

РОЗДІЛ VI. МІКРОБІОЛОГІЯ

UDC [579.811 : 57.018.6] : 546.4/7

INFLUENCE OF HEAVY METALS ON THE PIGMENT SYNTHESIZING ACTIVITY OF THE YEASTS *RHODOTORULA GLUTINIS* 1333

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Influence of heavy metals (Cu^{2+} , Zn^{2+} , Ni^{2+} , Cd^{2+} , Al^{3+} , Cr^{6+}) on the pigment synthesizing yeasts *Rhodotorula glutinis* 1333 has been studied. It was ascertained, that under the influence of the certain heavy metals ions concentrations yeasts may lose pigment synthesizing ability when growing in the solid nutrient medium (Sabouraud). It has emerged that the most toxic heavy metals for yeasts *Rh. glutinis* 1333 is Cr^{6+} (pigment genesis was blocked under the concentration of 10 mg/l chrome ions). The yeasts have turned out to be solid in regard to the aluminium ions (III) (only under the concentration of 400 mg/l the pigment synthesis was blocked). Yeasts ability to lose pigment under different heavy metals concentrations may be used in the bioindication researches.

Key words: *pigment synthesizing yeast, ions of heavy metals, growth.*

ВПЛИВ ВАЖКИХ МЕТАЛІВ НА ПІГМЕНТОСИНТЕЗУВАЛЬНУ АКТИВНІСТЬ ДРІЖДЖІВ *RHODOTORULA GLUTINIS* 1333

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Вивчений вплив важких металів (Cu^{2+} , Zn^{2+} , Ni^{2+} , Cd^{2+} , Al^{3+} , Cr^{6+}) на пігментосинтезувальні дріжджі *Rhodotorula glutinis* 1333. Встановлено, що під дією певних концентрацій іонів важких металів у дріжджів спостерігається втрата пігментосинтезувальної здатності при рості на твердому поживному середовищі (Сабуро). Найбільш токсичним важким металом для дріжджів *Rh. glutinis* 1333 виявився Cr^{6+} (пігментоутворення блокувалося при концентрації 10 мг/л іонів хрому). Стойкими дріжджі виявилися відносно до іонів алюмінію (ІІІ) (тільки при концентрації 400 мг/л блокувався синтез пігменту). Здатність дріжджів до втрати пігменту при різних концентраціях важких металів може бути використана в біоіндикаційних дослідженнях.

Ключові слова: *пігментосинтезувальні дріжджі, іони важких металів, рост.*

ВЛИЯНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ НА ПИГМЕНТСИНТЕЗИРУЮЩУЮ АКТИВНОСТЬ ДРОЖЖЕЙ *RHODOTORULA GLUTINIS* 1333

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Изучено влияние тяжелых металлов (Cu^{2+} , Zn^{2+} , Ni^{2+} , Cd^{2+} , Al^{3+} , Cr^{6+}) на пигментсintéзирующие дрожжи *Rhodotorula glutinis* 1333. Установлено, что под действием определенных концентраций ионов тяжелых металлов у дрожжей наблюдается потеря пигментсintéзирующей способности при росте на твердой питательной среде (Сабуро). Наиболее токсичным тяжелым металлом для дрожжей *Rh. glutinis* 1333 оказался Cr^{6+} (пигментообразование блокировалось при концентрации 10 мг/л ионов хрома). Стойкими дрожжи оказались по отношению к ионам алюминия (ІІІ) (только при концентрации 400 мг/л блокировался синтез пигмента). Способность дрожжей к потере пигмента при разных концентрациях тяжелых металлов может быть использована в биоиндикационных исследованиях.

Ключевые слова: *пигментсintéзирующие дрожжи, ионы тяжелых металлов, рост.*

INTRODUCTION

As is known, exceeding of the heavy metals (HM) concentrations in nature has an adverse effect on the ecological state of the environment, which may lead to the malfunction of physiological and biochemical processes taking place in living organisms [1, 2]. Main sources of HM, polluting environment, are metallurgy and galvanic shops of the industrial enterprises [3]. That is why the search for effective methods of environment pollution indications by HM has taken the first place recently. The surest and the most available methods of the anthropogenic violations diagnosis are based on a number of microbiological characteristics, because among all the representatives of the biota, microorganisms are the most sensitive to change of the medium [4]. So, the usage of the pigment synthesizing bacteria as bioindicators is a new and promising tendency [5]. Visual observation of the change of the pigment brightness under the influence of HM may serve as objective bioindicator of the environment pollution [6]. Thus, researches of the bacteria that we carried out aroused our interest to the research of the HM influence on the pigment synthesizing ability of the yeasts. In the literature accessible for us is mentioned only the fact that yeasts have the ability to sorb HM, and there is little information about the ability to change the pigment color in HM presence in the medium [7]. One of the richest in quality composition carotenoids are the yeasts *Rhodotorula glutinis*, which are able to synthesize phytoene, neurosporene, γ -carotene, β -carotene, ξ -carotene and torulene [8, 9]. It is colour saturation and stability of pigment data that determined the object of our research: to study the HM influence on the carotinoid synthesis of the yeasts *Rh. glutinis* 1333.

