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FRANCISZEK PĘDZIWILK
DANUTA OLEJNIK
HELENA GIEBEL
KRYSTYNA TROJANOWSKA

EFFECT OF MERCURY ON THE GROWTH OF THE YEAST *Kluyveromyces fragilis* CBS-379 AND ITS ACCUMULATION IN CELL BIOMASS *)

Institute of Microbiology, Biochemistry and Food Analysis, Agricultural University, Poznań

Key words: *Kluyveromyces fragilis*, mercury, yeasts biomass, growth of yeast.

The growth and fermentative ability of the yeast *Kluyveromyces fragilis* CBS-397 depending on the kind of medium and the degree of its contamination with mercury was studied. The ability to bind (accumulate) considerable quantities of mercury by yeast cell biomass was observed.

INTRODUCTION

The constant growth of industry, chemization of agriculture and expansion of motorization result in an uncontroled polution of man's environment with heavy metals. The toxic metals in the environment's biogeochemical cycle are consumed by plant and microorganisms and passed on to man via the food chain. The metals tend to accumulate in vitally important organs in man's organism triggering chronic toxic effects.

Microorganisms play an important role in cleaning environments contaminated with heavy metals. It is known that heavy metals may inhibit the growth of microorganisms (cell division inhibition) or disrupt the course of fermentative processes important in practical applications. On the other hand, microorganisms are capable of detoxifying heavy metals by their biotransformation or by bonding them, i.e. by "seizing" them from the substrate. The detoxification processes are directly related to

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the phenomenon of the microorganisms' immunity to relatively high concentrations of heavy metals [3, 7].

The aim of the present study was the determination of the effect of mercury contamination of the substrate on the growth and fermentative activity of the yeast *Kluyveromyces fragilis*. Also studied was the phenomenon of bonding and accumulation of mercury by yeast cell biomass.

MATERIAL AND METHODS

The yeast *Kluyveromyces fragilis* CBS-379, capable of fermenting lactose, were kept on agar slants: yeast water, peptone 0.5%, lactose 4%, agar 2.5%, pH 5.8. The yeast water was prepared as follows: 1000 ml of distilled water was added to 0.5 kg of baker's yeast and heated for 20 min in an autoclave at 1-at overpressure; after cooling, the flask containing the yeast suspension was placed in a refrigerator and stored till the next day. Yeast water (supernatant) was obtained after centrifuging the precipitated sediment.

Experimental cultures were grown on a whey medium (acid-rennin whey containing 4.2-4.6% lactose; K_2HPO_4 — 0.5%, KH_2PO_4 — 0.25%, ($NH_4/_2SO_4$ — 0.5%) and on malt wort (7° Baling).

Partial deporteinization of whey was done by heating it twice for 30 min in a Koch apparatus at pH 6.0. The sedimented proteins were removed by filtering.

Mercury was introduced into the medium in the form of the water solution $Hg/NO_3/_2 \cdot 1/2\ H_2O$ (pure) converting doses into Hg^{++} in mg/l medium. Yeast cultures in 750-ml Erlenmeyer flasks containing 300 ml medium were maintained on a shaker at 30°C. Yeast growth (population size) was determined by a direct cell count under a microscope in a Thom chamber, and by determination of dry cell mass. The cell biomass was separated from the liquid substrate by centrifugation at 3000 g, washed, weighed and dried.

Lactose in whey and in postfermentation fluids was determined by the method of Somogyi and Nelson [5, 6]. Mercury in yeast biomasses was determined by the method of flameless atomic absorption [1, 4]. All results given in the tables are mean values from at least three measurements.

