Bull. Environ. Contam. Toxicol. (1993) 51:651–656

Environmental Contamination and Toxicology

Resistance of Lactogenic Microorganisms and Mixed Ruminal Bacteria to Pentachlorobiphenyl (Delor 105)

E. Rybošová,[1] I. Breyl,[2] P. Siroka,[1] R. Nadaskay,[2] and P. Javorský[1]

[1]Institute of Animal Physiology, Slovak Academy of Sciences and [2]Institute of Zoohygiene and Veterinary Technique, 04001 Košice, Slovak Republic

Due to their multiple use in the whole range of industrial branches polychlorinated biphenyls (PCBs) belong to the most serious and extensively spread environmental pollutants. PCBs are toxic, mutagenic and teratogenic substances with proven bioaccumulation ability. These compounds have a negative impact on nearly every member of the biota (Kalmaz and Kalmaz 1979; Hooper et al. 1990). The nonpolar nature of PCBs is combined with a high lipophilicity and potential for bioconcentration. They are resistant to the physical and biological degradation. Most PCBs are chemically stable, low-volatile and water-insoluble compounds (Paris et al. 1978; Steen et al. 1978). The ruminants, as plant food consumers are, exposed to the impact of PCBs in contaminated areas despite the fact that their production has been discontinued. Residues of these substances detected in adipose tissue, liver, milk and milk products indicate that these substances still penetrate into the food chain by means of water and feedstuffs (Sanders and Chandler 1972; Breyl et al. 1990). Use of hydraulic mechanisms, silage pit coatings and protection of haylage towers by paints containing PCB substances can considerably contribute to the contamination of feeds.

Our study was aimed at the determination of PCB derivatives content in feed in a Velký Krtíš location with relatively high concentration of these compounds. We examined simultaneously the effect of Delor 105 (predominantly containing the pentachloroderivative of PCB, a primary component of protective coats of silage pits) on lactate producing strains Lactobacillus plantarum S15 and S20 isolated from silage. In view of the lack of data on sensitivity or tolerance of rumen bacteria to PCBs, the influence of Delor 105 on the mixed culture of sheep rumen bacteria was also investigated.

MATERIALS AND METHODS

Two lactogenic strains L. plantarum S20 and S15 were examined for their sensitivity to Delor 105. These silage strains were originally obtained from the culture collection of Research Institute of Animal Production in Nitra. L. plantarum S20 and S15 were grown on the MRS

* Send reprint requests to P. Javorský at the address above.

medium (de Man et al. 1960) aerobically. Delor 105 supplied by Chemko (Strážské, Slovak Republic) was dissolved in acetone and added aseptically to sterile media to obtain concentrations 0.2, 2.0 and 20 $\mu g\ mL^{-1}$ prior to inoculation. Cultures were incubated in 50 mL volume at 39°C without shaking. Media after 24 hours of incubation were used to determine the effects of Delor 105 on the production of lactic acid. Ruminal fluid collected from rumen-fistulated sheep was used as the source of inocula. Ruminal fluid was prepared anaerobically, under an O_2 - free CO_2 atmosphere (Bryant 1972). Fermentation and growth was detected in the 50 mL of RGC-rumen fluid-glucose-cellobiose medium by Holdeman et al. (1977). The medium containing 20% of clarified rumen fluid, was prepared anaerobically. This medium was used to assess the effects of Delor 105 (0.2 or 2.0 $\mu g\ mL^{-1}$) on the growth and production of volatile fatty acids of mixed ruminal microorganisms. Incubations were carried out in 100 mL flasks closed by butyl rubber stoppers, under an O_2-free CO_2 atmosphere. The control cultures contained no Delor 105 or an equivalent volume of acetone (1 mL). Growth was monitored by measuring the turbidity of the culture at a wavelength of 675 nm in a 1 cm cuvette. All treatments were done in triplicate.

The content of PCBs in individual feedstuffs (silage, haylage, roughage and concentrate feed) was determined after the extraction of feed (50 g) with 1:1 petroleum ether - ethylether mixture and extract purification by the acid hydrolysis (sulphuric acid) method, using Hewlett-Packard (5880 A) gas chromatograph, under the following conditions: column packing: 5 % OV 101 on Gas Chrom Q; 0.15-0.18 mm; the injector temperature 220 °C; the oven temperature 200 °C; the detector temperature 300 °C; using 1.5 m glass column, nitrogen as the carrier gas and ECD Ni 63 detector. Aroclor 1260 (Supelco, Switzerland) was used as a standard.