Thus, the aim of our study was to investigate the influence of heavy metals on the carotinoid synthesis of the yeasts *Rh. glutinis* 1333.

MATERIALS AND METHODS OF RESEARCH

The object of the research was pigment synthesizing yeasts *Rh. glutinis* 1333, which D.K. Zabolotny Institute of the microbiology and virology of National Academy of Sciences of Ukraine kindly gave us.

Solid nutrient medium Sabouraud was prepared on the base of the water with certain HM salt concentrations ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, CdCl_2 , $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{K}_2\text{Cr}_2\text{O}_7$). Thus, water model solutions contained such HM ions as Cu^{2+} , Zn^{2+} , Cd^{2+} , Ni^{2+} , Al^{3+} , Cr^{6+} . Nutrient medium Sabouraud without metals was used as a control. When Sabouraud set congeal, 18-days culture *Rh. glutinis* 1333 was seeded by solid lawn on it (0,2 ml per one Petri dish). Suspension density was $10^7/\text{ml}$ [10]. Yeasts incubated in the thermostat under the temperature 27-28°C. Results were calculated during 9 days of the cultivation. Visual observation and comparison of the experimental samples with the control was carried out. For the calculation of the color intensity difference between experimental and control samples, the Petri dishes with yeasts colonies were photographed, photos were loaded in the program Adobe Photoshop, indexes of the color model channels (Lab), and then the difference of the pigment color intensity was calculated in the program CIEDE 2000 [11].

RESULTS AND THEIR DISCUSSION

The results of the research showed that the yeasts *Rh. glutinis* 1333 react on certain metal concentrations' presence in the medium by the loss of pigment and by the growth delay (tables 1-6).

Found that between the total pigment synthesis inhibition and lack of growth there is a certain concentration range, which makes possible to use *Rh. glutinis* 1333 yeast for bioindication researches of pollution with heavy metals.

The influence of the ions Cu^{2+} on the pigment synthesis of the yeasts *Rh. glutinis* 1333 was remarked with the concentration of 200-300 mg/l, under which the growth of the small milky and big pale rosy colonies is observed during 9 days.

Under 350 mg/l concentration of the ions of the copper (II) on the 3rd day pigment-free colonies grew, and on the 6th and 9th day the poorly pigmented colonies appeared.

Table 1 – The influence of the ions Cu^{2+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration, Cu^{2+} , mg/l	3 ^d day		6 th day		9 th day	
	Growth*	Pigment**	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
100	+++	++++	++++	++++	++++	++++
200	++	±	++	±	++	±
300	+	±	+	±	+	±
350	+	-	+	±	+	±
400	-	-	+	-	+	±
425	-	-	+	-	+	-
450	-	-	-	-	-	-

Remarks (here and further): *growth: ++++ – confluent, +++ – good, ++ – moderate, + – weak, - – absent; **pigment genesis: ++++ – intensive, +++ – good, ++ – moderate, + – weak, - – absent, ± – pigment and pigment-free colonies are observed.

Copper concentration of 400 mg/l provoked weak growth of pigment-free colonies, on the 6th day, and on the 9th day the pale rosy colonies appeared. Under the concentration of 425 mg/l Cu^{2+} on the 6th and 9th day only the pigment-free colonies grew, and under 450 mg/l of the copper growth wasn't observed during 9 days.

Table 2 – The influence of the ions Zn^{2+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration Zn^{2+} , mg/l	3 ^d day		6 th day		9 th day	
	Growth	Pigment	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
50	+++	++	+++	++	++++	++
70	++	+	+++	++	+++	++
100	+	±	++	±	++	±
150	+	±	+	±	+	±
175	-	-	+	-	+	-
200	-	-	-	-	-	-

As we can see 50 and 70 mg/l concentrations of the zinc ions (II) provoked weak and moderate pigment genesis of the yeasts *Rh. glutinis* 1333. Under 100-150 mg/l Zn^{2+} concentrations over 9 days milky and pale rosy colonies grew. 175 mg/l concentration of the zinc ions in the medium provoked appearance of the pigment-free colonies on the 6th and 9th day. Under 200 mg/l Zn^{2+} concentration the growth wasn't observed.

