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A bacteriophage lytic enzyme gene as a marker for gene transfer and expression

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The plasmid pBR322 bearing the lytic enzyme gene of colitis bacteriophage was injected into 10 sets of 10 onion bulbs each, at multiple points, and germinated for 7 days. The lytic enzyme was detected in the extracts of 0–60% of the bulbs in each set by turbidometric assay. This lytic enzyme gene can be used as a marker gene for gene transfer in plants and animals and in chimaeric gene constructs to study regulation of gene expression.

VARIOUS genes of bacterial and animal origin have been used as reporter genes and positive selection markers in gene transfer experiments. The most commonly used reporter genes include genes for chloramphenicol acetyltransferase (CAT)[1,2], neomycin phosphotransferase (NPT II)[3,4], *Escherichia coli* β-glucuronidase (GUS)[5] and firefly luciferase[6]. CAT and GUS genes are widely used though they are poor selective agents. The NPT II gene is a good selective agent but the assay of the enzyme is not easy. These genes are also used in chimaeric gene constructs to determine promoter strengths and gene expression[7,8]. We report here the use of a lytic enzyme gene from a virulent phage of *E. coli*, the colitis phage[9] as a marker for gene transfer and expression.

The lytic enzyme gene was mapped on a 2.3 kb *Bam*HI fragment of the phage DNA and cloned in the plasmid pBR322[10,11]. The gene was completely sequenced and the amino acid sequence was derived. This enzymic protein was of 18.05 kd and contained 163 amino acids. The enzyme was purified using the cloned gene and the characteristics studied[10]. The pBR322 and the recombinant plasmid DNAs were prepared according to the method of Brinboim and Doly[12]. They were dissolved in 10 mM Tris-HCl, pH 7.6 and 0.5 mM $MgCl_2$ (TM buffer) and 0.5 μl portions containing 0.1 μg of DNA were injected into the lower one-third portion of onion (*Allium cepa*) bulbs of 2 cm diameter at about 100 points using a 10 μl Hamilton syringe. This facilitated the flooding of the DNA into the meristematic regions of the bulb. The injected onions were kept at 22°C for 60 min and then transferred to glass vials containing TM buffer and ampicillin, tetracycline and streptomycin (2 μg/ml each) so that the base of the bulbs is dipped in the buffer solution and incubated at 22°C in a BOD incubator for 7 days. Three to four whorls of the onions which are essentially nutritive, were peeled off. The injected region of the fast growing bulb, along with the stem and roots, was washed thoroughly with water and ground with 1 ml of TM buffer at 4°C in a mortar with a pestle. The unbroken cells and cell debris were centrifuged at 15,000 *g* and the supernatant fraction was collected and assayed for the enzymic activity.

Sensitized *E. coli* B cells prepared according to the method of Salser *et al.*,[13] were used as the substrate. The *E. coli* B cells were grown to mid-log phase in M9 medium, pelleted at 7000 *g*, suspended in TM buffer previously saturated with chloroform, washed with TM buffer and suspended in the same buffer to have an absorbance of about 0.6 at 450 nm. The enzyme activity was monitored by the decrease in $A_{450\,nm}$ after the addition of the extract.

The plasmid pBR322 or recombinant DNA were injected in sets of 10 bulbs at a time and incubated for 7 days at 22°C. The extract was prepared from each bulb and assayed for the lytic enzyme. This was repeated at least 10 times at different periods during the course of a year. The enzymic activity was absent in the extracts of all sets of onions that received the pBR322 DNA. In the extracts of two sets of onions and few onions in the rest of the 8 sets, which received the recombinant DNA, there was no detectable enzymic activity. However, in the extracts from 8 sets of onions, the enzymic activity was detected in bulbs, the number of which varied from 1 to 6. The results from a typical set of experiments are shown in Figure 1. The extracts from the pBR322 injected onions did not show any lytic enzyme activity (line 1). The extracts from onions into which pBR322 DNA bearing the lytic enzyme gene was injected showed significant enzymic activity (lines 2–4). However, the activity varied from bulb to bulb and experiment to experiment. A unit of enzyme activity is expressed as the amount of enzyme causing a decrease of 0.1 at $A_{450\,nm}$ per min at 30°C (ref. 13) which corresponds to 10 ng of the enzymic protein[10]. The enzyme activities varied from 0.5 to 50 units per bulb and represent 0.05–5.0 μg of the enzyme protein. The enzyme in these extracts was inactive on *Micrococcus lysodeikticus* cells