RESULTS AND DISCUSSION

As we know, the yeast *Kluyveromyces fragilis* is capable of fermenting lactose and is used in the production of β-galactosidase preparations and protein preparations (SCP), utilized for food and fodder, using whey

as the culture medium in the process [2, 9-11]. Bearing in mind also the practical aspect, we investigated the effect of mercury contamination of the substrate on the growth of yeast cell biomass in cultures on whey and malt wort media. In cultures on the whey medium the degree of lactose attenuation was also determined. The obtained data, presented in Table 1, indicate that already at 10 mg Hg^{++}/l medium there occurred a marked inhibition of yeast growth, both in the whey and in the malt wort medium, with growth in the latter almost completely stopped at 50 mg Hg^{++}.

The relatively high biomass yields obtained in the cultures on whey medium can be explained by the passing into the sediment of a part of the whey's proteins precipitated during the sterilization of the medium and subsequently centrifuged together with the yeast cells.

In samples with 50- and 100-mg additions of Hg^{++}/l, the inhibition of yeast growth on the whey medium was accompanied by an almost complete arrest of the fermentation process. It was only in samples with 10 mg Hg^{++}/l that after a temporary (up to 48 b) inhibition of lactose fermentation, the process resumed with increased speed and after 72 h incubation the degree of attenuation of lactose was nearly equal to that in controls without mercury additions.

The microscopic picture of cultures on whey and an malt wort was similar. Yeast cells in cultures containing growth-inhibiting doses of mercury (50 and 100 mg Hg^{++}/l) were untypical, small and deformed, filiform.

Initial assays of mercury in yeast biomass obtained from the culture on malt wort showed that the studied yeast strain absorbs considerable quantities of mercury from the substrate even when the level of this element in the medium is very low (Table 1). Hence, the amount of mercury accumulated in the biomass of the still normally developing yeast cells (microscope studies) may be very high.

This phenomenon may be dangerous insofar as yeast is widely used in the food and fodder industries. A separate experiment was performed to determine more precisely the amount of mercury adsorbed by yeast depending on the degree of substrate contamination and on medium kind.

Mercury doses increasing from 0.05 mg to 100 mg Hg^{++}/l fresh medium were introduced into *Kluyveromyces fragilis* CBS-397 cultures on whey and on malt wort; the additions were made regardless of the "endogenous" mercury found to occur in the fresh media (30 µg mercury l whey and 40 µg mercury l malt wort). After 48-h incubation mercury was assayed in the centrifuged and washed yeast biomass and in the post-fermentation fluid.

The obtained results are given in Tables 2 and 3. The amount of mercury bonded by yeast depended both on the mercury level in the medium and on the kind of medium.

Table 1. Effect of different mercury doses on lactose fermentation ability and cell biomass growt in the yeast *Kluyveromyces fragilis* CBS-397 cultured on shakers on whey and malt wort

Doses of Hg^{++} (mg/l culture)	Whey medium				Malt wort medium	
	incubation time (h)					
	24	48	72	72	72	72
	attenuated lactose (%)	attenuated lactose (%)	attenuated lactose (%)	biomass yield (dry mass) (g/l)	biomass yield (dry mass) (g/l)	mercury in biomass ($\mu g\ Hg^{++}/l$)
0	99.9	99.9	99.9	10.09	4.82	18
1	99.9	99.9	99.9	9.95	4.97	125
10	6.6	6.6	94.4	7.83	2.88	1725
50	2.2	2.2	4.4	7.12	0.44	—
100	4.4	4.4	6.6	7.94	0.67	—

Table 2. Bonding of mercury (Hg^{++}) taken from the substrate in cultures on whey medium by biomass of *Kluyveromyces fragilis* CBS-397 cells (incubation at 30°C for 48 h; cultures maintained on shaker)