Table 3 – The influence of the ions Cd^{2+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration Cd^{2+} , mg/l	3 ^d day		6 th day		9 th day	
	Growth	Pigment	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
20	+++	++	+++	++	+++	+++
50	++	++	+++	++	+++	+++
100	++	±	++	+	++	+
200	++	-	++	±	++	±
400	+	-	++	±	++	±
550	+	-	+	±	++	±
750	+	-	+	±	+	±
900	-	-	+	-	+	-
1000	-	-	-	-	-	-

The influence of the cadmium ions (II) on the pigment synthesis of *Rh. glutinis* 1333 was remarked with 20-50 mg/l concentration (moderate pigment genesis was observed). Under 100 mg/l concentration colonies were milky and pale rosy. 200-750 mg/l concentrations provoked appearance of the pigment-free colonies on the 3rd day, but on the 6th and 9th day pale rosy colonies were also observed. Under 900 mg/l concentration there was a weak growth of pigment-free colonies on the 6th and 9th day, and under 1000 mg/l there wasn't growth during 9 days. Thus, on the 3rd day of the cultivation absolute loss of pigment was observed under the concentration of Cd^{2+} that is by 734% lower than the concentration under which absolute blocking of the vital functions of the yeasts cells was observed.

Table 4 – The influence of the ions Ni^{2+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration Ni^{2+} , mg/l	3 ^d day		6 th day		9 th day	
	Growth	Pigment	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
25	+++	±	+++	±	+++	±
50	++	±	++	±	+++	±
75	+	-	+	±	+	±
125	-	-	+	-	+	-
150	-	-	-	-	-	-

Under 20-50 mg/l concentrations of the ions of nickel (II) the growth of the pigment colonies and pigment-free colonies was observed during 9 days. 75 mg/l Ni^{2+} concentration provoked appearance of the pigment-free colonies on the 3rd day, and on the 6th and 9th day pale rosy colonies were also observed. Under 125 ml/g concentration of the nickel ions the colonies were pigment-free, and under the concentration of 150 mg/l there wasn't growth for 9 days.

Table 5 – The influence of the ions Al^{3+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration Al^{3+} , мг/л	3 ^d day		6 th day		9 th day	
	Growth	Pigment	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
100	++++	±	++++	++++	++++	++++
200	+++	±	++++	++++	++++	++++
300	++	±	+++	++++	++++	++++
400	+	-	++	+++	+++	++++
450	+	-	++	++	+++	++++
500	+	-	+	±	++	++
600	-	-	-	-	+	-
700	-	-	-	-	-	-

Research showed that under the influence of the ions of aluminium (III) growth of pigment colonies and pigment-free colonies (under 100-300 mg/l concentration) and milky colonies (under 400-500 mg/l concentration) was observed on the 3rd day, but on the 6th and 9th day the culture *Rh. glutinis* 1333 restored the ability to synthesize the pigment to the rosy color. Pigment-free colonies appeared on the 9th day of the cultivation under 600 mg/l concentration of aluminium and under 700 mg/l growth wasn't observed at all. Thus, on the 3rd day of cultivation absolute loss of pigment was observed under the concentration of Al^{3+} that was by 20% lower than the concentration under which absolute blocking of the growth of *Rh. glutinis* 1333 was observed.

It was found that the most toxic HM for the yeasts *Rh. glutinis* 1333 is chromium (VI). It is known that Cr^{6+} is more toxic than Cr^{3+} . Under the concentration of 10 mg/l Cr^{6+} on the 3rd day the weak growth of the pigment-free colonies is observed, but on the 6th and 9th day pale rosy colonies appeared too.

Under the concentrations of 15-30 mg/l of the chromium ions on the 6th day of the cultivation the pigment-free colonies grew, and under 40-60 mg/l they appeared only on the 9th day of the cultivation. Under the concentration of 70 mg/l Cr^{6+} growth was absolutely inhibited.

