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AN Rtsl— DERIVATIVE PLASMID CONFERRING UV SENSITIVITY ON ESCHERICHIA COLI HOST

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SUMMARY: A deletion mutant was isolated from a kanamycin resistance R plasmid Rtsl. This mutant plasmid, pTW20, was found to enhance the lethal effect of UV irradiation on Escherichia coli host, especially at 42°C. A cloning experiment with pTW20 DNA demonstrated that the gene, puv, being responsible for the UV sensitivity was located on the kanamycin resistance gene containing BamHl fragment of pTW20. This fragment conferred a sensitivity to methyl methane sulfonate on its host along with the sensitivity to UV, suggesting that a reapir process of the host chromosome is impaired by the presence of puv.

A kanamycin resistance plasmid Rtsl is a naturally occurring temperature sensitive plasmid(1) with molecular weight of 140 mega daltons(2). This plasmid, belonging to T incompatibility group(3), characteristically confers pleiotropic temperature sensitivities on its host. Among the temperature dependent phenotypes, an inhibitory effect of Rtsl on the growth of the host at 42°C(4) has been a subject in which we are most interested. Similar effects on the hosts are observed with several mutants of ColVB(5) and F(T.Miki and T.Horiuchi, Jpn. J.Genet. 45:482, 1970), and also with other T incompatibility group plasmids(3).

Recently, we isolated several deletion mutants from Rtsl and found some of them to confer sensitivity to UV irradiation on E. coli host at 42°C. This is phenotypically the reverse of an effect endowed by ColIb(6) and the N incompatibility group plasmids(7, 8), which are known to protect the host from UV irradiation.

The present work reports on the characterization of an Rtsl derivative plasmid conferring UV sensitivity on its host and confirms the location of the gene being responsible for the sensitivity on a BamHl fragment of the plasmid.

MATERIALS AND METHODS

Bacterial strains and plasmids. Escherichia coli K-12 JC1557(F^- gal leu his arg met str) and W677(F^- thr leu thi) were employed as the host strains. pTW3 and pTW20 are derivative plasmids of Rtsl. The vector plasmid pACYC184(9) is resistant to chloramphenicol(Cp) and tetracycline(Tc). An EcoRl restriction site exists in the Cp gene and a BamHl site in the Tc gene.

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UV survival test. The method of Mortelmans and Stocker(10) was employed. Exponential cultures of bacteria in Penassay broth(PAB, Difco) at 37°C were washed and diluted into M9 salts. 0.01 ml of an appropriate dilution was spotted onto PAB agar plates. The plates were exposed to a UV lamp(Toshiba, 15-W) at a distance of 70 cm for varying times. Immediately after irradiation, they were wrapped in aluminum foil and incubated at 42°C and 37°C.

Preparation of plasmid DNA. Bacteria were cultured for isolation of plasmid DNA according to the method of Timmis et al.(11). JC1557(pTW20) was grown at 30°C in 500 ml of enriched M9 glucose medium containing 0.5% Casamino acids and 10 μg of thiamine per ml. When the optical density of the culture reached at 0.1, 50 μg of kanamycin per ml was added and cultivation was continued overnight. For preparation of vector plasmid and its recombinant plasmid DNAs, the plasmid$^+$ cells were grown in 50 ml of PAB at 37°C. At OD:1.0, 100 μg of spectinomycin per ml was added and grown overnight. From both types of culture plasmid DNA was isolated in Brij-cleared lysate(12). Cleared lysate of pTW20$^+$ cells were further concentrated by adding 10% polyethylene glycol 6000 (13). The cleared lysate was centrifuged in an ethidium bromide cesium chloride solution at 40,000 rpm for 36 hr at 20°C using a Beckman Type 65 Rotor. The covalently closed circular DNA was collected by puncturing the centrifuge tube, and ethidium bromide was extracted from the DNA solution with isoamyl alcohol. Finally, plasmid DNA was dialysed against 1,000 ml of 0.1 x SSC three times at 4°C.

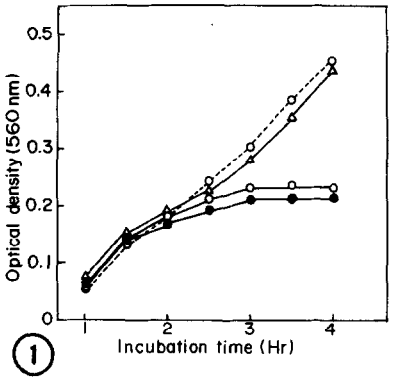
Manipulation of plasmid DNA. Digestion of plasmid DNA with EcoRl and BamHl restriction endonucleases(Takara Shuzo),ligation of digested DNA to vector plasmid with T4 ligase(BRL Inc.) and transformation of ligated DNA to E. coli were done by the method of Tanaka and Weisblum(14).

