A C T A A L I M E N T A R I A P O L O N I C A

Vol. IX (XXXV), No. 2 1985

FRANCISZEK PĘDZIWILK
KRYSTYNA TROJANOWSKA
HELENA GIEBEL
DANUTA OLEJNIK

THE EFFECT OF LEAD LEVEL ON THE GROWTH AND FERMENTATIVE ACTIVITY OF *LACTOBACILLUS ARABINOSUS* *)

Institute of Microbiology, Biochemistry and Food Analysis, Academy of Agriculture, Poznań

Key words: lead, inhibition of growth, fermentative activity, *Lactobacillus arabinosus*.

Lead in the form of $Pb(CH_3COO)_2$, introduced, into the culture medium in doses ranging from 1 to 2000 mg Pb/l, inhibited the growth and fermentative activity of *Lactobacillus arabinosus* ATCC 8014 in a relatively low degree. The medium was found to affect the growth-inhibitory ability of lead.

INTRODUCTION

Recent years have witnessed a growing interest in the effect of heavy metals on the microflora of natural environments [2, 4, 9, 7]. One such natural environment is the vegetation cover near public highways, very often contaminated with lead [4, 6, 10, 3, 5].

Assuming that the toxic effect of lead will be also reflected in the activity of the epiphytic microflora of plants, we studied the effect of this element on lactic acid bacteria which, as it is known, play a decisive role in the process of souring (silage) of fodder.

MATERIAL AND METHODS

The lactic acid bacteria *Lactobacillus arabinosus* ATCC 8014 (Syn. *L. plantarum*) were used in the study. Experimental cultures of the bacteria were maintained in test tubes (15 ml) and Erlenmeyer flasks

*) This research was partly financed by the government program 09.3. coordinated by the Institute of Plant Protection in Poznań.

(500 ml) on two media: fresh acidic-rennet whey (4.1% lactose) obtained from a municipal dairy and supplemented with 1 g Difco yeast extract, pH 6.8, and a medium according to M.V.A. containing peptone, yeast extract, glucose and mineral salts [8].

Lead was introduced into the experimental cultures in the form of water solutions $Pb(CH_3COO)_2 \cdot 3H_2O$ in doses increasing from 1 to 2000 mg Pb/l medium. Inoculates were prepared from a 24-h culture (37°C) of *Lactobacillus arabinosus* ATCC 8014 on the medium according to M.V.A. The bacteria were centrifuged from the liquid medium, washed with a physiological salt solution, and then a suitably diluted suspension containing ca. 500 cells per 1 ml, was prepared. The inoculum thus obtained was introduced in 1-ml portions to the 15-.1 liquid medium in test tubes and to 15-ml medium with agar for cultivation on Petri dishes. The medium in flasks (500 ml) was inoculated with 25 ml of the suspension. The effect of lead on the growth of lactic acid bacteria was studied in dish cultures on media solidified with agar (2%). 1 ml of the inoculate (ca. 500 cells) was placed on each dish and then 15 ml of liquefied medium with a suitable dose of lead was added. After 48 h of incubation at 37°C the emergent colonies were counted.

The fermentative activity of *Lactobacillus arabinosus* ATCC 8014 was determined on the basis of measurements of acidity corresponding to the amount of lactic acid accumulated in the liquid medium.

After 24 and 48 h incubation at 37°C, 5 ml of the culture was titrated with 0.1 n NaOH against bromomethyl blue as indicator.

The content of lead in the sediments and in post-fermentation fluids was determined by the atom-adsorption flame spectro-photometry technique using a Perkin-Elmer apparatus [1].

All results given in this paper are mean values from three repetitions in two series of the conducted experiments.

RESULTS AND DISCUSSION

The results of the study are presented in Tables 1 and 2. Lead added to media solidified with agar in the form of lead acetate inhibited the growth of bacteria in various degrees depending on the kind of medium. The effect of the medium on the growth-inhibitory capabilities of lead was clearly marked, and it was more evident in the medium according to M.V.A. than in the more complex whey medium, also solidified with agar. In the former case the bacteria growth inhibition was apparent already when 1 mg Pb was added to 1 l of the medium, and when the Pb dose was increased to 5 mg l, inhibition was as high as almost 35%; in the whey medium the 5-mg Pb l dose caused an inhibition of ca. 11% and only at 100 mg Pb l there occurred a sizeable drop in the number of colonies

Table 1. Effect of lead on the growth and fermentative activity of *Lactobacillus arabinosus* ATCC 8014 cultivated on medium according to M.V.A.

Pb dose (mg/l culture)	Measured degree of bacteria culture acidity				No. of bacteria colonies on dish after 48 h (37°C)
	after 24 h		after 48 h		
	pH	lactic acid (%)	pH	lactic acid (%)	
0	5.0	0.2362	4.35	0.9040	314
1	5.5	0.0787	4.50	0.5175	265
5	5.9	0.1462	4.40	0.9225	205
50	5.1	0.2925	4.40	0.9337	207
100	5.2	0.2362	4.35	0.9112	199
300	5.1	0.2925	4.35	0.9337	180
500	5.2	0.2812	4.35	0.9787	169
750	5.3	0.2700	4.30	0.9562	195
1000	5.3	0.2362	4.30	0.9337	189
2000	5.3	0.2137	4.45	0.9040	124

Table 2. Effect of lead on the growth and fermentative activity of *Lactobacillus arabinosu* ATCC 8014 cultivated on whey medium

