

Isolation and Characterization of Chitin-Glucan Complexes from the Mycothallus of Fungi Belonging to *Russula* Genus

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Abstract

Composition and sorption properties with respect to heavy metals ions of chitin-glucane complexes (CGC) isolated from the mycothallus (fruit body) of fungi belonging to *Russula* genus those have been harvested within the zones with different levels of man-caused pollution. The amount of insoluble CGC isolated does not exceed 1.1 %; the content of chitin (CHT) is not higher than 0.65 %. In going from a non-contaminated zone to contaminated areas the content of CGC and CHT in mycothallus biomass is observed to increase for all the three species. The sorption capacity of CGC with respect to lead (II) and copper (II) ions is not high reaching the values of 107 and 123 $\mu\text{mol/g}$, respectively; however, a high selectivity (100 %) is exhibited for the sorption of copper (II) ions. For the CGC under investigation we observed a tendency towards an increase in the sorption capacity of CGC with the increase biotope contamination with heavy metals. The investigations performed could result in employing abundant fungi with the limited food and taste value belonging to the genus *Russula* (mushrooms such as russules) in order to obtain sorption materials.

Key words: fungi of the genus *Russula*, chitin-glucane complexes, sorption copper (II) and lead (II)

INTRODUCTION

The cell wall of fungi is formed on the basis of a polysaccharide complex and is determined by the system of microfibrilles built-in into an amorphous matrix. The role of “reinforcing structures” could be played by microfibrilles of chitin-glucan complexes (CGC). The role of a “cementing substance” could be played by proteins, lipids, soluble polysaccharides. The separation of an amorphous matrix by means of a chemical method results in the formation of polysaccharides [1] complicated with respect to their structure and composition. Owing to the polysaccharide groundwork that exhibits a non-trivial structure such organic material obtained is considered to offer excellent possibilities with respect to the sorption of heavy metal ions [2]. The

mentioned property is extensively enough studied by the example of fungi belonging to the phyla such as *Zygomycota*, *Ascomycota*, *Deuteromycota* [3].

The content of chitin (CHT) in fungi is subject to considerable fluctuations within the range from 0.2 to 26 % not only for fungi belonging to different genera, but also for fungi of the same species. So, the basidial fungi (the phylum *Basidiomycota*) contain a lower amount of CHT, amounting up to 12 %, than the imperfect fungi (the phylum *Deuteromycota*) those contain up to 26 % of CHT [1]. As far as the basidial fungi (*Basidiomycetes*) are concerned, the content of CHT in fungi *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* those belong to the order *Agaricales* is lower [4] than it is for such fungi as *Phanerochaete sanguinea* and *Ganoderma applanatum*, belonging to the order *Phyllophorales* [5].

The sorption properties with respect to heavy metal ions use to be exhibited not only at the level of polysaccharides isolated. The living biomass of fungi is also capable of accumulating them. The studies concerning the bioaccumulation of heavy metals by a fungal mycelium demonstrate that fungus *Agaricus acrosporus* can absorb up to 1.1 mmol of a metal per 1 g of a sorption material, and the living system mass uses to absorb a much greater amount of a metal, than it is observed for a dead system [6]. Development of fungi in the environment with an increased content of heavy metals promotes the process of metal bioaccumulation in fungal cell wall [7]. It is known, that the presence of toxic metals causes the structure of polysaccharide groundwork to change [1]. The role of CHT and its amounts in the sorption of metals is not quite clear, since the acetamide groups occurring within its structure represent weak complex forming agents.

The goal of the present work consisted in the investigation concerning the structure and amount of CGC isolated from mushrooms belonging to the genus *Russula* those have been harvested within a non-contaminated territory as well as in the territories contaminated with heavy metals. Sorption properties of CGC obtained for heavy metal ions (Cu^{2+} and Pb^{2+}) as well as the sorption selectivity with respect to copper (II) ions in the series of its standard satellites (Co^{2+} , Ni^{2+} , Zn^{2+}) have been simultaneously investigated.

RESEARCH OBJECTS AND METHODS

The subject of inquiry of this work was presented by fungi belonging to the order Agaricomycetes, the genus *Russula* (mushrooms such as russules). These mushrooms range in rather variegated types of forests, all of them are capable of forming mycorrhiza with trees of Russian forests, amounting to about 45 % of total mushrooms occurring in the forests of Russia [8]. Russules grow immediately on a contaminated soil, as apposed to tree fungi belonging to the order *Aphyllorphorales* those grow on a possible intermediate absorber of metal ions such as wood. The choice of the aforementioned genus is caused by a wide natural occurrence

and productivity of fungi belonging to this genus. This fact allows one to harvest enough fruit bodies of such fungi within any habitat. In order to take into account the variation of CGC amount in fruit bodies of mushrooms belonging to this genus we gathered the three mushroom species such as *R. vesca* Fr. (with light pileus), *R. consobrina* Fr. (with brown pileus) and *R. integra* (L). Fr. (with dark red pileus). We selected young fruit bodies not damaged by insects or by corruption processes.