RESULTS

Isolation of Rtsl deletion mutants. E. coli W677(Rtsl) was treated with nitrosoguanidine for isolating mutant strains that produce an excess amount of extracellular DNase(15). Several mutants were obtained and found these strains harboring deletion derivatives of Rtsl. Among them, plasmids pTW10 and pTW20 were found to confer UV sensitivity on their hosts, especially at 42°C. As pTW10 killed its host to some extent at 42°C without UV irradiation, this plasmid was inappropriate to investigate the mechanism of the UV sensitivity. Hence, pTW20, which is the smallest derivative(41 Mdal) of Rtsl, was chosen for further study. A spontaneous deletion mutant of Rtsl, pTW3(60 Mdal), was also used.

Growth of plasmid$^+$ cells. Growth of JC1557 harboring the Rtsl derivative at 42°C is shown in Fig.1. The pTW3 and pTW20 showed similar inhibitory effects on host cell growth. In contrast, pTW201-BK consisting a BamHl fragment of pTW20 and vector plasmid pACYC184(described below) did not inhibit the growth of the host. At 30°C, growth of the cells harboring any plasmid was comparable to that of the plasmid$^-$ cells.

UV sensitivity of plasmid$^-$ cells. As shown in Fig. 2, pTW20 enhanced the lethal effect of UV irradiation on JC1557 host remarkably at 42°C. Whereas, JC1557(pTW3) showed an intermediate level of UV sensitivity between the pTW20$^+$



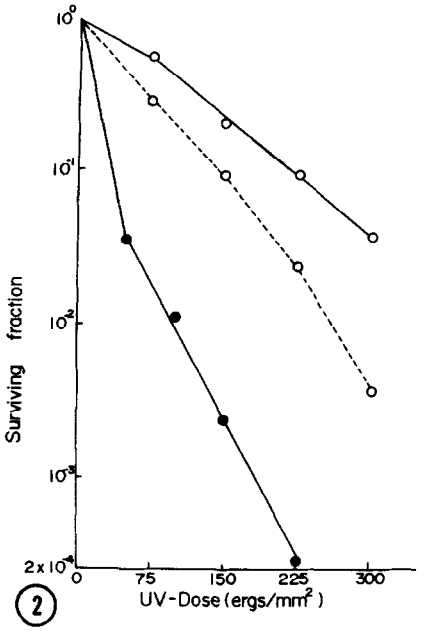


Fig. 1. Growth curve of E. coli JC1557 with or without plasmid in broth at 42°C. Bacteria were grown in PAB at 30°C. At a cell density of about 6×10^8 per ml, the culture was diluted two-hundred-fold into fresh PAB and continued to grow at 42°C. Growth was monitered densitometrically using spectrophotometer (Coleman Junior II). ---○--- JC1557 R⁻, ——●—— JC1557(pTW3), ——○—— JC1557(pTW20), ——△—— JC1557(pTW201-BK).

Fig. 2. UV survivals of JC1557 with or without plasmid at 42°C. UV irradiated cells on PAB agar plate were incubated in dark at 42°C. The number of colonies developed after 18hr(at least) of incubation were scored. For pTW3⁺ cells the plate was incubated 24hr, since the development of the colonies was retarded. ——○——JC1557 R⁻, ---○--- JC1557(pTW3), ——●—— JC1557(pTW20).

and the plasmid⁻ cells. These suggest that the increase in lethality of the pTW20⁺ cells after UV irradiation at 42°C is mainly due to UV irradiation, but is not to high temperature, since the extent of the inhibition of the host growth by pTW20 at 42°C was not greater than that by pTW3, as shown in Fig. 1. To distinguish the effect of UV irradiation from that of high temperature, pTW20 DNA was dissected with restriction endonucleases, and the effects of the cloned fragments on UV sensitivity and growth of the host were examined.