Lactic acid was determined directly in the culture samples collected after 24 hour of cultivation according to Pryce (1969).

Volatile fatty acids were determined by gas-liquid chromatography using a Perkin-Elmer gas chromatograph (model 8500) equipped with microprocessor. A stainless steel column (length 1.75 m; i.d. 1.9 mm) was packed with 5% Carbowax 20M-TPA/0.5% H_3PO_4 on Supelcoport (100/120 mesh). Nitrogen at a flow rate of 28 $mL\ min^{-1}$ was used as a carrier gas. The oven temperature was 132°C, the FID (flame-ionization detector) temperature 250°C and the injector temperature 220°C. The pressure of hydrogen was 0.91 $kg\ cm^{-2}$ and the air pressure approximately 1.39 $kg\ cm^{-2}$. The samples were prepared as follows: A 4 mL sample of each culture mixture was transferred to a test-tube. Crotonic acid (1 mL of solution) was added to each test-tube as an internal standard. These mixed solutions after half an hour at room temperature were centrifuged at 3500 rpm for 15 min. A 0.2 μL of each centrifuged solution was analyzed and a concentrations of volatile fatty acids was calculated using response factors, which were calculated from a standard mixture of volatile fatty acids with the same concentration of crotonic acid as that, in the analyzed samples.

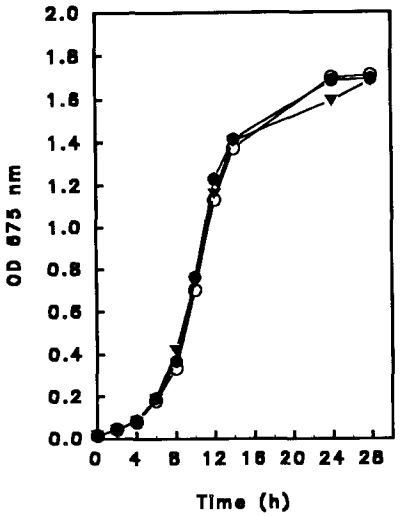

Figure 1. Effect of Delor 105 on growth of L. plantarum 20 S cultured in MRS medium. Curves: • control; ° control 1 mL acetone; ▾ 20 μg mL^{-1} Delor 105.

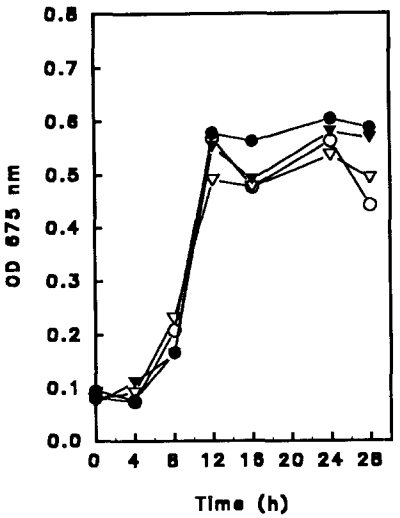

Figure 2. Effect of Delor 105 on growth of mixed ruminal microorganisms cultured in RGC medium. Curves: • control; ° control 1 mL acetone; ▾ 0.2 μg mL^{-1} Delor 105; ▽ 2 μg mL^{-1} Delor 105.

RESULTS AND DISCUSSION

The average content of PCBs determined in individual feeds was as follows: The highest values of PCBs were detected in silage, amounting to 0.502 mg kg^{-1} (149), and in haylage, equal to 0.059 mg kg^{-1} (66). Relatively low values of PCBs were detected in the roughages feed 0.014 (147) and concentrate feed 0.006 mg kg^{-1} (29). The numeric values in the brackets represents the amount of different investigated samples from area of V. Krtíš. The content of PCBs measured in contaminated silage samples suggests that the daily consumption of approx. 30 kg of such contaminated silage can introduce approx. 15 mg of polychlorinated biphenyls into a cattle organism. This assumption served as a basis for calculations of Delor 105 concentrations used in experiments dealing with its influence on lactogenic bacteria and on the mixed culture of rumen bacteria of sheep (0.2 μg mL^{-1}).