The gathering of fruit bodies was carried out within the impact region of atmospheric emissions from Middle Ural copper-smelting plant located at the suburb of the Revda city (the Sverdlovsk Region). As the result of long-standing atmospheric pollution, a powerful gradient of toxic loading has been formed: the content of heavy metals is from one to two orders of magnitude higher (to a maximum extent more than 400 times higher) than the background level near to the plant, decreasing up to a background level at the distance of several kilometers [9, 10]. In this territory there are long-term complex studies carried out concerning forest ecosystem response to environmental pollution, where a background zone (30 km to the west from the plant), a buffer zone (4 km to the west) and an impact zone (1.5 km to the west) are distinguished. Owing to the industrial contamination, there are various metals in soil, mainly copper and lead. The content of copper in the forest litter amounted to 134.3 $\mu\text{g/g}$ for the background zone, 3635.7 $\mu\text{g/g}$ for the buffer one and 5164.1 $\mu\text{g/g}$ for the impact zone; the content of lead therein was equal to 83.5 $\mu\text{g/g}$, 1516.1 $\mu\text{g/g}$ and 1532.1 $\mu\text{g/g}$, respectively [9, 10]. The MPC level of corresponding metals in soil are equal to 3 mg/kg for Cu and 32 mg/kg for Pb [11].

The habitat of mushrooms represented a fir forest. The time of gathering was August, 2007. The determination of species was carried out employing the keys by B. P. Vasilkov [12] and by M. V. Vishnevsky [13] as well as using a mini-atlas "Mushrooms" [14].

The isolation of CGC was carried out by means of a standard procedure: a raw biomass (~10 fruit bodies) was treated twice with a 8 % KOH solution at 90 °C during 1 h. Then the mixture was processed with 6.7 % HCl solution

at a room temperature during 3 h. The product was then filtered and dried at 70 °C in order to gain the constancy of the mass. The composition was characterized by means of elemental analysis performed employing a Perkin Elmer automatic analyzer (the USA). The identification of CGC products isolated was carried out with the help of Fourier transform IR spectroscopy using an attenuated total internal reflection unit. For comparison we used a reference CGC isolated from the mycelium of the fungus *Aspergillus niger* (from the collection of the Biology faculty of the Ural State University).

The sorption properties were estimated keeping a sorbent in a solution containing copper (II), nickel (II), cobalt (II), zinc (II) sulphates with the concentration of 0.025 mol/L at pH 4.5, via the subsequent analysis of a filtrate for the residual amount of metal ions employing an Optima 4300 DV atomic emission spectrometer. Lead was sorbed in a similar manner; however, this procedure was performed using acetate solutions (the concentration equal to 0.025 mol/L, ammonia acetate buffer, pH 6.5).

RESULTS AND DISCUSSION

A fraction equal to about 60 % of the biomass of a mushroom usually falls on the cell wall [1]. During the treatment of mycelium with alkali solution one can observe the biomass disintegration to occur with the separation of protein, fatty and alkali-soluble carbohydrate components from “reinforcing” microfibrilles of chitin-glucan complexes those are not dissolved under these conditions.

The content of CGC, CHT as well as the content of CHT in CGC for the mushrooms under investigation is presented in Fig. 1. As seen, the amount of CGC insoluble in alkali (% with respect to the raw biomass) at the maximum is somewhat higher than 1 %. In going from the background zone to the buffer one and then to the impact zone, the amount of CGC was been observed to increase for all the three species: from 0.14 to 0.40 % for *R. vesca*, from 0.26 to 1.06 % for *R. consobrina* and from 0.26 to 1.1 % for *R. integra*. For the fungus *A. niger* (the collection culture) the content of CGC in the row biomass amounts up to 0.93 %, the