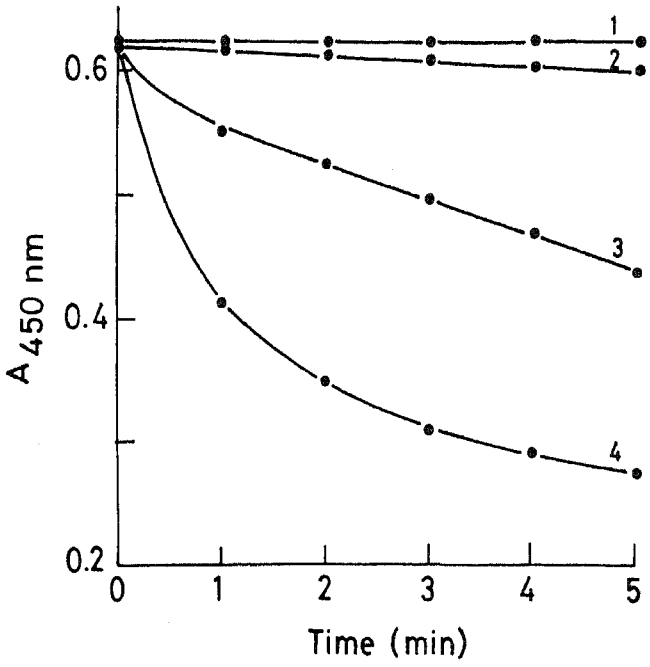

Figure 1. Lytic enzyme activities in the extracts of onion bulbs. The pBR322 or the recombinant DNA, 10 μg in 50 μl of TM buffer, was injected into each onion bulb in a set of 10, which were grown at 22°C for 7 days. The extract was prepared and the enzyme was assayed as described in the text. The extract (100 μl) was added to 1 ml of the sensitized *E. coli* cells and the decrease in $A_{450\ nm}$ was recorded. Lytic enzyme activity in extracts from bulbs injected with: lane 1, pBR322 DNA, and lanes 2-4, pBR322 bearing the lytic enzyme gene.

and was inactivated by 5 mM EDTA showing the specificity of the colitis phage lytic enzyme. The variations in the expression of the genes as shown by the enzymic activities in different onion bulbs in different experiments may be due to variations in the competence of the onions and the extent of injury to the tissues during injection. The gene transfer by direct injection of DNA into the large onion tissues at several points is very convenient and is comparable to the injection of DNA into the developing floral tillers[14, 15].

The fate of the injected DNA which is being expressed is yet to be analysed in detail. However, the injected DNA may not exist as free plasmid in the onion cells as it could not be detected by transformation of *E. coli* HB101 cells. Since prokaryotic promoter is not recognized by the eukaryotic RNA polymerase, it is likely that the lytic enzyme gene is integrated into the organellar or nuclear DNA at positions downstream to the promoter and transcribed by the RNA polymerase present in the onion cells.

The transfer of the lytic enzyme gene and the assay of its product, the enzyme, are simple and sensitive and hence the lytic enzyme gene can be used as a marker in gene transfer in plants and animals. The lytic enzyme gene can also be used in chimaeric gene constructs to study the regulation of gene expression at the level of transcription and translation to identify the promoters, assess their strengths and tissue specificity.

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