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

one blood culture isolate were lysotyped, their plasmids extracted and analyzed after restriction enzyme digestion. All isolates were of lysotype 10, and all had an identical 72 kb plasmid. Apart from these two cases, STM of lysotype 10 has never been isolated in Switzerland (1978–1984), and the particular plasmid pattern has not been observed among 89 strains analyzed in 1982/83.

Urogenital mycoplasma and *Neisseria gonorrhoeae*: an association?

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It is known that sexually transmitted diseases may occur in pair simultaneously. Thus, Neisseria gonorrhoeae urethritis in 40–45% of the cases is associated with *Chlamydia trachomatis*. This high proportion suggests the administration of a tetracycline active against both organisms.
We observed that the growth of *Neisseria gonorrhoeae* or the presence of its antigens, demonstrated by an immunoenzymatic assay, is frequently followed by the isolation of *Mycoplasma hominis* or *Ureaplasma urealyticum* together or separately. Our results show the percentage of this association and the possible nutritional or biological explanation.
The pathological role of the association and the effects of treatments are discussed.

Temperature-induced hand inversion in *Bacillus subtilis* macrofibers

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Study of the behavior of helical *Bacillus subtilis* macrofibers growing at given temperatures or submitted to a temperature up- or downshift reveals that: 1) cell wall conformation is temperature dependent 2) wall upwelling is required for helix hand inversion. Left to right inversion – but not right to left – requires synthesis at the new temperature of a specific amount of wall. Thus, the two processes appear to be asymmetrical. Relaxation motions induced by lysozyme or by autolysins indicate that: 1) peptidoglycan plays a key role in helical deformation 2) cell wall is under stress. Macrofiber behavior following inhibition of wall or protein synthesis reveals the involvement of a surface-acting wall protein(s) in development of left-handed fibers. Protease digestion of this protein(s) suggests that it is involved in the establishment of wall stress.

Characterization of NAD-dependent variants of *Pasteurella multocida* isolated from pneumonic lesions in swine

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Organisms with morphological and cultural characteristics of *P. multocida*, but showing symbiotic growth with *Staphylococcus epidermidis* like *Haemophilus parasuis* were isolated from lesions of enzootic pneumonia in two pigs. In subsequent investigations they were found to require NAD but no serum or other enrichment for growth. Colonies were very mucoid and confluent but they did not adhere to the agar. Biochemical characteristics other than NAD-dependence were similar to those of *P. multocida*; positive reactions: oxydase, catalase, ornithin decarboxylase, acid produced from glucose, sucrose, xylose, sorbitol, mannitol; negative reactions: indole, urea, esculin, ONPG, no acid from arabinose, rhamnose, lactose, trehalose, maltose, inositol, adonitol, dulcitol, salicin. Antibiograms were similar to those of *P. multocida* isolates in swine. However, no line of precipitation was observed against antisera to *P. multocida* types 1 to 16 of Heddelston. We suppose that these organisms may represent variants of *P. multocida* rather than a new species of the genus *Haemophilus*.

Bacterial metabolism and toxicity of halogenated anilines

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The microbial degradation of a number of ring-halogenated anilines frequently used in pesticide chemistry was investigated in pure cultures. A *Moraxella* sp. which grew on fluoro-, chloro- and bromosubstituted anilines as sole sources of carbon and nitrogen was isolated from soil. The pathway of degradation was determined by analysis of catabolic intermediates and enzymatic activities. The substituted anilines were converted by an oxygenase with a broad substrate specificity to the corresponding catechols, which were further metabolized through the ortho-cleavage pathway.
In addition to the catabolic studies, the growth inhibitory effect of various anilines on *Moraxella* sp. growing on a complex medium was also determined. The degree of inhibition ranged from 5% (addition of 1 mM aniline) to 100% (addition of 1 mM 3,4-dichloroaniline) and was correlated with the pKa and the octanol/water distribution coefficient of the anilines.

Immunocytochemical localization of protein and DNA components of the bacterial nucleoid

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The structural organization of the bacterial nucleoid was studied by colloidal-gold protein A immunolabeling of thin sections of rapidly frozen *Escherichia coli* cells embedded in K4M. Our previous results with cryosubstituted *E. coli* showed a new aspect of the nucleoid: ribosome-free spaces, filled with grainy and fine stranded material, were identified as DNA-containing by specific Osmium-ammines staining (Hobot et al., J. Bact., in press). In this study, specific immunolabeling with sera from MRL/Mp mice and monoclonal anti-DNA IgGs localize double stranded and single stranded DNA, respectively, within the ribosome-free areas. Polyclonal rabbit antibodies specific for two bacterial proteins believed identical to 'histone-like' proteins Hu and HLP I, were similarly used to study their role in the presumed 'nucleosomic' structure of the nucleoid. The significance of the intracellular distribution of these DNA-binding proteins, relative to ss and ds DNA, is discussed in the context of the transcriptionally active bacterial genomic structure.

Chlorhexidin effects on pulmonary tissue in guinea pigs. Initial results

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With the object of observing pathological effects of chlorhexidin solutions on alveoli, we placed guinea pigs in artificial ventilation. The chlorhexidin solutions were added to the humidor of the respirator; we tested 10 guinea pigs with a 0.06% solution, 10 others with a 20% solution and 10 animals with steril distilled water. The guinea pigs having very well survived, we were able to cut off their lungs after 24 h of experimenting in view of a morbid anatomical observation. The results showed a focal mesothelial hyperplasy, very moderate, without specific significance. We never observed a lesion on the air cells of the lungs.