Doses of Hg^{++} (mg/l culture)	Biomass yield (g dry mass/l culture)	Mercury determined in yeast biomass		Mercury content in postculture fluids (μg Hg^{++}/l)	Amount of mercury bonded in yeast biomass (%)
		μg Hg^{++} in biomass from 1000 ml of culture	μg Hg^{++} (1 g biomass/ /dry mass)		
0 (0.03 mg/x)	11.92	5.01	0.42	25	16.7
0.05	9.99	25.78	2.58	50	32.2
0.1	10.16	54.80	5.39	80	42.1
0.5	10.31	259.33	25.14	240	48.9
1.0	10.43	583.66	55.94	340	56.7
10.0	9.43	5 901.15	652.60	4 500	58.8
50.0	9.33	31 563.88	3 381.84	18 000	63.1
100.0	9.28	65 318.88	7 036.14	34 000	65.3

*) Mercury found in fresh whey

Table 3. Bonding of mercury (Hg^{++}) Taken from the substrate in cultures on malt wort by biomass of *Kluyveromyces fragilis* CBS-397 cells (incubation at 30°C for 48 h; cultures maintained on shaker)

Doses of Hg^{++} (mg/l culture)	Biomass yield (g dry mass/l culture)	Mercury determined in yeast biomass		Mercury content in post-culture fluids (μg Hg^{++}/l)	Amount of mercury bonded in yeast biomass (%)
		μg Hg^{++} in biomass from 1000 ml of culture	μg Hg^{++} (1 g biomass/dry mass)		
0 (0.04 mg)*)	3.37	22.6	6.70	20	56.5
0.05	3.15	52.3	16.60	32	58.1
0.1	3.16	118.2	37.32	40	84.4
0.5	3.18	442.7	139.05	120	81.9
1.0	3.18	860.0	270.15	200	82.6

*) Mercury found in fresh whey

In control samples containing no mercury additions it was found that in cultures on whey only 16.7% of the substrate's "endogenous" mercury was bonded by the yeast, whereas in malt wort cultures as much as 56.6% of the mercury passed from the medium to the yeast biomass. In the whey cultures the yeast cell biomass bonded from 32.2% of mercury (at 0.05 mg Hg^{++} addition per 1 medium) to 65.3% (100 mg Hg^{++} added per 1).

The biomass of the malt wort culture, on the other hand, bonded from 58.1% (0.05 mg Hg^{++}/l) to as much as 82.6% mercury in the case of 1 mg Hg^{++} added to 1 l of the medium. At higher doses of mercury (10, 50 and 100 mg Hg^{++}/l) the yeast growth on malt wort was almost completely stopped.

The contamination level of yeast biomass from the cultures on whey was up to over 7 mg Hg/g dry mass, whereas mercury content in biomasses from the malt wort cultures attained only 0.27 mg Hg/g dry mass (for the addition of 1 mg Hg^{++}/l medium). As already mentioned, at higher doses of mercury the yeast did not grow on malt wort. In attempting to account for the lower "immunity" of yeast towards mercury in malt wort cultures, one must note that the biomass obtained from the wort cultures contained almost exclusively yeast cells, while more than half of the biomass obtained from whey cultures consists of precipitated whey proteins. Proteins, as we know, may also bond mercury and this may be seen as a form of detoxification, in this case of a fluid substrate for yeast [8].

The presence of protein components of the medium may thus partly explain the greater "immunity" of yeast to the toxic effect of mercury in cultures on whey.

In view of the surprisingly large amounts of mercury bonded by yeast cell biomass, we performed additional experiments with cultures on partly deproteinized whey.

Studying the effect of mercury on the growth of biomass, the increase of cell number and the degree of lactose attenuation (Table 4), we also assayed the amount of mercury bonded (accumulated) in the yeast biomass (Table 5).

In cultures with small doses of mercury no changes in fermentation activity (degree of lactose attenuation) were found and there were also no clear differences in cell biomass accretion. A marked drop in cell biomass yield together with a slightly lower degree of lactose attenuation degree was observed only at the level of 1.0 mg Hg^{++}/l. At higer mercury doses (10, 50 and 100 mg Hg^{++}/l medium) there occurred a large decrease of cell number (Table 4). Very interesting results were obtained for the accumulation of mercury by yeast cell biomass in cultures on deproteinized whey (Table 5).

