phenotype which does not depend on recombinant plasmid.

In four independent experiments based on the second method four plasmids (pGam1, pGam2, pGam4, pGam5) were isolated which can stably transfer enhanced radiation-resistance during the transformation. These plasmids can not enhance radiation-resistance of the Gam^r444 strain, but one of the plasmids (pGam1) makes the strain Gam^r445 more radioresistant. After introduction of the pgam plasmids into recA13 and rpoH15 recipients radiation-resistance of these strains is not elevated. Therefore expression of mutations Gam^r cloned on pGam depends on the two stress systems (SOS and heat-shock regulons). The same dependence was shown for the strain Gam^r444 [2,3]. Two plasmids (pGam2 and pGam4) supress post-irradiation DNA degradation in the γ-irradiated recA cells and probably a code for an recA-independent inhibitor of DNA degradation. After introduction of the pGam plasmid into the strain 12Δ20γ cured from the resident plasmid p12Δ20γ we obtained 12Δ20γ/pGam transformants which are more radioresistant than the parent 12Δ20γ. Therefore radioprotective effects of mutations Gam^r cloned in the strain 12Δ20γ and in the plasmids pGam are additive. The levels which are nearly as high as for the strains Gam^r444 were obtained after introduction of the pGam plasmids into a recipient grpE deficient in GrpE heat-shock protein and constitutively expressing other heat-shock proteins. It is possible that the strains 12Δ20γ also contain cloned recessive mutation Gam^r affecting the regulation of heat-shock protein expression.

Our data showed that we have succeded in the cloning of mutant alleles Gam^r enhancing radioresistance of the E. coli wild type cells and that radioresistance of Gam^r mutant is due to two different mutations (dominant and recessive).

Radioresistance of E.coli strains, *kGy.*

(3D doses giving $S = 10^{-3}$)

AB1157	1,1	AB1157/pGam2	1,7	12Δ 20γ /pGam4	3,4
Gam^{r}444	5,0	AB1157/pGam4	2,1	12Δ 20γ p/Gam5	3,1
Gam^{r}445	2,6	AB1157/pGam5	1,8	grpE/pGam1	4,2
12Δ 20γ	2,3	Gam^r 445/pGam1	3,6	grpE/pGam2	3,7
AB1157/pGam1	2,4	12 Δ 20γ/pGam1	3,4	grpE/pGam4	4,2

References

1. Bresler S.E., Verbenko V.N., Kalinin V.L. //Genetika. 1980. V.16. P.1753.
2. Bresler S.E., Verbenko V.N., Kalinin V.L. //Genetika. 1984. V.20. P.746.
3. Bresler S.E., Verbenko V.N., Kalinin V.L. //Genetika. 1986. V.22. P.2658.