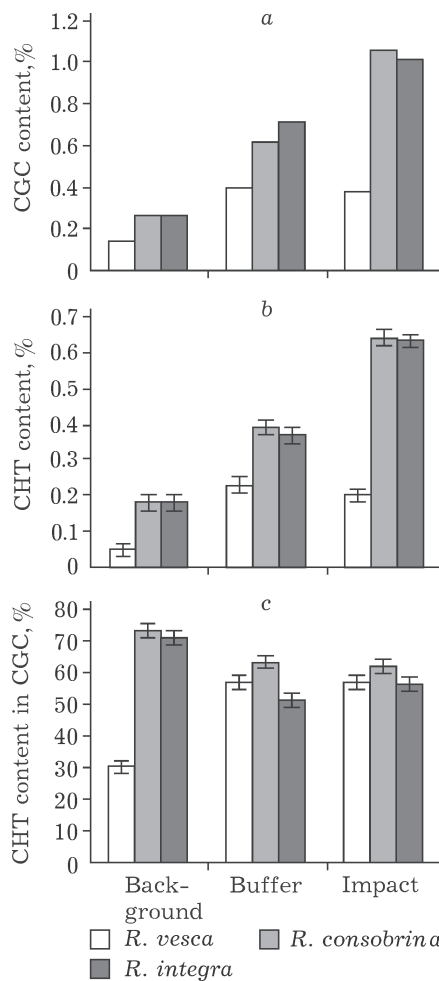


Fig. 1. Content of CGC (a), CHT (b) and the content of CHT in CGC (c) with respect to the dry solid matter of fungal biomass, for different mushrooms species.

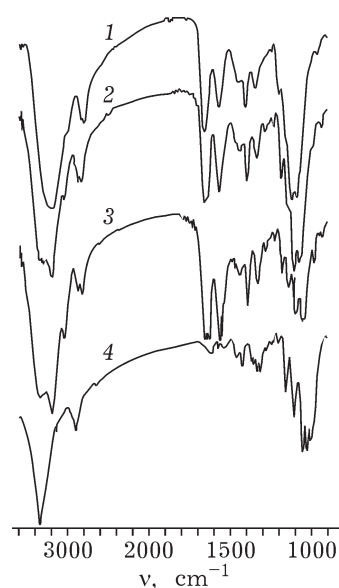


Fig. 2. IR reflection spectra: 1 – CGC isolated from mushroom belonging to *Russula* genus; 2–4 – the spectra for reference CGC isolated from the fungus *Aspergillus niger* (2), for chitin (3) and for glucan (4).

content of CHT therein is equal to 0.46 %, the content of CHT in dry solid CGC matter amounted to 50 %.

The identification of CGC isolated was carried out with the help of a reflection IR spectroscopy method. The comparison of CGC isolated from three fungal species those grew within the three ecological zones demonstrate the appearance of the spectra to be almost identical (Fig. 2, spectrum 1), which indicates their similar structure. The coincidence of CGC spectrum with the spectrum of reference CGC (see Fig. 2, spectrum 2), isolated from fungus *A. niger*, indicates that the alkali-insoluble part obtained really represents CGC, containing chitin and glucan components approximately in the same proportions. The presence of the chitin component results in the presence of absorption bands at 1650, 1552 and 1376 cm^{-1} (see Fig. 2, spectrum 1) those correspond to the vibrations of amide groups such as Amide I, Amide II and Amide III, respectively. For chitin, the wave number values of the same absorption bands are equal to 1653, 1558 and 1376 cm^{-1}

(see Fig. 2, spectrum 3). The presence of the glucan component is indicated by the presence of absorption bands in the spectra within the wave number range of 3400–2800 and 1160–1020 cm^{-1} those correspond to the groups of vibrations for N–H, O–H, C–H and C–O, C–C bonds manifested both for chitin (see Fig. 2, spectrum 3), and for glucan (see Fig. 2, spectrum 4). The comparison of relative intensities for absorption bands at 1653 and 1069 cm^{-1} in the spectrum of chitin (see Fig. 2, spectrum 3) where every pyranose ring is related to one acetyl group, with corresponding absorption bands at 1650 and 1068 cm^{-1} in CGC spectra (see Fig. 2, spectrum 1) demonstrates that for the latter the amount of the acetyl groups is lower than the amount of the pyranose rings.

