THE EFFECT OF THE HERBICIDE CIPC
ON RHODOTORULA GLUTINIS CELLS

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It had been known before [2, 8, 9, 10] that the herbicides of the carbamates group strongly affect physiology of microorganisms. The aim of the present work was to gain an insight into the action of the herbicide CIPC (isopropyl-N/3-chlorphenyl carbamate) on the cells of soil yeast *Rhodotorula glutinis*, as a model of soil microflora. Attention has been paid to the following questions: the effect of the CIPC on the growth of the cells, the pigment production by yeast, the content of nucleic acids and lipids, the sorption of some dyes by the cells and the penetration of the CIPC into the cells. The action of the herbicide CIPC on the yeast cells with the action of the detergent of sodium taurocholate has been compared.

METHODS

The yeast strain *Rhodotorula glutinis*, isolated from soil [10] in the Rider medium, during 1-6 weeks, was cultivated at 30°C. The herbicide CIPC, technically pure (50% of active ingredient), in the dose of 100 ppm, or CIPC analytically pure, in the dose of 50 ppm, or sodium taurocholate of 10 ppm was added to the culture medium. The growth of yeast cells was determined nephelometrically.

The pink pigment produced by yeast has been obtained from the cells by means of extraction with 96% ethanol and determined spectrophotometrically.

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The determinations of nucleic acids content in the yeast cells with different methods were carried out. The yeast cells after 2-4 weeks of incubation were harvested by centrifugation, washed twice with phosphate buffer pH 7, once with distilled water and dried in vacuo at 0°C. Dried cells were disintegrated mechanically by grinding with acid-washed sand for 20 min at 5°C, then DNA was isolated according to the Kirby method [7] and determined gravimetrically. The nucleic acids (RNA + DNA) were also isolated by the method of Marmur [12] or the method of Schneider [15]. Then the RNA amount was determined colorimetrically by the method of Mejbaum [13] and DNA by the method of Dische [6] modified by Burton [4]. The content of nucleic acids (RNA + DNA) was determined spectrophotometrically. The microscopic observations of nucleoids of the yeast cells have been carried out according to the method of Tulasne Vendrely [3].

Intracellular lipids of the yeast cells were obtained using the method described by Deinema [5] and determined gravimetrically.

The effect of CIPC and sodium taurocholate on the sorption of some dyes by the yeast cells was determined by the following method:

The cells were harvested by centrifugation, washed twice with phosphate buffer of pH 7 and suspended in the water solution (0.001%) of a dye. Each sample contained the same number of yeast cells. After half an hour the cells were centrifuged and the quantity of a dye in the supernatant was determined spectrophotometrically. The following dyes have been used: Janus green, methylene violet, acridine orange, neutral red, trypaflavine, methylene blue, erythrosine and eosine. In the next experiment the yeast cells in the medium without the herbicide, were incubated for 16 days. Then the yeast cells were centrifuged and suspended in the suspension of the CIPC technically pure in the doses of 1000, 10 and 0.1 ppm, or in the solution of sodium taurocholate in the doses of 1000 and 100 ppm. After an hour the yeast cells were centrifuged and their sorption ability of methylene blue was determined. Simultaneously the microscopic observations of the yeast cells coloured with methylene blue were carried out.

To test the penetration of CIPC into the yeast cells, the yeast have been incubated in the media containing CIPC technically or analytically pure in doses 100 and 50 ppm, respectively. After 1-2 weeks of incubation the cells were centrifuged and washed twice with ethanol (96%), to remove the CIPC from their surface. Then, the air-dry cells were weighed and the quantity of CIPC were determined according to the Lowen's method [10]. The presence of CIPC in the yeast cells was
determined biologically with buckwheat test by the method described by Sobieszczański [14] and by thin layer chromatography according to the method of Abbott [1].

RESULTS AND DISCUSSION

CIPC technically pure in the dose of 100 ppm inhibited the growth of yeast, while the CIPC analytically pure in the dose of 50 ppm delayed it (Fig. 1). Sodium taurocholate in the dose of 10 ppm showed only a slight effect on the growth of yeast.

