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SENSITIVITY OF ANAEROBIC RUMEN FUNGI TO PENTACHLOROPHENOL AND PENTACHLOROBIPHENYL

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Abstract

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Sensitivity of 16 strains of rumen fungi belonging to *Neocallimastix frontalis*, *Neocallimastix joyonii*, *Piromonas communis* and *Sphaeromonas communis* to pentachlorophenol and pentachlorobiphenyl was studied. In all strains the utilization of substrate and visible production of fungal biomass were depressed by increasing concentrations of pentachlorophenol (from 1 to 10 μg/ml). Contrary to this, all strains were resistant to pentachlorobiphenyl at concentration of 50 μg/ml.

Rumen fungi, pentachlorophenol, pentachlorobiphenyl

Pentachlorophenol (PCP) and polychlorinated biphenyls (PCBs) are priority pollutants in the environment. PCP is extensively used as wood preservative, herbicide, insecticide and fungicide. PCBs found widespread industrial use in the past as a component of paints and hydraulic liquids. Ruminants are constantly at risk of exposure to PCP and PCBs, in spite of the fact, that PCBs production was stopped several years ago. Residues of haloaromatic compounds in milk, liver and adipose tissue, indicate that these products still migrate into food-producing cycle. The contamination of feeds is probably the most important route of livestock exposure to PCP and PCBs.

Shull and McCarthy (1979) showed that PCP inhibited the rumen fermentation *in vitro*. Low concentration of PCP (10 μg/ml) decreased cellulose fermentation and lowered production of propionate. Yokoyama et al. (1988) examined the sensitivity of fourteen rumen bacterial species to PCP in pure cultures. Cellulolytic strains were highly sensitive while other strains were more resistant. To our knowledge, the effect of PCBs on rumen microorganisms was not investigated probably due their lower acute toxicity.

The effect of PCP and PCBs on rumen anaerobic fungi was not studied either. Anaerobic fungi form an integral component of the rumen cellulose-digesting microbial population, which is presumably more sensitive to toxic substances than other groups of microorganisms. This work therefore presents results on the sensitivity of rumen fungi from different ruminants to pentachlorophenol and pentachlorobiphenyl.

Materials and Methods

Organisms

Sixteen strains belonging to four fungal species were examined. Origin of isolates is presented in Table 1. All strains were isolated in this Institute except strain AA_1, which was obtained in cooperation with the Institute of Physiology in Alma-Ata (U.S.S.R.). Experimental animals (a cow, a sheep and a camel) were provided with rumen fistulas. Strains DSC_1 and DSC_2 were isolated from the rumen of a fallow-deer. After being shot, the animal's GIT was cut open and the rumen contents transferred to the laboratory in vacuum flask. Isolation techniques were those of Hungate (1969) as modified by Joblin (1981). Isolated strains were subcultured every 3 or 4 days in broths containing glucose.

Medium

Complex medium for growth and maintenance of fungi was medium 10 of Caldwell and Bryant (1966), except that glucose (4 g/l) was the only sugar present and 10 % (v/v) of clarified rumen fluid was added. The pH was adjusted to 7.0-7.2. Medium was prepared anaerobically using cysteine−HCl (0.05 %) as reducing agent.

Chlorinated compounds

Pentachlorophenol, analytical grade, was obtained from Fluka (Buchs, Switzerland). Pentachlorobiphenyl, technical grade, was supplied by Chemko (Strážske, Czechoslovakia). Pentachlorophenol was dissolved in sterile ethanol and added aseptically to sterile media to obtain concentrations 0, 1, 2, 5 and 10 μg/ml. Pentachlorobiphenyl was dissolved in dimethyl-sulphoxide and added to media to obtain concentrations of 0, 5, 10, 20 and 50 μg/ml.

Culture conditions

Incubations were carried out in 20 ml flasks closed by butyl rubber stoppers, under O_2-free CO_2 atmosphere. The medium (15 ml) was inoculated by 1 ml of 3 days-old culture of a fungus. Cultures were done in triplicate. Inoculated cultures were grown at 39 °C for 4 days.

Substrate utilization

Residual glucose was determined by the glucose oxidase-peroxidase method. A commercial Bio-La-Test "Oxochrom Glucose" (Lachema, Brno, Czechoslovakia) was used. Residual glucose was expressed relatively to the initial glucose concentration and plotted agains concentration of pentachlorophenol and pentachlorobiphenyl. The IC_{50} was the concentration of the chlorinated compound in which only 50 % of the initial glucose was utilized within 4 days of incubation interval by a fungus.

