

Rhine and are generally considered to be recalcitrant. Knackmuss' group, however, has managed to obtain biodegradation of a few naphthalene sulfonic acids as carbon sources for growth (degradation rate about 1 mkat/kg of protein) and the first reaction is desulfonation.

We decided to test whether the sulfur rather than the carbon in 7 'non-biodegradable' sulfononaphthalenes (2-4 substituents) was available to microorganisms. Sulfur-limited batch enrichments were inoculated with washed material from industrial sewage plants. Substrate-dependent growth with substrate disappearance was observed with each substrate.

Strain Z63 utilized SO_4^- or e.g. 2-amino-5-hydroxy-7-sulfononaphthalene with a growth yield of about 3 kg of protein/mole of S (degradation rate about 20 $\mu\text{kat/kg}$ of protein). The observations, that the sulfur but not the carbon of these compounds is readily available to microorganisms from native environments, lead us to believe that desulfonation and ring-degradation reactions occur naturally with different specific activities in different organisms.

Microbial degradation of benzenesulfonic acid and its derivatives

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Sulfonated aromatic compounds (e.g. metabolites of dyestuffs) are observed to be major pollutants of rivers and lakes. We have enriched for organisms that are able to utilize as sole source of carbon and energy for growth benzenesulfonate, its 2-amino-, 4-amino-, 4-hydroxy-, 4-methyl- or 4-carboxy-derivative. Pure cultures (18) were isolated. All were bacteria.

One organism, OS-1, isolated to utilize 2-amino-benzenesulfonate, also metabolized benzenesulfonate and 4-methyl-benzenesulfonate. The specific growth rates on these substrates were 0.11, 0.19 and 0.07 h^{-1} , respectively. Each substrate was utilized quantitatively with growth yields of about 5 g of protein/mole of C. Sulfite tended to accumulate towards the end of growth and was then oxidized to sulfate which accumulated stoichiometrically.

Cell extracts of strain OS-1 were prepared and NAD(P)H-dependent substrate disappearance and sulfite release were observed. Specific activities in non-optimized assays were 0.1 and 0.3 mkat/kg of protein for benzenesulfonate and 2-amino-benzenesulfonate, respectively.

ELISA test application on food intoxication at CHUV (Centre Hospitalier Universitaire Vaudois)

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The use of ELISA test (recommended by Prof. Dr H. Fey from the University of Bern), confirmed the presence of staphylococcus enterotoxins strains in prepared dishes served at CHUV, which have been suspected to be the cause of food intoxication. 88 samples have been analyzed according to the standard criteria meaning the research of *Staphylococcus*, the *Salmonella* and the *Clostridium perfringens*. 10 samples have been revealed as highly infected by staphylococcus coagulase positive. 4 samples contained the strains which showed, due to ELISA test, the capacity of producing a great quantity of enterotoxin D.

On the other hand, the strains produced from the nose of some CHUV kitchen's employees with the same lysotype as the strains produced by the infected food, showed also the capacity of producing a great quantity of enterotoxin D.

Anaerobic degradation: catabolism of xylene under denitrifying conditions

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The microbial degradation of m-xylene (1,3-dimethylbenzene) under denitrifying conditions was studied in a perfusion column filled with river sediment material. This column represented a typical river water/ground water infiltration system. After several months of adaptation, as much as 0.3 mM m-xylene at a flow rate of 2.4 cm/h was completely degraded in a column with a total length of 26 cm. Using radiolabeled substrate, 80% of the [^{14}C] m-xylene was mineralized to $^{14}\text{CO}_2$. The conversion of m-xylene was coupled with a reduction of NO_3^- to NO_2^- . No rapid metabolism of m-xylene was observed upon substitution of NO_3^- by either oxygen or sulfate. Studies to elucidate the mechanism of the anaerobic m-xylene metabolism are in progress. The crucial step in the anaerobic degradation appears to be the introduction of a functional group followed by a conversion to an intermediate such as a phenol.

Surface proteins and Western Blot analysis of *Listeria monocytogenes*

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Most clinical isolates of *Listeria monocytogenes* (Lm) belong to only two serotypes, i.e. 1/2b and 4b, which does not allow sufficient discrimination for epidemiological studies. Disc SDS PAGE of Lm after light sonication showed about 50 different bands, including eight major bands. All the 17 known serotypes presented similar patterns. A 160 kd protein had a shorter migration for serotypes 4ab, 6a, 6b. Differences in intensity have also been found in 25 kd doublet. Serotype 1/2c is characterized by a dense band at 36 kd. Most of the bands found on the SDS-PAGE react with rabbit anti 4b antiserum at Western Blot analysis. With this technique, four major immunodominant components were found, with apparent mol. wt of respectively 40 kd, 50 kd, 70 kd and 95 kd.

In conclusion, SDS-PAGE of surface proteins in Lm might be a useful tool for epidemiological studies.

New Antibiotics and Resistance

DNA homology of a transferable *Clostridium difficile* resistance determinant with transposon TN551

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Clostridium difficile, described as a causative agent of antibiotic associated pseudomembranous colitis, shows resistance to many antimicrobial agents. Resistance to either clindamycin/erythromycin (CC'/Ery^r) or tetracycline was found to be transferable to susceptible *C. difficile* strains. Our present results suggest that the two resistance determinants are located on the chromosome. Further results, achieved by DNA-DNA hybridization experiments, indicate DNA homology of the CC'/Ery^r determinant with transposon Tn551 which is found in *Staphylococcus aureus*. Tn551 belongs to a group of related transposable elements coding for resistance to macrolide, lincosamide and streptogramin B in a variety of gram positive bacteria. — Experiments are in process to determine whether the CC'/Ery^r determinant may

originally have been transferred to *C. difficile* from unrelated gram positive bacteria such as staphylococci or enterococci, species which might have come into close contact with *C. difficile*.

