2. Biologically Contained Recombinant Bacteria That Degrade Chlorobenzoate

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Objectives of research

It is important to gain the public acceptance for the release of genetically engineered microorganisms in natural ecosystems for agricultural and environmental applications.

To control the viability of recombinant bacteria in the environment, we constructed recombinant bacteria which can degrade chlorobenzoate and whose growth can be controlled by temperature.

Results and discussion

A 9.0-kb *Sac*I fragment containing a region involved in the degradation of 3-chlorobenzoate (3CBA) in *Alcaligenes eutrophus* NH9 was cloned into *Pseudomonas putida* PaW8 by electroporation. The chlorobenzoate-degradation activity of the recombinant *P. putida* was confirmed in liquid medium and on agar plate. Two recombinant systems in *P. putida* which can degrade chlorobenzoate and whose growth can be controlled by the temperature were constructed (Fig. 1). In system 1, the *Sac*I fragment and growth-controlling plasmid (PST28) were introduced into *P. putida* separately with different replication origins (RSF1010*ori* and pSa*ori*). In system 2, all the genes were cloned in one plasmid. Deleterious genes which could be induced at 35℃ ~38℃ by λ PR promoter and temperature-sensitive cI repressor (cI857) and control the growth of *P. putida* were screened in each system using deleterious gene screening vectors (pPSV311 and pSV1). Chromosomal DNA extracted from *Escherichia coli* JM109 or *Streptomyces lividans* TK24 was partially digested with *Sau*3AI and used in shotgun cloning of deleterious genes in each vector. The ability of the deleterious genes to control the growth of host cells was evaluated (Fig.2). It was necessary to screen the deleterious genes in each system independently, since copy number of screening vector might influence the efficiency of the deleterious gene.

Characterization of the deleterious genes and construction of more practical recombinant bacteria for use in the environment are in progress.

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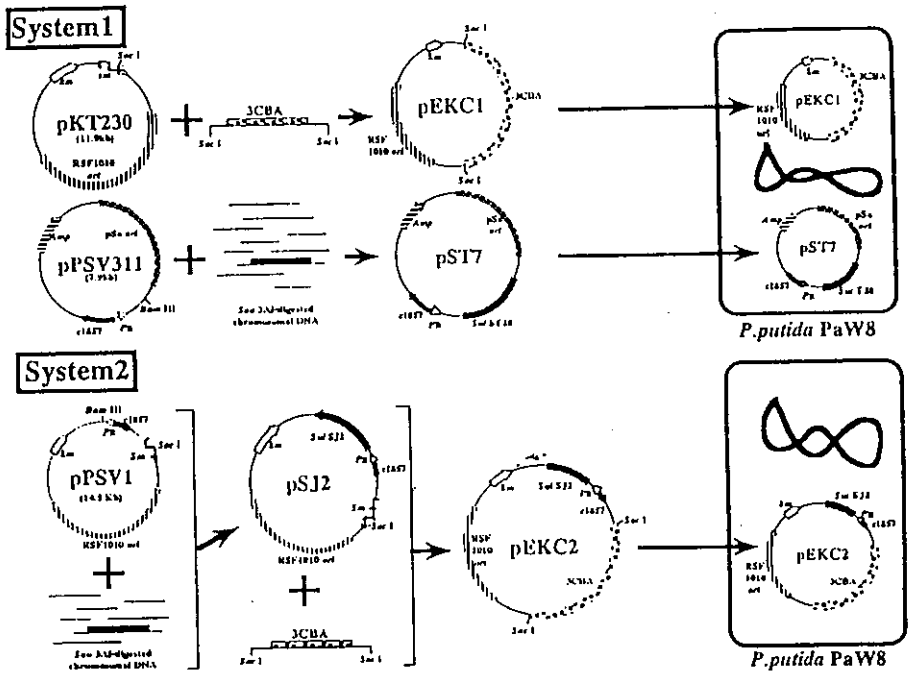

Fig. 1 Scheme for the constructions of biologically contained recombinant bacteria (*Pseudomonas putida* PaW8) that degrade 3-chlorobenzoate.