Results and Discussion

The results obtained are summarized in Table 1. Generally, strains of rumen fungi were susceptible to pentachlorophenol (IC_{50} from 2.9 to 9.8 μg/ml) and resistant to pentachlorobiphenyl ($IC_{50} > 50$ μg/ml).

Table 1

Sensitivity of strains of anaerobic rumen fungi to pentachlorophenol (PCP) and pentachlorobiphenyl (PCB)

Fungus	Inhibitory concentration IC_{50} (μg/ml)	
	PCP	PCB
Neocallimastix frontalis		
PC_3	4.1	r
CB_3	4.8	r
UCF_{12}	7.0	r
Neocallimastix joyonii		
PNg	5.0	r
PM	6.4	r
P_1	3.8	r
P_4	7.9	r
AA_1	7.9	r
Piromonas communis		
G_3	2.9	r
PC_1	3.9	r
PC_2	5.1	r
PC_3	4.1	r
Sphaeromonas communis		
DSC_1	4.5	r
DSC_2	6.7	r
SC_2	9.8	r
SC_3	6.4	r

Strains PC_3, CB_3, PNg, PM, P_1, G_3, PC_2 and PC_3 were isolated from a cow fed hay, silage and concentrate. Strains UCF, PC_1, SC_2 and SC_3 were isolated from a hay fed sheep. Strains AA_1 was isolated from a hay fed camel. Strains DSC_1 and DSC_2 were isolated from a fallow-deer.

r, resistant, $IC_{50} > 50$ μg/ml

The sensitivity of rumen fungi to pentachlorophenol is similar to sensitivity of rumen bacteria, which was reported by Yokoyama et al. (1988). The data of Yokoyama et al. suggest that the adverse effect of pentachlorophenol on rumen microorganisms may be the result of its role as an uncoupler of electron transport and as a protonophore. It is known that fungi are sensitive to ionophores (Marounek and Hodrová 1989) probably due to the interruption of fungal endomembrane function (Weete et al. 1989). Contrary to rumen bacteria, differences in sensitivities of different strains of rumen fungi to PCP are relatively small. Also PCBs can inhibit membrane-associated functions, in spite of the fact that these highly lipophilic compounds are not able to form anions and transfer ions through membranes. PCBs tend to accumulate in the hydrophobic interior of cell membranes. Blakemore and Cerey (1978) reported the inhibition of growth of two marine bacteria by very low concentration (10 μg/ml) of PCBs. Inhibition was probably caused by impaired transport of nucleic acid precursors through the membrane of affected cells (Blakemore 1978). Pentachlorobiphenyl in this study, however, influenced neither the utilization of substrate nor the visible growth of fungal cultures. Anaerobic fungi represent a significant part of fibre--degrading population in the rumen. From the point of view of effective fermentation, the observed resistance may be an important factor, due to the high possibility of livestock exposure to PCBs. The findings may be relevant in terms of evaluation of similar anaerobic degradation of lignocellulosic materials in pollutant stressed environment.

Citlivost anearobních bachorových hub k pentachlorfenolu a pentachlorbifenylu

Zjišťovali jsme účinek pentachlorfenolu a pentachlorbifenylu na 16 kmenů anaerobních bachorových hub, řadících se k druhům *Neocallimastix frontalis*, *Neocallimastix joyonii*, *Piromonas communis* a *Sphaeromonas communis*. U všech kmenů zvyšující se koncentrace pentachlorfenolu (1—10 μg/ml) inhibovala utilizaci substrátu a viditelnou produkci biomasy. Inhibiční účinek pentachlorbifenylu při koncentraci 50 μg/ml jsme nezjistili.

Чувствительность анаэробных рубцовых грибков к пентахлорфенолу и пентахлорбифенилу

Нами проводились исследования воздействия пентахлорфенола и пентахлорбифенола у 16 штаммов анаэробных грибков рубца, относящихся к видам *Neocallimastix frontalis, Neocallimastix joyonii, Piromonas communis* и *Sphaeromonas communis*. У всех штаммов повышающаяся концентрация пентахлорфенола (1 - 10 мкг/мл) ингибировала утилизацию субстрата и явную продукцию биомассы. Ингибирующее действие пентахлорбифенола при концентрации 50 мкг/мл нами не было установлено.

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