Similarly as in the case of non-deproteinized whey (Table 2) the amount of bonded mercury depended here on the amount of mercury in-

Table 4. Effect of different mercury doses on growth and lactose fermentation ability of *Kluyveromyces fragilis* CBS-397 cultured on shakers on whey medium (deproteinized whey); incubation at 30°C for 72 h

Doses of Hg^{++} (mg/l culture)	Biomass yield (dry mass) (g/l)	Attenuated lactose (%)	Cell number in ml culture
(0.03 mg)*)	3.75	99.9	7.3×10^8
0.05	3.79	99.0	6.9×10^8
0.1	3.68	99.0	6.9×10^8
0.5	3.89	99.3	6.3×10^8
1.0	2.83	98.3	4.2×10^8
10.0	1.02	7.2	3.0×10^8
50.0	1.03	7.2	2.2×10^8
100.0	1.63	11.9	2.0×10^8

*) Mercury found in fresh whey

Table 5. Bonding of mercury (Hg^{++}) by *Kluyveromyces fragilis* CBS-397 cell biomass in cultures on whey medium (deproteinized whey); incubation at 30°C for 72 h

Doses of Hg^{++} (mg/l culture)	Biomass (dry mass) (g/l culture)	Assayed mercury content		
		biomass from 1000 ml culture (μg)	biomass (μg/g dry mass)	per cent of bonded mercury
(0.03 mg)*)	3.75	3.56	0.95	11.8
0.05	3.79	52.11	13.75	65.1
0.1	3.68	69.55	18.90	53.5
0.5	3.89	384.44	98.83	72.5
1.0	2.83	784.33	277.15	76.1
10.0	1.02	3 387.38	3 320.97	33.7
50.0	1.03	18 555.14	18 014.70	37.0
100.0	1.63	37 300.64	22 883.84	37.2

*) Mercury found in fresh whey

troduced into the substrate. The percent of mercury bonded by the biomass increased with the increase of the amount of added marcury but only up to the dose of 1 mg Hg^{++}/l when the accumulation reached 76%. At higher doses such as 10, 50 and 100 mg Hg^{++}/l the figure dropped to a low 33.7% which may be explained by the considerably reduced yeast cell biomass growth. Given the lower biomass yield in yeast cultures on

deproteinized whey, there was a large increase of the absolute amount of mercury bonded by the yeast per 1 g dry mass: it was up to 18.0 mg Hg^{++}/g dry mass of yeast biomass at the dose of 50 mg Hg^{++}/l and as much as 22.8 mg Hg^{++}/g dry mass in yeast culture with an addition of 100 mg Hg^{++}/l medium.

Although in yeast cultures on deproteinized whey containing low mercury doses (e.g. 0.5 and 1.0 mg Hg^{++}/) there is no visible drop either of the fermentation activity or the biomass yield, over 70% of the mercury nevertheless passes from the medium to the biomass attaining in the latter levels ranging from over 100 to ca 277 μg Hg^{++}/g dry mass.

In the case of direct utilization of yeast biomas as fodder, such a mercury contamination may be dangerous. From the ecological point of view, it would be interesting to uncover the mechanism of mercury bonding by yeast cells.

The planned subsequent stage of these studies will incorporate a broader look at the interactions between microorganism, heavy metal and environmental factors.

CONCLUSIONS

1. The toxicity of mercury towards the investigated yeast depends not only on the degree of contamination but to a large extent also on the kind of substrate. Whereas in cultures on malt wort and partly deproteinized whey the growth of yeast was inhibited already when 10 mg Hg^{++} was added to 1 l of medium, the yeast in cultures on non-deproteinized whey still continued to develop at the level of 100 mg Hg^{++}/l.