In vitro beta-lactamase induction in *E. coli* by ceftriaxon

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In a broth culture of *E. coli* K12 921 the concentration of ceftriaxon was increased stepwise during several passages. This leads to a selection of substrains with MIC's increasing from 0.06 µg/ml to 2.5 µg/ml. The reduced susceptibility of these newly obtained strains is accompanied by the occurrence of a chromosomally encoded β-lactamase (pI 8.6). Similar results were obtained using ampicillin. The MIC increased from 2.5 µg/ml to > 250 µg/ml. However, this strain also showed an increased resistance to ceftriaxon (MIC 2.5 µg/ml), which was rather surprising. Further treatment of this strain with increasing ceftriaxon concentrations over several passages, resulted in a substrain with a MIC for ceftriaxon of 100 µg/ml. This increase could be correlated with an augmented β-lactamase secretion. These findings do have a clinical importance. A ceftriaxon therapy of an *E. coli* infection may fail if the patient has been treated before with ampicillin or another similar β-lactamase antibiotic.

Outer membrane proteins in a chloramphenicol-resistant strain of *Pseudomonas aeruginosa*

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The outer membrane of a clinical isolate of *P. aeruginosa* resistant to chloramphenicol (CM), and its derivative sensitive to the drug were analyzed by polyacrylamide gel electrophoresis. A major outer membrane polypeptide with an apparent mol. wt of 50,000 daltons found in sensitive cells is almost lacking in the resistant cells, thus supporting the view of altered outer membrane permeability to CM in this strain. In vitro polypeptide synthesis experiments demonstrated that the ribosomes of the resistant strain were sensitive to the action of CM. Cell-free extracts of the sensitive mutant acetylated CM as well as its resistant parent strain. Moreover, intact cells of the resistant strain inactivated CM 9.06 times less than the sensitive mutant. The later accumulated ¹⁴C-CM two times more than the resistant strain. These results clearly indicated that the resistance of the clinical isolate of *P. aeruginosa* to CM was due to reduced permeability towards the drug.

Induction and reversion of methicillin-resistant *Staphylococcus aureus* (MRSA) by antibiotics

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Induction and reversion were defined as an increase or decrease in MIC. After incubation with sub-inhibitory or inhibitory concentrations of methicillin and thienamycin, induction occurred which was reversible, depending on duration of growth without antibiotic. Population analysis showed MRSA to consist of several populations of differing sensitivities, but the populations of methicillin-sensitive strains (MSSA) were more homogeneous (similar sensitivity of all bacteria). The greater resistance of induced MRSA seems to be due to selection of more resistant populations, rather than to induction of metabolic processes. Reversion is then only a matter of overgrowth of the faster-growing sensitive population and depends on duration of growth, as demonstrated.

In vivo acquired resistance to beta-lactam compounds and fluoroquinolones: an experimental model

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To reproduce in vivo acquired resistance to new expanded-spectrum cephalosporins and fluoroquinolones, a murine peritonitis model has been developed. 2 h after i.p. challenge with *Enterobacter cloacae* (E.c.) - 10⁸ CFU - animals received a single antibiotic shot = Ceftriaxone (CTX): 50 mg kg⁻¹ b.wt; Pefloxacin (PFX): 25 mg kg⁻¹ b.wt; Amikacine (AMK): 15 mg kg⁻¹ b.wt. 24 h later, peritoneal E.c. populations were analyzed on Szibalski gradient agar. With CTX, shift towards resistance multiplied the MICs by a factor of 100-1000 (34/35); hyperproduction of beta-lactamase, and altered OMPs PAGE patterns were observed in all resistant variants (4 E.c. strains tested). With PFX, a shift was also observed, but to a lesser extent, and less frequently. AMK did not shift significantly E.c. populations.

Resistance of *Klebsiellae* to cephalosporins. Particular properties of beta-lactamases isolated from *K. oxytoca*

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K. pneumoniae and *K. oxytoca* are usually resistant to penicillins, and this resistance is normally associated with a beta-lactamase production. *K. pneumoniae* which produce a penicillinase are virtually sensitive to all cephalosporins, whereas *K. oxytoca* are more or less resistant to these antibiotics. We have studied beta-lactamase production of 10 strains of *K. oxytoca* isolated in Switzerland. All strains produce a single beta-lactamase as shown by iso-electric focusing. Four different patterns have been obtained with major bands at pI: 5.2, 5.7, 6.0 and 6.3. The specific activities of the crude extracts were condensed between 15 and 9000 mU/mg. This did not seem to be related to the pI. The kinetic constants were determined for a large set of beta-lactam antibiotics and the four enzymes showed the similar properties:

- hydrolysis of most of the tested beta-lactams, including the methoxy-imino-cephalosporins, such as cefuroxim and the 3rd generation of cephalosporins: cefoperazon, cefotaxim and ceftriaxon,
 - cephamycins (cefoxitin and cefotetan), moxalactam and ceftazidim are very resistant to hydrolysis,
 - all the enzymes are very sensitive to the action of clavulanic acid.
- These properties are very different from those observed with other enterobacteria.

Intrafamilial long-time epidemiology of drug-resistance factors and other plasmids in fecal *E. coli*

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We investigated fecal coliforms of five small families whether and when they contain R-factors and other plasmids. We paid particular attention to know at which moment after birth the first drug resistance factor appears in the coliforms of babies. To determine the intrafamilial plasmid movement we used the identification of R-factors, colicinogenic factors and cryptic plasmids. To identify the hot-strains we used the methods of serotyping, colicinotyping and lysotyping and also the properties for lactose fermentation, hemolysis and mobility.