The composition of the compounds isolated was determined with the help of elemental analysis (Table 1). As seen, the composition of HGK depends on mushroom species. The content CHT in the raw biomass has also demonstrated an increase for the micothallus of all the three species: from 0.04 to 0.23 % for

TABLE 1
Parameters of CGC isolated from fungi belonging to *Russula* genus

Fungal species	Zone	Content, %			Chemical formula
		C	H	N	
<i>A. niger</i>	Background	<u>42.67</u>	<u>6.54</u>	<u>3.61</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.49}(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_{0.51}(\text{H}_2\text{O})_{0.81}$
		42.65	6.71	3.61	
<i>R. vesca</i> (with light pileus)	Background	<u>42.06</u>	<u>6.12</u>	<u>2.25</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.7}(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_{0.3}(\text{H}_2\text{O})_{0.78}$
		42.04	6.66	2.23	
	Buffer	<u>43.54</u>	<u>6.27</u>	<u>4.08</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.43}(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_{0.57}(\text{H}_2\text{O})_{0.63}$
		43.55	6.64	4.05	
	Impact	<u>43.24</u>	<u>6.36</u>	<u>4.05</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.43}(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_{0.57}(\text{H}_2\text{O})_{0.71}$
		43.24	6.67	4.02	
<i>R. consorbinia</i> (with brown pileus)	Background	<u>42.59</u>	<u>6.49</u>	<u>4.87</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.27}(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_{0.73}(\text{H}_2\text{O})_{1.02}$
		42.56	6.81	4.86	
	Buffer	<u>43.25</u>	<u>6.35</u>	<u>4.39</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.37}(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_{0.63}(\text{H}_2\text{O})_{0.75}$
		43.27	6.70	4.38	
	Impact	<u>42.46</u>	<u>6.33</u>	<u>4.23</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.38}(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_{0.62}(\text{H}_2\text{O})_{0.95}$
		42.48	6.78	4.24	
<i>R. integra</i> (with dark red pileus)	Background	<u>42.74</u>	<u>6.45</u>	<u>4.78</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.29}(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_{0.71}(\text{H}_2\text{O})_{0.96}$
		42.73	6.79	4.77	
	Buffer	<u>42.13</u>	<u>6.25</u>	<u>3.54</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.49}(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_{0.51}(\text{H}_2\text{O})_{0.94}$
		42.15	6.76	3.57	
	Impact	<u>44.06</u>	<u>6.55</u>	<u>4.06</u>	$(\text{C}_6\text{H}_{10}\text{O}_5)_{0.44}(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_{0.56}(\text{H}_2\text{O})_{0.50}$
		44.05	6.58	4.04	

Note. The numerator denotes found value, the denominator stands for calculated value.

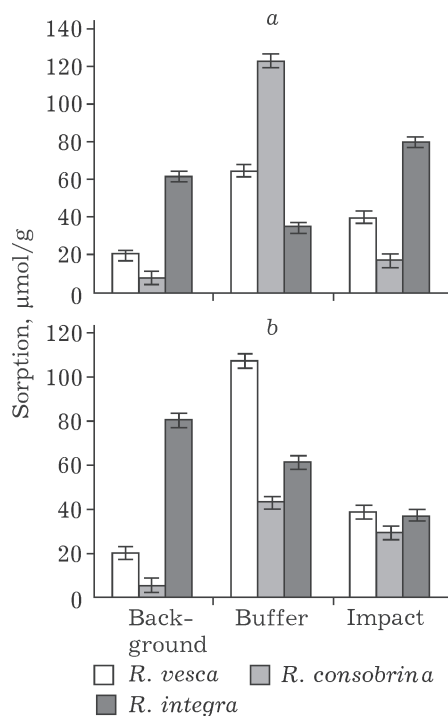


Fig. 3. Sorption capacity of CGC: a - Cu^{2+} , b - Pb^{2+} .

R. vesca, from 0.19 to 0.65 % for *R. consobrina* and from 0.18 to 0.62 % for *R. integra*. In going from a non-contaminated zone to contaminated zones one can observe a decrease in the chitin fraction in dry CGC for the mushrooms containing mainly chitin component such as *R. consobrina* and *R. integra*, from 73 to 62 % and from 71 up to 56 %, respectively, whereas mushroom the such as *R. vesca* containing mainly glucan component exhibits an increase from 30 to 57 % (see Table 1).

The sorption capacity of CGC isolated with respect to lead and copper ions is characterized by maximal values of 107 and 123 $\mu\text{mol/g}$, respectively (Fig. 3). For the species *R. vesca* and *R. consobrina* the sorption capacity of CGC exhibits the greatest value in the buffer zone both with respect to copper, and with respect to lead, whereas for the species such as *R. integra* no certain regularity is observed.

The sorption capacity of CGC for the mushroom *R. vesca* with respect to copper (II) ions in going from the background zone to the buffer one increased from 20 to 65 $\mu\text{mol/g}$, whereas in passing to the impact zone one can observe a decrease in this value down to 40 $\mu\text{mol/g}$. A similar tendency has been revealed for *R. consobrina*. For the CGC of the mushroom *R. integra*

the sorption capacity in going from the background zone to the buffer one has decreased from 62 to 35 $\mu\text{mol/g}$, whereas in going to the impact zone this value has increased up to 80 $\mu\text{mol/g}$.