![Fig. 1. The effect of CIPC and sodium taurocholate on the growth of yeast](image)

1 — control cells. Cells influenced by:
2 — CIPC technically pure in the dose of 100 ppm,
3 — CIPC analytically pure in the dose of 50 ppm,
4 — sodium taurocholate in the dose of 10 ppm

It was observed that the yeast cells in the presence of CIPC, or detergent in the dose of 100 ppm, did not produce the pink pigment very characteristic for the control cells (Fig. 2). The yeast cells in the medium with sodium taurocholate in the dose of 10 ppm produced this pigment, but after 4 weeks of incubation they lost it, while the loss of pigment in the control culture was noted after about 6 weeks.

The amount of the nucleic acids, isolated from the yeast cells influenced by CIPC analytically pure, was about 4 times less than the control (Fig. 3). The quantitative determinations of RNA and DNA showed that in the cells incubated in the presence of CIPC there were less RNA and less DNA (Table 1). The microscopic observations confirmed it.

The determination of intracellular lipids of the yeast cells showed, that the CIPC caused the decrease of the lipids content (Table 2). It was also found that the cells influenced by CIPC were about 2.5 times lighter than the control cells (Fig. 4). This could be explained by the change
Fig. 2. The effect of CIPC and sodium taurocholate on pigment production by yeast. Explanation as in Fig. 1.

Fig. 3. The effect of CIPC on the nucleic acids content in yeast cells. 1 — control cells, 3 — cells influenced by CIPC analytically pure in the dose of 50 ppm.

<table>
<thead>
<tr>
<th>Object</th>
<th>Nucleic acid %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA</td>
</tr>
<tr>
<td>Control cells</td>
<td>100</td>
</tr>
<tr>
<td>Cells influenced by CIPC analytically pure</td>
<td>74,4</td>
</tr>
<tr>
<td>Cells influenced by CIPC technically pure</td>
<td>96,0</td>
</tr>
</tbody>
</table>
The effect of the herbicide CIPC...

Table 2

<table>
<thead>
<tr>
<th>Object</th>
<th>Intracellular lipids</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 g wet weight</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of cells</td>
<td></td>
</tr>
<tr>
<td>Control cells</td>
<td>0.0326</td>
<td>100</td>
</tr>
<tr>
<td>Cells influenced by CIPC analytically pure</td>
<td>0.0081</td>
<td>25</td>
</tr>
<tr>
<td>Cells influenced by CIPC technically pure</td>
<td>0.0143</td>
<td>44</td>
</tr>
</tbody>
</table>

of cellular structure caused by this herbicide. The cells influenced by CIPC absorbed more of some dyes than the control cells. The methylene blue was absorbed to the most extent (Fig. 5). The number of living cells incubated in the presence of CIPC was not smaller than the number of control cells, so the increase of absorbed dye by the cells influenced by CIPC could be considered as a result of this herbicide action. It was worth noting that all the dyes absorbed by the cells to a greater degree belonged to the basic dyes. Two acid dyes: eosine and erythrosine were not absorbed by the cells incubated in the medium with CIPC. This could be explained by some changes of electrostatic surface charge of the cells influenced by the herbicide. The quantity of the methylene — blue absorbed by the cells depended on the age of the cells (Fig. 6). Maximal methylene blue sorption was after 2 weeks of incubation, but the cells influenced by CIPC absorbed 20% more of the dye, while the cells influenced by sodium taurocholate 15% more than control cells.
Fig. 5. Sorption of various dyes by yeast cells

1 — dyes absorbed by control cells. Dyes absorbed by cells influenced by CIPC technically pure in the dose of 100 ppm: 2 — Janus green, 3 — methylene violet, 4 — acridine orange, 5 — neutral red, 6 — trypaflavine, 7 — methylene blue

Fig. 6. Sorption of methylene — blue by yeast cells after different incubation time

explanation as in Fig. 1

yeast cells incubated in the control medium without the herbicide and treated with CIPC or sodium taurocholate for an hour, showed also the increase in the methylene blue sorption, but to a smaller degree (Fig. 7).

Generally, the effect of CIPC on the dye sorption by the yeast cells was similar to the effect of the sodium taurocholate, but the herbicide was affected stronger than the detergent.