2. An almost complete inhibition of lactose-fermentation ability of the yeast *Kluyveromyces fragilis* CBS-379 occurred in cultures on partly deproteinized whey already when 10 mg Hg^{++} were added per 1 l medium, whereas in cultures on non-deproteinized whey the effect occurred only when the addition was as much as 50 mg Hg^{++}/l medium.

3. It was found that yeast biomass is able to permanently bond (accumulate) considerable amounts of marcury. The leval of accumulated mercury attained 7 mg/g dry mass of yeast obtained from cultures on non-deproteinized whey, and as much as almost 23 mg Hg^{++}/g dry substance of yeast from cultures on deproteinized whey.

4. Mercury was found to be present in the initial substrates: malt wort (40 μg Hg^{++}/l) and fresh whey (30 μg Hg^{++}/l).

LITERATURE

1. Deitz F. D., Sell J. L., Bristol D.: J.AOAC 1973, 56, 378.
2. Dickson R. C., Dickson L. R., Markin J. S.: J. Bacteriol., 1979, 1, 51.
3. Gadd G. M., Grieffiths A. J.: Microbial Ecology 1978, 4, 303.

4. Kędzierski B.: Prace Instytutu i Lab. Bad. Przem. Spoż., 1975, 4, 457.
5. Nelson N.: J. Biol. Chem., 1944, **153**, 375.
6. Somoggi N.: J. Biol. Chem., 1952, **195**.
7. Summers A. O.: Ann. Rev. Microbiol., 1978, **32**, 637.
8. Ramamoorthy S., Kushner D. J.: Microbial Ecol., 1975, **2**, 162.
9. Vananuvat P., Kinsella J. E.: J. Fd. Sci., 1975, **40**, 336.
10. Wasserman A. E., Hampson J. W., Alvare N. F.: J. Water Pollution Control Federation 1961, **23**, 1090.
11. Wierzbicki L. E., Kosikowski F. V.: J. Dairy Sci., 1973, 1, 26.

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Authors address: 60-623 Poznań, Mazowiecka 48

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

WPŁYW RTĘCI NA WZROST DROŻDŻY *KLUYVEROMYCES FRAGILIS* CBS-379 ORAZ JEJ AKUMULACJA W BIOMASIE KOMÓRKOWEJ

Instytut Mikrobiologii, Biochemii i Analizy Żywności, AR, Poznań

Streszczenie

Przeprowadzono badania nad wpływem skażenia podłoża rtęcią na wzrost i aktywność fermentacyjną drożdży *Kluyveromyces fragilis* CBS-379. Przedmiotem badań było również zjawisko wiązania i akumulacji rtęci przez biomasę komórek drożdżowych. Hodowle doświadczalne drożdży prowadzono na pożywce serwatkowej (serwatka nieodbiałczona oraz częściowo odbiałczona) i na brzeczce słodowej. Stwierdzono, że toksyczność rtęci względem badanych drożdży zależy nie tylko od stopnia skażenia, ale również w znacznej mierze od rodzaju podłoża. Gdy na przykład na brzeczce słodowej oraz serwatce odbiałczonej wzrost drożdży był hamowany już przy 10 mg Hg^{++} na litr, to w hodowlach na serwatce nieodbiałczonej drożdże rozwijały się jeszcze przy zawartości 100 mg Hg^{++} na litr pożywki. Prawie całkowite zahamowanie zdolności fermentowania laktozy następowało przy 10 mg Hg^{++} na litr w hodowlach na serwatce odbiałczonej i dopiero przy 50 mg Hg^{++} na litr w hodowlach na serwatce nieodbiałczonej. Stwierdzono, że biomasa drożdżowa ma zdolność wiązania (kumulacji) znacznych ilości rtęci. Poziom związanej rtęci dochodził do 7 mg na gram suchej masy drożdży uzyskanych z hodowli na serwatce nieodbiałczonej i aż prawie 23 mg na gram suchej masy drożdży pochodzących z hodowli na serwatce częściowo odbiałczonej.