There were not variations in the sensitivity of L. planturum S20 and S15 to Delor 105. Both L. plantarum S20 and S15 were resistant to 0.2, 2.0 and 20 μg mL^{-1} Delor 105 (Fig.1). The addition of 1 mL acetone solution directly to the media slightly depressed the growth of tested strains. Lactic acid production of L. plantarum S20 and

Table 1. Lactic acid concentrations of L. plantarum S20 and S15 grown with graded concentrations of Delor 105.

Medium conc. ($\mu g\ mL^{-1}$)	Concentration of lactic acid ($mg\ mL^{-1}$)	
	L. plantarum S20	L. plantarum S15
Control	6.43	13.03
Control[a]	4.63	11.83
Delor 105 (0.20)[b]	4.63	11.60
Delor 105 (2.00)[b]	4.90	11.80
Delor 105 (20.0)[b]	4.83	11.73

[a] A 1 mL of acetone was added.
[b] Delor 105 was added in a 1 mL of acetone. Values are the averages of triplicate .

S15 were not appreciably affected by increasing concentrations of Delor 105, in comparison to the acetone control. Lactic acid concentration was decreased by the addition of acetone to the medium (Table 1).

The mixed ruminal microorganisms cultured in RGC medium were resistant to 0.2 and 2 $\mu g\ mL^{-1}$ Delor 105. Acetone solution slightly decreased the growth in comparison to culture containing no Delor 105 and acetone (Fig.2). Acetate and propionate concentrations were decreased in cultures of mixed ruminal microorganisms with 2 $\mu g\ mL^{-1}$ of Delor 105, in comparison to the control, but in comparison to the control containing acetone the concentrations were unchanged (Table 2). Butyrate concentration was increased by the addition of acetone or 2$\mu g\ mL^{-1}$ Delor 105 to the medium. Total volatile fatty acid concentration was slightly decreased by the addition of 1 mL acetone or 2 $\mu g\ mL^{-1}$ Delor 105 to the medium.

Bacteria manifested a wide range of PCB exposure symptoms ranging from growth inhibition to growth stimulation, depending upon the bacterial species, type and concentration of PCB being tested. Of 100 estuarine bacterial and fungal isolates examined 26 were inhibited to some extend by high ($mg\ L^{-1}$ range) concentrations of Aroclor 1242 (Bourquin et al. 1975). A strong negative correlation between amylase and gelatinase production and growth inhibition was noted. Bourquin and Cassidy (1975) observed bacteriostatic effect by 10 $mg\ L^{-1}$ of Aroclor 1242 in four estuarine bacterial isolates.

The mechanism of the inhibitory effect of PCB on the metabolism of bacteria is not known. A possible explanation for inhibition of a pseudomonades by 10 $\mu g\ L^{-1}$ Aroclor 1254 was the partially defected adenine transport (Blakemore 1978). Stimulation of bacterial growth by PCBs has been reported by Sayler et al. (1977). In an estuarine pseudomonades, its growth rate was stimulated by 10 $\mu g\ L^{-1}$ Aroclor

Table 2. Volatile fatty acid concentrations of mixed ruminal microorganisms cultured in RGC medium containing Delor 105.

Medium concen. (μg mL^{-1})	Concentration mM 100 mL^{-1}			
	Acetate	Propionate	Butyrate	Total fatty acids
Control	1.311	0.687	0.301	2.299
Control[a]	1.203	0.517	0.346	2.065
Delor 105 (0.2)[b]	1.369	0.716	0.293	2.377
Delor 105 (2.0)[c]	1.236	0.530	0.363	2.128

[a] A 1 mL of acetone was added.
[b] Delor 105 was added in 0.1 mL of acetone.
[c] Delor 105 was added in 1 mL of acetone. Values are the averages of triplicate.

1254 and oxygen consumption was stimulated through the 500 μg L^{-1} level.