It was found that CIPC penetrated into the yeast cells. After 16 days of incubation the yeast cells contained 9.5 µg of CIPC technically pure or 5.0 µg of CIPC analytically pure in 10 mg of dry weight. The presence of CIPC in the yeast cells was also indicated by thin layer chromatography and biologically with buckwheat test (Fig. 8).
CONCLUSIONS

1. The CIPC technically pure, which is being applied in agriculture, affects stronger the growth of yeast than the CIPC analytically pure.
2. The yeast cells in the presence of the CIPC, both technically and analytically pure, do not produce the pigment.
3. The CIPC, both technically and analytically pure, causes the changes in the cellular structure.
4. The action of the herbicide CIPC technically pure on the sorption of methylene blue by yeast cells is similar to the action of the detergent, however, the herbicide action is stronger.
5. The CIPC penetrates in the cells but CIPC technically pure does that to a greater degree.
Streszczenie

Badano wpływ herbicydu CIPC — preparatu technicznie czystego (50% substancji aktywnej) i analitycznie czystego, na komórki drożdży glebowych Rhodotorula glutinis.

Działanie herbicydu porównywano z działaniem detergentu taurocholanu sodu. Stwierdzono, że CIPC technicznie czysty w ilości 100 ppm hamował wzrost drożdży, podczas gdy CIPC analitycznie czysty oraz detergent opóźniały podział komórek.

Komórki drożdży hodowane w obecności herbicydu lub detergentu w ilości 100 ppm nie produkowały różowego pigmentu, charakterystycznego dla kultury kontrolnej. Herbicyd powodował zmiany w budowie komórki, jak zmniejszenie się ilości kwasów nukleinowych, zwłaszcza DNA, oraz ilości lipidów wewnątrzkomórkowych. Komórki drożdży hodowane w obecności herbicydu lub detergentu w ilości 100 ppm nie produkowały różowego pigmentu, charakterystycznego dla kultury kontrolnej. Herbicyd powodował zmiany w budowie komórki, jak zmniejszenie się ilości kwasów nukleinowych, zwłaszcza DNA, oraz ilości lipidów wewnątrzkomórkowych. Komórki drożdży hodowane w obecności herbicydu zarówno technicznie, jak i analitycznie czystego były około 2,5 raza lżejsze od komórek kontrolnych. Ponadto stwierdzono, że CIPC technicznie czysty powodował znaczne zwiększenie ilości sorbowanych przez komórki barwników zasadowych, a zwłaszcza blękitu metylenowego. Wpływ detergentu na zwiększenie się sorbcji blękitu metylenowego był około 10 razy słabszy.
Stwierdzono, że herbicyd przenika do komórki drożdży, przy czym preparat technicznie czysty znajdowano w ilości około 2 razy większej od ilości CIPC analitycznie czystego.

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ВЛИЯНИЕ ГЕРБИЦИДА НА КЛЕТКИ ДРОЖЖЕЙ RHODOTORULA GLUTINIS

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Резюме

Изучали влияние гербицида CIPC, технически и аналитически чистого на клетки почвенных дрожжей Rhodorula glutinis.

Действие гербицида сравнивали с дестергентом таурохолат натрия. Установлено, что 100 ppm CIPC технически чистого задерживали рост дрожжей, тогда как аналитически чистый CIPC и дестергент замедляли деление клеток.

Клетки дрожжей инкубированные в присутствии гербицида или дестергента с концентрацией 100 ppm не производили розового пигмента, очень характерного для этих микроорганизмов.

Гербицид вызывал изменения в структуре клеток, в частности снижал количество нуклеиновых кислот, в первую очередь DNA, а также липидов. Вес клеток инкубированных в присутствии гербицида, как технически так и аналитически чистого, был на около 2,5 раза меньше, чем вес контрольных клеток.

Установлено также, что технически чистый CIPC повышал количество основных красителей сорбированных клетками дрожжей, особенно метиленовой синей.

Действие дестергента на повышение сорбции метиленовой синей оказалось на около 10 раз слабее.

Установлено, что гербицид проникает в дрожжевую клетку, однако количество технически чистого препарата в клетках было на около 2 раза больше, чем количество аналитически чистого CIPC.