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ABSTRACTS

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PSEUDOMONAS - AN EFFICIENT SYSTEM FOR GENE CLONING AND MANIPULATION

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The tremendous biochemical versatility, the pathogenecity towards plants and animals and the sudden emergence of antibiotic resistance in Pseudomonas evoked a lot of interest among microbiologists and molecular geneticists. These factors and the finding that there was very poor or no expression of Pseudomonas genes in the commonly used cloning system Viz., Escherichia coli led to the development of efficient Pseudomonas cloning systems. Now, very well defined host strains derived from a number of Pseudomonas spp. viz. P.aeruginosa, P.putida, P.acidovorans, P.fluorescens, P.cepacia, P.stutzeri, P.syringae etc. are available. A number of cloning vectors with properties such as varying host range, transformation frequency, copy number, and transposon transfer ability have been developed. Using these tools it has been possible, in recent years, to analyse the gene organisation, especially by transposon mutagenesis and manipulate the expression and regulation of a wide

variety of genes and operons, particularly those involved in detoxification/degradation of toxic xenobiotics and their recalcitrant residues.

P.aeruginosa PAO 1162, PAO 2003, AC 161, P.putida KT 2440 and strains of many other species have been found to be very good hosts. They show a transformation efficiency of 10^3 - 10^5 transformants/ug DNA, though these values are much lower that those obtained with E.coli K 12.

Most of the cloning vectors that have been developed for Pseudomonas are the so called broad host range vectors which can be propagated in a wide range of Gram negative bacteria. RSF 1010, R 1162, R 300B etc. are best characterised broad host range, high copy number cloning vectors belonging to IncQ and IncP4 group plasmids. IncP1 goup plasmids, RK2, RP1, RP4, R68 are broad host range but low copy number cloning vectors. These vectors have been found to be more stable than the former group. pKT231, a 13 Kb plasmid vector was one of the earliest to be developed and extensively used. This has the resistance markers for kanamycin and streptomycin and contains unique clevage sites for XhoI, ClaI, SmaI/XmaI, PvuI and HindIII within the kanamycin^r gene. Cloning of DNA fragments in these sites results in insertional inactivation of kanamycin resistance.

Cloning at EcoRI, HpaI, SstI or SstII sites usually results in the inactivation of streptomycin resistance. This enables the easy scoring of transformant clones containing recombinant plasmids. Another interesting and a very useful feature of this vector is that it can be mobilised or transmitted at very high frequencies from where it resides to another. Gram negative bacterium, provided the donor strain concomitantly carries a conjugatory plasmid that can supply conjugal transfer functions. Conjugation as a means of gene transfer is quite advantageous, mainly because unlimited amounts of DNA in the plasmid can be transferred from the donor to the recipient cells. This becomes more relevant as the catabolic pathways for many of the xenobiotic degradation consist of several genes and are borne by huge plasmids.

Construction of gene banks in Pseudomonas hosts have not been possible as cosmid vectors such as those used for cloning in E. coli were not available. However, several broad host range cosmid vectors based on RSF1010 - or RK2 type replicons can be packaged into lambda heads and used to construct gene banks in E.coli which could, then, be transferred to Pseudomonas hosts.

Genetic markers such as lac Z gene encoding B-galactosidase and xylCAB genes of TOL plasmid

pWWO specifying the enzymes that catabolise toluene and xylenes to benzoate and toluates have been inserted to the vectors for studying the expression and regulation of cloned genes. Promoter probe vectors such as RIb679A, pKT240 have been found to be useful in detecting promoter sequences in cloned DNA.

Expression vectors pMMB22, pNM185 have been developed. The former vector contains E.coli tac hybrid promoter and the $lacI^{g}$ repressor gene. The expression of the cloned gene is induced by lac system inducers such as IPTG upto 20 times from the basal levels. pNM185 contains TOL plasmid meta clevage pathway gene promoters and the gene of their positive regulator, XylS. The cloned gene could be induced by benzoates.

These tools have been successfully made use of for constructing hybrid metabolic pathways with novel activities through the assembly of the genes taken from different pathways and from different micro-organisms.

In conclusion it could be said that these genetic engineering tools will prove to be very efficient for the construction of microbial strains for degradation of a number of, hitherto, highly recalcitrant xenobiotic residues.