Isolation and Characterization of Plasmids Carrying the *dut* Gene of *Escherichia coli**

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The enzyme deoxyuridine 5'-triphosphate hydrolase (dUTPase, E.C. 3.6.1.23) produces dUMP, the immediate precursor of thymidine nucleotides. An important function of the enzyme may be to keep the intracellular concentration of dUTP at a low level, thus reducing the incorporation of uracil into DNA.[1] This study was made in order to construct *Escherichia coli* strains that overproduce dUTPase, thus facilitating enzymological investigations of the enzyme. By using restriction enzyme fragments from the transducing phage λ*dspoT*447 [2] and the plasmid pBR322,[3] we have constructed plasmids carrying the dUTPase gene *dut*.

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Some members of a set of λ transducing phages carrying the genes *pyrE* and *spoT* were found to increase the amount of dUTPase in induced lysogenes.[4] This finding allowed a tentative allocation of the *dut* gene to a *Pst*I + *Eco*RI fragment also harboring the *pyrE* gene [2] (Fig. 1). Thus selection for *pyrE*$^+$ plasmids would allow isolation of plasmids carrying the *dut*$^+$ gene. Another selective system was based upon the use of a phenotype caused by *dut* mutation; a *dut* Δ (*xth*–*pnc*A) strain, BW286,[5] is viable at 25 °C but unable to grow at 42 °C.

Selection of dut$^+$ *after BamHI digestion.* A partial digest of λ*dspoT*447 DNA (5 μg) and a complete digest of plasmid pBR322 DNA (1 μg) with restriction enzyme *Bam*HI were ligated and used to transform [6] strain BW286 selecting for temperature resistance at 42 °C on LB plates [7] containing 100 μg/ml ampillicin. Of the transformants two were picked for further study and shown to be *dut*$^+$. The plasmids from those strains were characterized by cutting with different restriction enzymes.

Plasmid pKK3 (Mw 8.5 Md) was shown to have two *Bam*HI fragments, 5.8 Md and 2.7 Md, respectively. Digestion of pKK3 with *Pst*I gave a number of fragments, most of which could be identified as *Pst*I fragments of λ*dspoT*447 DNA.[2] However, two of them, 2.5 Md and 2.0 Md in size, could not be reconciled with the *Pst*I cleavage pattern of λdspoT447. One of these two fragments should contain DNA from λ*dspoT*447 corresponding to a

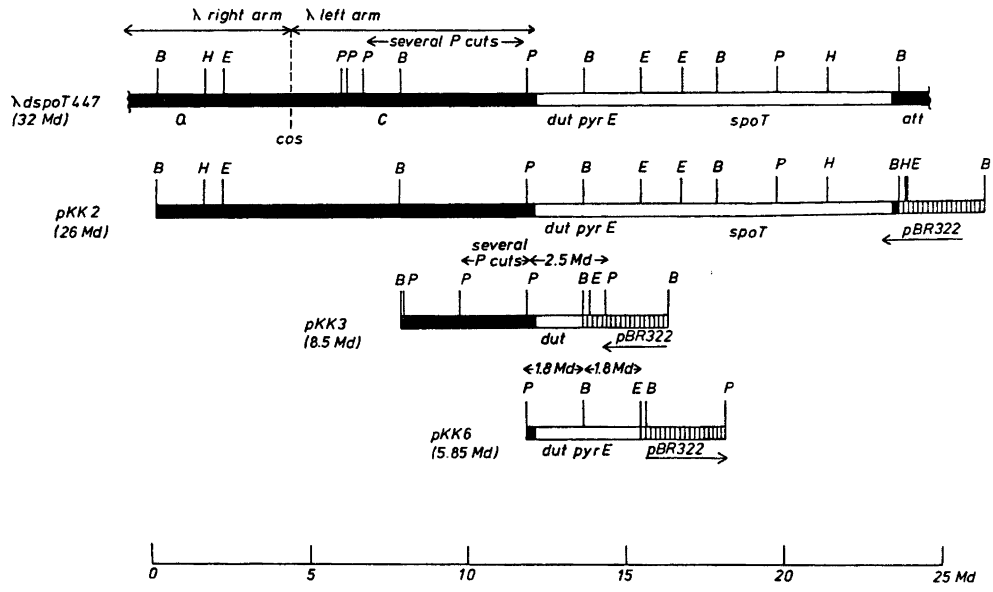

Fig. 1. Restriction endonuclease map of λ*dspoT*447 and *dut* plasmids. B, P, H and E: Restriction endonuclease sensitive sites of *Bam*HI, *Pst*I, *Hind*III and *Eco*RI. Closed bars: λ DNA. Open bars: *E. coli* DNA and striped bars: pBR322 DNA.

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Table 1. Escherichia coli strains harboring *dut*$^+$ plasmids.

Bacterial strain	Plasmid	Host strain	
		Designation	Relevant genotype
KK969	pKK2	BW286	*dut*$^-$, *ΔXth*
KK970	pKK3	BW286	*dut*$^-$, *ΔXth*
KK975	pKK6	NF929	*dut*$^+$, *pyrE*$^-$

*Pst*I + *Bam*HI fragment of 1.8 Md. Since the size of *Pst*I + *Bam*HI fragments of pBR322 is 2.0 Md and 0.7 Md,[8] we conclude that the 2.5 Md fragment carries the *dut*$^+$ gene and is composed of 1.8 Md from the *Pst*I – *Bam*HI fragment of λ*dspoT*447 and 0.7 Md of pBR322. This conclusion was verified by cleavage with both *Bam*HI and *Pst*I. Thus, plasmid pKK3 is a *dut*$^+$ plasmid generated from a *Bam*HI cut in the *pyrE* gene and has the fragments oriented as shown in Fig. 1.

Plasmid pKK2 (Mw 26 Md) was characterized by cutting with restriction enzymes *Bam*HI, *Hind*III and *Bam*HI + *Hind*III. The plasmid was shown to be a *dut*$^+$, *pyrE*$^+$ and *spoT*$^+$ plasmid with the orientation as shown in Fig. 1.

Selection of pyrE$^+$ *after PstI + EcoRI digestion.* Since plasmid pKK2 carries the *pyrE*$^+$ gene it was chosen for a *Pst*I + *Eco*RI digestion experiment. After digestion of pKK2 DNA (4 μg) and pBR322 DNA (2 μg) with *Pst*I and *Eco*RI the *pyrE* mutant, NF929, was transformed selecting for the *pyrE*$^+$ character (growth on minimal medium *without* uracil). One transformant was picked and shown to be resistant to tetracycline and sensitive to ampillicin. The plasmid from this strain, pKK6, was analyzed by restriction enzyme digestion. The digestions gave the following fragments: With *Pst*I one fragment of 5.85 Md, with *Pst*I + *Eco*RI fragments of 3.6 and 2.25 Md and with *Bam*HI + *Pst*I fragments of 2.05, 2.0 and 1.8 Md. Plasmid pKK6 has the fragments oriented as shown in Fig. 1.

Enzyme production. Strains carrying the *dut*$^+$-plasmids are listed in Table 1. They have been found to overproduce dUTPase (KK970 about 10-fold and KK975 about 15-fold). They are now being used for purification of the enzyme in large amounts for investigations of its molecular properties.

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