Cloning of pTW20 DNA. The pTW20 DNA was cleaved into six fragments with EcoR1 restriction endonuclease(Fig.3A), and all of the fragments were cloned to the vector plasmid pACYC184. Of the six hybrid plasmids, only pTW201(E-1 fragment of pTW20 plus pACYC184) was found to confer UV sensitivity and kanamycin resistance on JC1557 host. Consequently, the E-1 fragment(11.7 Mdal) was dissected further with BamH1(Fig.3B), and the effects of UV irradiation was studied.

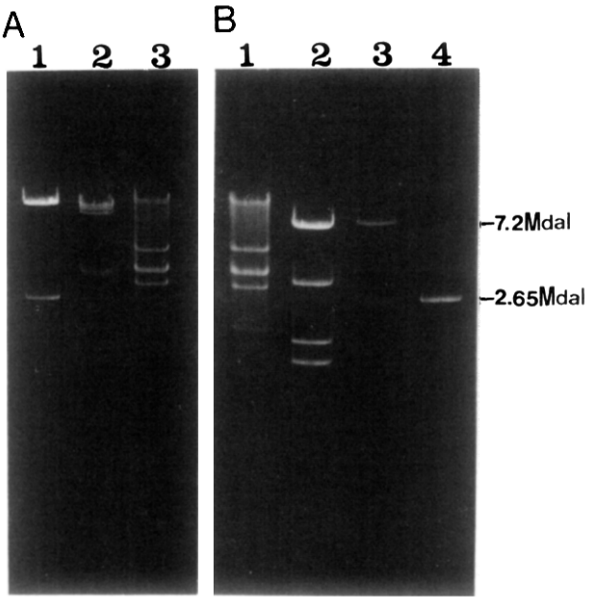


Fig. 3. Restriction enzyme analysis of pTW20 and its cloned DNA.
A) DNA digested with EcoRI was electrophoresed in a 0.8% agarose gel for 4hr at a constant current of 20mA. 1. pTW201 DNA[E-1 fragment(11.7 Mdal) of pTW20 plus pACYC184(2.65 Mdal)], 2. pTW20 DNA, 3. λ DNA, which is shown as a molecular weight standard.
B) DNA digested with BamHI, but λ DNA shown was digested with EcoRI. 1. λ DNA, 2. pTW201 DNA, 3. pTW201-BK(7.2 Mdal BamHI fragment of E-1 plus pACYC184) 4. pACYC184 DNA.

As shown in Fig. 4, pTW201-BK(7.2 Mdal BamHI fragment of E-1 plus pACYC184) conferred a strong UV sensitivity on JC1557 host along with kanamycin resistance. It should be stressed that although the increase in UV sensitivity by pTW201-BK was remarkable at 42°C, it was also observed at 37°C. In addition, this plasmid did not inhibit host cell growth at 42°C, as shown in Fig. 1. These indicate that pTW20 confers UV sensitivity on its host, and the gene being responsible for the sensitivity is located on the 7.2 Mdal BamHI fragment of pTW20. We designated the gene puv(p denotes plasmid).

Sensitivity to methyl methane sulfonate. JC1557 with or without pTW201-BK was seeded onto PAB agar plate containing 0.04% of MMS, and the number of colonies developed on the plate was compared with that on drug free plate. All the plasmid$^-$ cells retained the colony forming ability on the MMS$^+$ plate. In contrast, only 8% and 1% of the plasmid$^+$ cells grew in the presence of this concentration of MMS at 37°C and 42°C, respectively. Thus, pTW201-BK conferred MMS sensitivity on its host, and the sensitivity was also enhanced at 42°C.

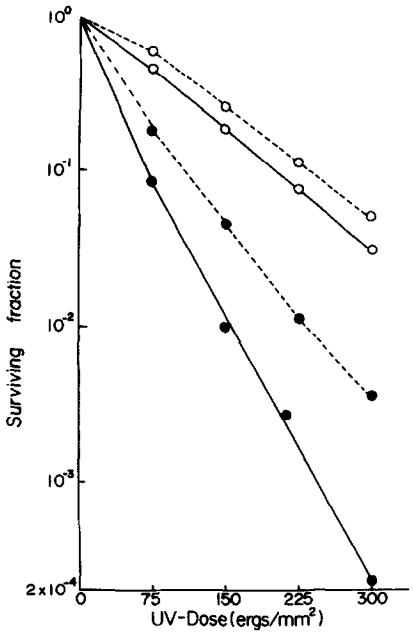


Fig. 4. UV survivals of JC1557 with cloned fragment of pTW20 or vector plasmid pACYC184 at 37°C and 42°C. Experimental procedures were the same with those described in the legend to Fig. 2, but the plates after UV irradiation were incubated at 37°C and 42°C. ---○--- JC1557(pACYC184) at 37°C, ——○—— JC1557(pACYC184) at 42°C, ---●--- JC1557(pTW201-BK) at 37°C, ——●—— JC1557(pTW201-BK) at 42°C.

