Cloning of autonomously replicating DNA-sequences (*ars*) from *Methanococcus vannielii* in yeast

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DNA fragments of the archaebacterium *Methanococcus vannielii* were analyzed for autonomously replicating sequences (*ars*) in the eukaryote *Saccharomyces cerevisiae*. The hybrid plasmid Yip5, which is composed of pBR322 and the yeast *URA3* gene, but lacks a functional origin of replication of yeasts, was used for the selection of methanogenic DNA fragments, providing origins of replication in an uracil auxotrophic yeast. Total DNA of *Methanococcus vannielii* was digested with *Bam*HI or *Bam*HI/*Hind*III and cloned using Yip5 as a vector in *E. coli*. Clones of *E. coli* containing recombinant plasmids were screened for the ability to transform the uracil auxotrophic yeast strain to prototrophy. Several independent transformants contained low copy number autonomously replicating plasmids which by hybridization and restriction analysis were shown to be composed of pBR322, the yeast *URA3* gene and DNA fragments from *Methanococcus vannielii*. Transformation of *E. coli* using selection for the pBR322 marker with these plasmids was successful as was the reintroduction into yeast. Two recombinant plasmids containing *ars* from the methanogen, pET598 and pET599, were further analyzed and characterized.

Organization of catabolic genes of TOL plasmid pWWO of *Pseudomonas putida*

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TOL plasmid pWWO specifies catabolic pathway for toluene and xylene degradation. Hybrid plasmids containing the pathway genes werre constructed using pBR322-based narrow host range vectors and RSF1010- and R388-based wide host range vectors, and subsequently mutagenized with transposon Tn*1000* or Tn*5*. The resulting insertion mutant plasmids were examined for their ability to express catabolic enzymes. The physical location of the insertions in each Tn*1000* and Tn*5* derivative plasmids was determined by restriction endonuclease cleavage analysis. This information permitted the construction of a precise physical and genetic map of the pathway genes. To analyze multienzyme genes for toluate 1,2-dioxygenase, complementation tests were carried out in *Escherichia coli* and in *P. putida* which defined three genes for this enzyme.

Cloning of DNA sequences encoding the *Rsr* I restriction-modification system of *Rhodopseudomonas sphaeroides* 630

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The only reported isochizomer of the well-characterized type II restriction enzyme *Eco*RI is *Rsr* I from *R. sphaeroides* 630. Using the *Eco*RI genes as a hybridization probe, we have found that the *Rsr* I and *Eco*RI restriction-modification (r/m) systems share some homology. To allow a more detailed comparison of the two r/m systems, a library of *R. sphaeroides* 630 DNA was constructed in λ L47.1 and several recombinant phage that express the *Rsr* I modification gene, and hence are totally resistant to the action of *Eco*RI endonuclease in vivo and in vitro, have been isolated. Restriction mapping and Southern hybridization experiments on the phage DNA have localized the *Rsr* I gene sequences to a 4.8 kb *Hind*II-*Sal*I fragment. Unlike the *Eco*RI genes, the *Rsr* I gene may not be plasmid-borne.

Construction of a broad host range vector for the regulated expression of cloned genes in a range of gram-negative bacteria

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TOL plasmid pWWO carries a positively-regulated *meta*-cleavage pathway operon that encodes enzymes for the complete degradation of benzoate and toluates by *Pseudomonas putida*. We have recently characterized the operon promoters, whose activation by benzoate and toluates is mediated by the product of a regulatory gene, *xylS*. Comparison of these with several other promoters that cause constitutive expression of the operon allowed the derivation of a consensus nucleodite sequence for a *P. putida* promotor which is significantly different from that of *Escherichia coli*.
The *meta*-cleavage operon promoters and their regulatory gene have been inserted in plasmid pKT231 in order to construct a broad host range expression vector. Analysis of the expression of a test gene cloned in this vector confirmed that the vector promoters function in a regulated fashion in a wide variety of gram-negative bacteria, including soil and water isolates, as well as plant and animal pathogens.

Use of Tn*5-751* for cloning the *arcABCD* gene cluster involved in fermentative growth of *Pseudomonas aeruginosa*

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P. aeruginosa is able to utilize L-arginine as the energy source for growth under anerobic, nitrate-free conditions. Mutations in the *arcABCD* cluster specifying the inducible arginine deiminase pathway enzymes abolish fermentative growth on arginine. The recombinant transposon Tn*5-751* (carrying kanamycin and trimethoprim resistance determinants separated by a single *Eco*RI site) was used for insertional mutagenesis of the *P. aeruginosa* chromosome. Several *arc*: :Tn*5-751* mutants were isolated and their DNA was restricted with *Eco*RI. Restriction fragments carrying either resistance determinant for Tn*5-751* plus flanking parts of the *arc* region were cloned separately in *Escherichia coli*. Subcloning allowed the reconstitution of the entire *arc* cluster on a 5.5 kb fragment, which complemented the *arc,A,B,C,D* mutants previously mapped by transduction. In *E. coli*, the *arc* cluster specified very low activities of the three deiminase pathway enzymes; strong vector promoters enhanced *arc* expression up to 100fold.

Posters

Conjugal septicemia: *Salmonella typhimurim* (STM) in a couple with AIDS

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The two patients, husband (30y) and wife (20y) from Zaire, both had AIDS, as defined by immunosuppression (skin anergy, multiple opportunistic infections, lymphopenia and a low ratio of OKT4 to OKT8 lymphocytes) and the presence of serum antibodies to LAV and HTLV3. STM were repeatedly isolated from both feces and blood in both patients, and persisted in spite of prolonged amoxicillin therapy which produced bactericidal serum levels at $> 1:16$ dilution. From each patient, one stool and