In our experiments with lactogenic strains L. plantarum S20 and S15 we determined no effect of Delor 105 in concentrations 0.2 -20 μg mL^{-1} on growth and lactic acid production. Delor 105 (2 μg mL^{-1}) was 10-fold higher in experiments with mixed cultures of rumen bacteria than present in the rumen during the consumption of PCB contaminated silage (0.502 mg kg^{-1}). Despite this fact, the mixed culture of rumen bacteria was resistance to this concentration of Delor 105. A strong resistance to pentachlorobiphenyls was also recorded in rumen strains of fungi by Hodrová and Marounek (1991). All tested strains of rumen fungi were resistant to the amount as high as 50 μg mL^{-1} of PCBs.

The rumen bacteria (Yokoyama et al. 1988) and rumen fungi (Hodrová and Marounek 1991) are more sensitive to pentachlorophenol. According to these authors the adverse effects of PCP on ruminal microorganisms may be the result of its role as both an uncoupler of electron transport and a protonophore. The resistance of mixed rumen microorganisms to PCBs could be partly explain by those lipophilic character. The PCBs derivates consumed by ruminants are probably dissolved in ruminal lipids and pass through the gastrointestinal tract by rumen-entero-hepatic circulation without significant contact with rumen microorganisms. Although the PCBs concentrations detected in silage did not affect the silage preservative lactogenic bacteria and mixed ruminal bacteria, their cumulative toxic effects on animal and human organisms are still of considerable importance.

Acknowledgments. This research was financially supported by Grant Agency of the Slovak Academy of Sciences (GA SAV 63/91) and partially by the Ministry of Agriculture of Slovak Republic.

REFERENCES

Blakemore RP (1978) Effects of polychlorinated biphenyls on macromolecular synthesis by a heterotrophic marine bacterium. Appl Environ Microbiol 35:329-336

Bourquin AW, Kiefer LA, Berner NH, Crow S, Ahern DG (1975) Inhibition of estuarine microorganisms by polychlorinated biphenyls. Rev Ind Microbiol 16:256-261

Bourquin AW, Cassidy S (1975) Effect of polychlorinated biphenyl formulations on the growth of estuarine bacteria. Appl Microbiol 29:125-127

Breyl I, Nádaskay R, Sokol J, Augustinský V (1990) The residues of chlorinated hydrocarbons and polychlorinated biphenyls (PCB) in milk and milk products. Vet Med (Praha) 35:179-186

Bryant MP (1972) Commentary on the Hungate techniques for culture of anaerobic bacteria. Am J Clin Nutr 25:1324-1328

Hodrová B, Marounek M (1991) Sensitivity of anaerobic rumen fungi to pentachlorophenol and pentachlorobiphenyl. Acta Veter (Brno) 60:335-338

Holdeman LV, Cato EP, Moore WEC (1977) Anaerobe laboratory manual, 4 th edition, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blackburg, Virginia.

Hooper SW, Pettigrew CA, Sayler GS (1990) Ecological fate, effects and prospects for the elimination of environmental polychlorinated biphenyls (PCBs). Environ Toxicol Chem 9:655-667

Kalmaz EV, Kalmaz GD (1979) Transport, distribution, and toxic effects of polychlorinated biphenyls in eco-system. Ecol Model 6:223-251

Man JC de, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130-135

Paris DF, Steen WC, Baughman GL (1978) Role of physico-chemical properties of Aroclor 1016 and 1242 in determining their fate and transport in aquatic environments. Chemosphere 4:319-325

Pryce JD (1969) A modification of the Barker-Summerson method for the determination of lactic acid. Analyst 94:1151-1152

Sanders HO, Chandler JH (1972) Biological magnification of polychlorinated biphenyl (Aroclor 1254) from water by aquatic invertebrates. Bull Environ Contam Toxicol 7:257-263

Sayler GS, Shon M, Colwell RR (1977). Growth of an estuarine Pseudomonas sp. on polychlorinated biphenyls. Microbial Ecol 3:241-255

Steen WC, Paris DF, Baughman GL (1978) Partitioning of selected polychlorinated biphenyls to natural sediments. Water Res 12:655-657

Yokoyama MT, Johnson KA, Gierzak J (1988) Sensitivity of ruminal microorganisms to pentachlorophenol. Appl Environ Microbiol 54:2619-2624

Received January 25, 1993; accepted April 22, 1993.