DISCUSSION

We showed in this study that an Rts1 derivative plasmid pTW20 conferred a strong UV sensitivity on E. coli host, and that the gene puv being responsible for the sensitivity was located on the 7.2 Mdal BamH1 fragment of pTW20. At the beginning of this work, we suspected the enhancement of UV sensitivity by the Rts1 derivatives being related to the temperature dependent inhibitory effect on host cell growth, since the UV effect was demonstrated remarkably at 42°C. However, these two effects on the host were discriminated from each other, as shown in the experiment with the cloned fragments of pTW20.

Following two questions have newly arisen from the cloning experiment. One is why was the enhancement of UV sensitivity by the 7.2 Mdal fragment remark able at 42°C, though it did not show the inhibitory effect on the host growth at 42°C. This may be simply ascribed to a temperature dependency of the expression of the gene puv. Alternatively, an unknown factor on the fragment might affect the expression of puv. To resolve this, further dissection of the 7.2 Mdal fragment is needed. The other question is which fragment of pTW20 has the function to inhibit host cell growth at 42°C. Any EcoR1 fragment of pTW20 cloned to pACYC184 did not show a discernible inhibitory effect on the host. Therefore,

the responsible gene for the effect might straddle on an EcoRI restriction site, or multiple genes are concerned with the effect.

The increase in UV sensitivity of E. coli host by pTW20 suggests that a repair process of the host chromosome is impaired by the presence of puv. This notion is supported from the finding that pTW201-BK$^+$ cells were sensitive to MMS. It is of interest to note that the sensitivity of the host to MMS increased at 42°C as well as that to UV. It is presently unknown which process of the repair pathway of the host is affected by puv. If excision repair is involved, there could be a possibility that pTW201-BK would increase the mutation frequency of its host, especially at higher temperature.

Finally, we must mention whether the extracellular DNase, of which production is determined by RtsI and its derivatives(15), is concerned with the sensitivity to UV. A correlation between them is, however, unlikely, since E. coli W677 harboring a mini-pTW20(E-1 fragment plus E-6:Rep fragment of pTW20) does not produce DNase extracellularly(Y. Ito, unpublished data).

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REFERENCES

1. Terawaki, Y., Takayasu, H., and Akiba, T. (1967) J. Bacteriol. 94, 687-690.
2. Ishihara, M., Kamio, Y., and Terawaki, Y. (1978) Biochem. Biophys. Res. Commun. 82, 74-80.
3. Coetzee, J. N., Datta, N., and Hedges, R. W. (1972) J. Gen. Microbiol. 72, 543-552.
4. Terawaki, Y., Kakizawa, Y., Takayasu, H., and Yoshikawa, M. (1968) Nature(London) 219, 284-285.
5. Koyama, A. H., and Yura, T. (1975) J. Bacteriol. 122, 80-88.
6. Howarth, S. (1965) J. Gen. Microbiol. 40, 43-55.
7. Drabble, W. T., and Stocker, B. A. D. (1968) J. Gen. Microbiol. 53, 109-123.
8. Tweats, D. J., Thompson, M. L., Pinney, R. J., and Smith, J. T. (1976) J. Gen. Microbiol. 93, 103-110.
9. Chang, A. C. Y., and Cohen, S. N. (1978) J. Bacteriol. 134, 1141-1156.
10. Mortelmans, K. E., and Stocker, B. A. D. (1976) J. Bacteriol. 128, 271-282.
11. Timmis, K. N., Cabello, F., and Cohen, S. N. (1978) Molec. Gen. Genet. 162, 121-137.
12. Clewell, D. B., and Helinski, D. R. (1969) Proc. Natl. Acad. Sci. U.S.A. 62, 1159-1166.
13. Humphreys, G. O., Willshaw, G. A., and Anderson, E. S. (1975) Biochim. Biophys. Acta. 383, 457-463.
14. Tanaka, T., and Weisblum, B. (1975) J. Bacteriol. 121, 354-362.
15. Matsumoto, H., Kamio, Y., Kobayashi, R., and Terawaki, Y. (1978) J. Bacteriol. 133, 387-389.