Table 6 – The influence of the ions Cr^{6+} on the pigment synthesizing ability of the yeasts *Rh. glutinis* 1333

Ions concentration Cr^{6+} , mg/l	3 ^d day		6 th day		9 th day	
	Growth	Pigment	Growth	Pigment	Growth	Pigment
Control	++++	++++	++++	++++	++++	++++
10	+	-	++	±	+++	±
15	-	-	++	-	+++	±
20	-	-	++	-	+++	-
30	-	-	+	-	+++	-
40	-	-	-	-	++	-
60	-	-	-	-	+	-
70	-	-	-	-	-	-

The results of the calculation of the difference in pigment genesis intensity between control and experiment showed that with the increase of the HM ions concentration in the nutrient medium it grows (tables 7 – 12).

Table 7 – The estimation of the pigment color intensity on the concentration row Cu^{2+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day						6 th day						9 th day					
	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a
Control	66	22	31		42	29	32		42	31	33							
100	60	23	28	5,5±0,02	46	21	22	6,1±0,4	38	22	22	6,3±0,8						
200	50	22	35	14,9±0,05	56	18	28	15,1±0,6	24	15	24	16,6±0,9						
300	49	24	30	15,6±0,9	57	20	24	15,7±0,08	22	20	26	16,7±1,1						
350	48	24	20	18,0±1,2	55	12	30	16,7±1,1	21	26	25	16,9±0,7						
400	-	-	-	-	58	13	24	18,3±1,2	23	15	22	17,2±1,2						
425	-	-	-	-	59	14	31	18,8±0,8	16	21	27	20,4±1,1						

Remark (here and further): L, a, b – indexes of the color model channels CIE Lab; dE – difference of the color intensity between control and experiment, calculated by means of computer program CIEDE 2000.

Table 8 – The estimation of the pigment color intensity on the concentration row Zn^{2+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day						6 th day						9 th day					
	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a
Control	66	23	33		42	30	32		42	31	33							
50	55	25	24	11,2±0,7	54	23	24	12,5±0,4	55	25	24	13,5±0,4						
70	48	19	30	16,7±0,9	55	25	30	13,1±0,09	56	28	30	14,0±0,6						
100	50	25	26	15,2±0,4	55	17	20	14,7±1,1	58	26	22	16,8±1,1						
150	51	24	20	15,6±1,0	56	18	22	15,3±0,3	59	24	25	17,4±1,2						
175	-	-	-	-	59	12	30	20,4±0,7	61	23	26	19,3±0,9						

Table 9 – The estimation of the pigment color intensity on the concentration row Ni^{2+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day						6 th day						9 th day					
	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a
Control	67	23	33		42	32	32		42	29	32							
25	51	25	26	15,0±0,7	58	28	25	16,3±0,3	57	28	23	15,7±0,1						
50	50	18	25	15,7±1,1	54	12	30	17,0±1,1	58	28	30	16,0±1,1						
75	50	22	28	15,5±0,5	58	18	26	17,5±0,9	57	18	20	16,3±0,9						
125	-	-	-	-	57	10	29	20,2±1,2	60	25	21	18,7±0,8						

Table 10 – The estimation of the pigment color intensity on the concentration row Cd^{2+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day						6 th day						9 th day					
	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a	b	dE	L	a
Control	67	22	33		42	30	33		41	31	30							
20	55	23	26	11,2±0,9	54	20	26	12,8±0,4	48	21	22	8,2±0,09						
50	53	19	30	12,4±0,7	53	16	20	13,2±0,9	47	17	25	9,2±0,8						
100	51	22	28	14,5±1,1	54	13	27	15,3±0,06	57	28	23	16,3±0,5						
200	50	24	20	17,3±1,2	56	21	20	15,3±1,1	56	18	30	16,9±1,1						
400	49	22	21	17,6±0,05	57	23	22	15,8±0,08	57	19	23	17,0±0,5						
550	48	25	24	18,4±0,8	58	20	26	16,7±1,1	58	28	30	17,0±1,1						
750	47	21	22	19,3±1,1	58	23	20	17,1±0,5	56	15	26	17,4±0,5						
900	-	-	-	-	61	20	24	19,5±0,2	61	24	28	20,2±1,2						