Sui ST28 and *Sui* SJ2 are deleterious genes screened by shotgun cloning. Abbreviations: PR, λ PR promoter; cI857, temperature-sensitive λ cI repressor gene; Km, kanamycin resistance gene; *Sm*, streptomycin resistance gene; *Amp*, ampicillin resistance gene; RSF1010ori, region for replication of plasmid RSF1010 (IncQ); pSaori, region for replication of plasmid pSa151 (IncW).

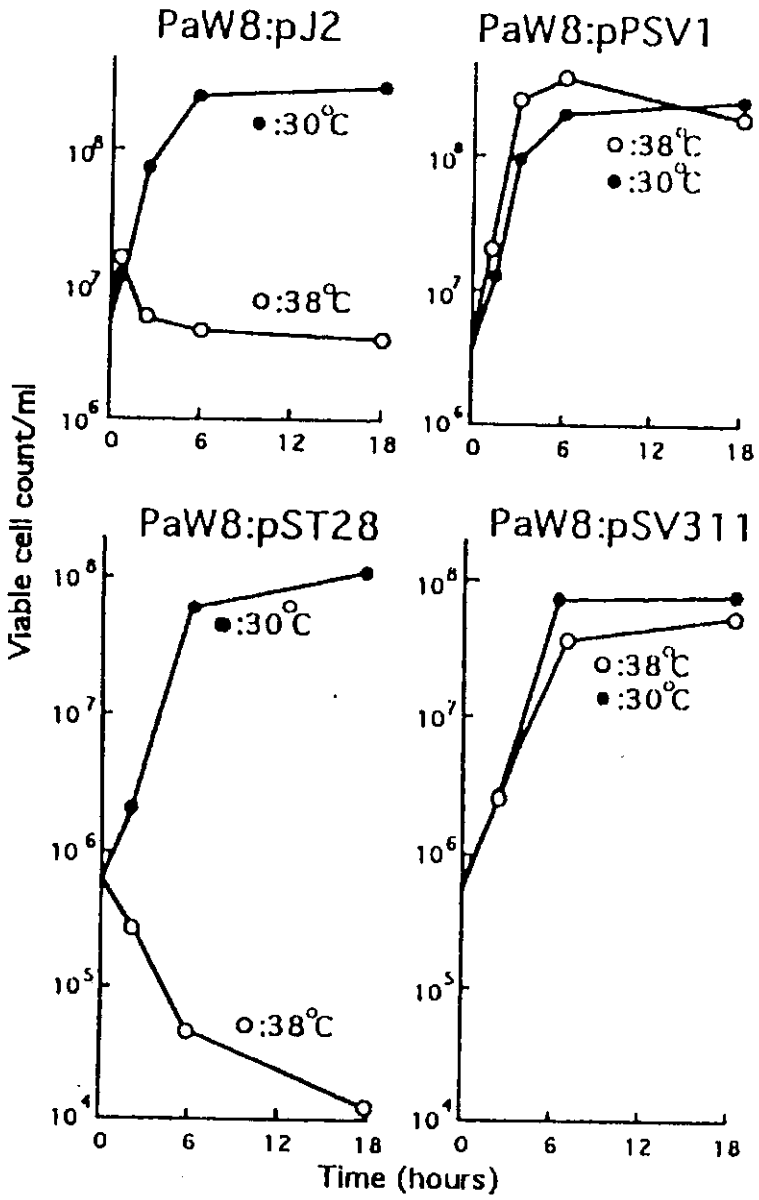

Fig. 2 Induction of deleterious genes in the deleterious gene clone of *P. putida* PaW8 constructed with pPSV311 (PaW8:pST28) and pPSV1 (PaW8:pJ2). Deleterious gene clones grown in liquid medium at 30℃ were harvested at the early exponential growth phase. The culture was divided into two portions (0 Time); one was incubated at 30℃, and the other at 38℃. At various time a small portion of the culture was collected and viable cell count/ml was determined.