Pb dose (mg/l culture)	Measured degree of bacteria culture acidity				No. of bacteria colonies on dish after 48 h (37°C)
	after 24 h		after 48 h		
	pH	lactic acid (%)	pH	lactic acid (%)	
0	5.8	0.0506	4.6	0.1237	394
1	6.0	0.0337	5.0	0.0450	379
5	6.0	0.0393	5.4	0.0618	348
50	5.9	0.0562	5.2	0.0787	351
100	5.8	0.0393	5.2	0.0731	260
300	5.8	0.0450	4.9	0.0675	241
500	5.75	0.0450	5.3	0.0618	245
750	5.75	0.0731	5.5	0.0618	358
1000	5.75	0.1125	5.5	0.0900	286
2000	5.70	0.1012	5.6	0.1237	310

appearing on the dishes. However, even at 2000 mg Pb 1 medium the growth of the bacteria and their fermentative activity (increase of acidity) were marked. What is still more remarkable, at the highest doses of lead the acidity of the culture (amount of produced lactic acid) equalled that of the controls, and in the whey medium already after 24 h incubation it was even much higher than in the cultures without lead.

In order to explain this phenomenon, an additional experiment was performed (results are given in Table 3). 150 mg Pb/500 ml medium were introduced into flasks containing the medium according to M.V.A. Lactic

acid bacteria (*Lactobacillus arabinosus*) were added to two flasks and those, together with the other two containing the medium without bacteria, were incubated at 37°C for 72 h. In the flasks without bacteria a sterile sediment appeared. After incubation the sediments from all four flasks were centrifuged.

Table 3. Binding of lead by bacterium cell biomass or by the precipitating (passing to the sediment) components of culture medium. *Lactobacillus arabinosus* ATCC 8014 cultures maintained on medium according to M.V.A. Incubation time—72 h, temperature—37°C

Material	Experiment	Pd added (mg/500 ml)	Centrifuged sediment precipitated from the sterile medium or biomas+protein sediment from bacterium culture (g dry mass)	Pb content in fluid after centrifugation of sediment (supernatant) (mg/500 ml)	Pb content in sediment or biomass (mg)
Sterile medium according to M.V.A. without bacteria (500 ml)	I	150	0.934	0.28	150.3
	II	150	0.892	0.34	150.6
Bacterium culture on medium according to M.V.A. (500 ml)	I	150	3.400	3.87	146.1
	II	150	3.680	3.06	148.2

The lead content in sediments and in the fluids was assayed and it turned out that almost all the lead in bacteria cultures and the entire lead content in the medium without bacteria passed from the medium to the centrifuged sediment.

The conclusion arises that lead introduced into the medium precipitates almost completely together with the medium's protein components and the biomass of bacteria cells. There thus occurs a kind of detoxication of the culture medium for microorganisms.

CONCLUSIONS

1. The presence in the medium of lead in the form $Pb(CH_3COO)_2$ in doses from 1 to 2000 mg Pb/l inhibits the growth and fermentative activity of *Lactobacillus arabinosus* ATCC 8014 in a relatively low degree.

2. It was found that the medium affects the capability of lead to inhibit the growth of bacteria. The inhibitory effect was less pronounced on whey with agar than on the medium according to M.V.A.

3. Lead introduced into the liquid medium almost entirely precipitated together with the medium's protein components, hence its high content in the centrifuged sterile sediments and bacterium cell biomasses.

LITERATURE

1. Anal. Meth. der Atom-Absorption Spectrophotometric, Perkin-Elmer, November 1974.
2. Babich H., Stoltzky: Appl. Microbiol., 1978, 23, 55.
3. Barudi W., Bielig H. J.: Z. Lebensm. Unters. Forsch., 1980, 170, 254.
4. Bewley R. J. F.: Appl. Environ. Microbiol., 1980, 40, 1053.
5. Crump D. R., Barlow P. J.: Environ. Pollut. Ser. B., 1982, 3, 181.
6. Farmer J. G.: J. Sci. Food Agric., 1979, 30, 816.
7. Gadd G. M., Grieffiths A. J.: Microb. Ecology 1978, 4, 303.
8. Methods of Vitamin Assay, Intersience Myer Freed: Publishers, a division of John Wiley and Sons, New York, London, Sydney 1966.
9. Summers A. O.: Ann. Rev. Microbiol., 1978, 32, 637.
10. Wheeler G. L., Rolfe G. L.: Environ. Pollut., 1979, 18, 265.

Manuscript received: Decembre 1982
Authors address: 60-623 Poznań, Mazowiecka 48

F. Pędziwilk, K. Trojanowska, H. Giebel, D. Olejnik

WPŁYW POZIOMU OŁOWIU NA WZROST I AKTYWNOŚĆ FERMENTACYJNĄ *LACTOBACILLUS ARABINOSUS*

Instytut Mikrobiologii, Biochemii i Analizy Żywności, Akademia Rolnicza, Poznań

Streszczenie

Badano wpływ różnych dawek ołowiu (od 1 do 2000 mg Pb na litr podłoża) na wzrost i aktywność fermentacyjną *Lactobacillus arabinosus* ATCC 8014. Stosowane dawki ołowiu wprowadzone w formie $Pb(CH_3COO)_2 \cdot 3H_2O$ w stosunkowo małym stopniu hamowały wzrost oraz aktywność fermentacyjną bakterii w hodowlach na dwóch pożywkach białkowo-cukrowych: na pożywce wg M.V.A. [8] oraz na pożywce serwatkowej.

Stwierdzono wpływ pożywki na ograniczające wzrost bakterii działanie ołowiu. Ołów wprowadzony do płynnego podłoża prawie w całości wytrącał się z białkowymi komponentami pożywki przechodząc do osadu. Zachodził zatem swoisty proces detoksykacji podłoża hodowlanego.