Table 11 – The estimation of the pigment color intensity on the concentration row Al^{3+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day				6 th day				9 th day			
	L	a	b	dE	L	a	b	dE	L	a	b	dE
					40	28	30		40	31	31	
Control	67	24	32		41	22	25	3,1±0,03	43	23	26	4,6±0,4
100	50	22	28	15,4±0,04	40	24	22	3,8±0,09	44	21	25	6,0±0,03
200	49	23	25	16,7±0,2	42	21	23	4,2±0,6	43	18	24	7,0±0,9
300	48	24	27	17,5±1,1	49	23	24	9,0±0,7	45	20	27	7,2±0,5
400	49	20	21	17,1±0,9	52	20	21	12,5±0,9	45	20	28	7,4±0,4
450	50	28	20	17,5±0,08	56	22	26	16,0±0,1	53	19	22	13,9±1,0
500	49	28	21	18,1±0,5	-	-	-	-	59	24	28	19,3±1,1
600	-	-	-	-	-	-	-	-	-	-	-	-

Table 12 – The estimation of the pigment color intensity on the concentration row Cr^{6+} in *Rh. glutinis* 1333

Metal concentration, mg/l	3 ^d day				6 th day				9 th day			
	L	a	b	dE	L	a	b	dE	L	a	b	dE
					42	28	32		42	32	34	
Control	67	23	31		42	28	32		42	32	34	
10	48	22	25	17,6±1,1	57	21	22	15,7±0,9	57	19	22	16,4±0,9
15	-	-	-	-	59	20	25	17,5±1,1	58	21	24	16,9±0,07
20	-	-	-	-	59	24	22	17,6±0,7	59	23	26	17,5±1,1
30	-	-	-	-	58	15	30	17,8±0,8	58	15	29	18,5±0,5
40	-	-	-	-	-	-	-	-	61	20	24	19,6±1,2
60	-	-	-	-	-	-	-	-	62	18	23	20,9±1,3

So, intensive pigment genesis of *Rh. glutinis* 1333 was remarked on the 3rd day of the cultivation in the presence in the medium of 100 mg/l of the copper ions and on the 6th and 9th day under 100-450 mg/l of aluminium that corresponds to the indexes of dE from 3, 1±0, 03 to 7, 4±0, 4. It should be noticed, that the yeasts are able to restore completely the ability to synthesis carotinoids on the 9th day of cultivation in the presence in the medium of up to 450 mg/l of the aluminum ions (III). The difference of the color intensity of the pigment-free colonies under the influence of HM varied from 17,1±0,5 to 20,9±1,3. The most long term inhibit effect on the pigment synthesis had cadmium ions (II), on the 3rd day of the cultivation under 200-750 mg/l concentration dE was within the limits 17,3±0,2 – 19,3±1,1. dE of the pigment-free colonies also varied under the influence of chrome (VI) from 17,5±1,1 to 20,9±1,3.

Thus, the research has shown that the yeasts *Rh. glutinis* 1333 are able to react on the HM presence in the nutria medium by the blocking of the pigment synthesis and by the growth inhibiting from certain concentration levels, because of that further research of the pigment synthesizing yeasts with the purpose of its recommendation for research in the field of the environment pollution by HM turns out to be interesting.

CONCLUSIONS

1. The research has shown that yeasts *Rh. glutinis* 1333 react on the certain metal concentrations presence in the medium by pigment loss and by growth inhibiting.
2. It has emerged that the most toxic HM for yeasts *Rh. glutinis* 1333 is Cr^{6+} (pigment genesis was blocked under the concentration of 10 mg/l chrome ions), the yeasts have turned out to

be solid in regard to the aluminium ions (III) (only under the concentration of 400 mg/l the pigment synthesis was blocked).

3. Obtained results make it possible to recommend the yeasts *Rh. glutinis* 1333 for the usage in the bioindication research of the degree of environment pollution by HM.

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УДК 614.21:614.88]:[579.63:616-085.33](477.64-25)

АНТИБИОТИКОРЕЗИСТЕНТНОСТЬ ДОМИНИРУЮЩИХ МИКРООРГАНИЗМОВ В ЗАПОРОЖСКОЙ БОЛЬНИЦЕ СКОРОЙ ПОМОЩИ

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Проведен микробиологический мониторинг отделений Запорожской больницы скорой помощи. Выявлены доминирующие микроорганизмы: *E. faecalis*, *P. aeruginosa*, *A. baumannii*, *E. coli*, *S. aureus*, *K. pneumonia*, их антибиотикорезистентность к основным группам атибактериальных препаратов: пенициллинам, цефалоспоринам, карбопенемам, гликопептидам, аминогликозидам, фторхинолонам, макролидам. Определена эмпирическая антибиотикотерапия для: *E. faecalis*, *P. aeruginosa*, *A. baumannii*, *E. coli*, *S. aureus*, *K. pneumonia*.

Ключевые слова: антибиотикорезистентность, доминирующие микроорганизмы.