The character of changing the sorption capacity of CGC with respect to lead (II) ions in the case of going from the background zone to the buffer one and further to the impact zone for the mushrooms such as *R. vesca* and *R. consobrina* was similar reaching the maximal values of 107 and 43 $\mu\text{mol/g}$, respectively. For the CGC of the mushroom such as *R. integra* the sorption capacity decreases from 80 to 37 $\mu\text{mol/g}$. There are data presented in the literature concerning the CGC sorption capacity for the fungus *Agaricus macrosporus* (belonging to the order *Agaricales* as well as the genus *Russula*) [6]. The maximal extraction level for lead and copper obtained by means of a dead mycellium at pH 4.5 amounts to 124 and 303 $\mu\text{mol/g}$, respectively, i.e. the sorption level with respect to lead is of the same order of magnitude, whereas copper is sorbed to a less extent.

Within the series of nickel (II), cobalt (II), copper (II) and zinc (II) ions the selectivity reaches 100 %: the sorption is observed only for copper (II) ions. The chitin-glucan complex isolated from *A. niger* can sorb other ions, too.

The regularities observed indicate that the natural selection in a habitat contaminated with heavy metal ions, to all appearance, promotes the formation of fungi populations with thicker cell walls (with a higher content of CGC) those exhibit a better sorption of toxic metals. Biological mechanisms wherewith fungi can survive in the presence of heavy metal ions were described in the literature [15, 16]. These mechanisms include the biosorption by cell walls, pigments or extracellular polysaccharides owing to the complex formation, which biosorption results in the decrease of the permeability; in addition the mechanisms include endocellular metal accumulation and distribution with the subsequent metal transformation *via* oxidation, reduction or precipitation. The adaptive mechanism consists in the fact that the response to the increase in the concentration of metal ions the fungi *Aspergillus* sp. is presented by a fast growth with the subsequent stage of dying off. It follows herefrom that an efficient manifestation of the protective biological

TABLE 2

Calculated sorption capacity for chitin-glucan complexes with respect to heavy metals

Fungal species	Zone	Sorption capacity, $\mu\text{mol}/100\text{ g}$ of row biomass	
		Cu^{2+}	Pb^{2+}
<i>R. vesca</i>	Background	2.8	0.8
	Buffer	24	22.5
	Impact	16	15.2
<i>R. consorbina</i>	Background	2.1	1.3
	Buffer	76	27
	Impact	18	31
<i>R. integra</i>	Background	16	21
	Buffer	25	44
	Impact	88	41

mechanism is provided by an increase in the amount of biomass, including the mass fraction of the cell wall. It is not inconceivable that in the presence of increased heavy metal concentrations in soil the adaptive mechanisms are started those are also connected with the change in the character of fungal cell wall synthesis.

Table 2 demonstrates the results of calculation for the sorption of copper (II) and lead (II) ions by chitin-glucan complexes in the fruit bodies of mushrooms belonging to *Russula* genus performed basing on the content of CGC in the raw biomass and the adsorption of ions by the CGC isolated. One can see that all the three species exhibit the potentiality of retaining heavy metals due to cell wall CGC, and this ability is more pronounced for contaminated zones than for the background territory. Thus, there are some bases for considering the CGC of cell walls in mushrooms of *Russula* genus to participate in the adaptation to heavy metal impact.

Alongside with the bioaccumulation of copper and lead ions by mushrooms which results in the prevention of heavy metal penetration into the endocellular space due to the barrier properties of the chitin-glucan part of a cell wall, the growth of a mushroom ceases at a certain value of ions concentration [17], to all appearance, owing to the inhibition of chitin-synthetase just within the cell wall itself [1]. It turns out that there are two mutually exclusive processes proceeding simultaneously such as producing a great amount of the biomass

and the inhibition of the former. The apparent contradiction disappears when it is assumed that there is an additional adaptive mechanism which provides removing toxic metal ions from the cells via new biochemical reactions. Indeed, the fact that fungi grow on the mineral of pyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$) was described in the literature, resulting in the pyromorphite to be processed into lead oxalate [18]. This fact indicates testifies the possibility of an adaptive biochemical mechanism occurring to provide the removal of a toxic metal from the cells of a fungus.

Though the amount CGC contained in the mushrooms under investigation is insignificant, the way to isolate them (i.e. the method for determining their amount) is simple, it does not require for employing any special equipment, as compared to the case of determining trace amounts of heavy metals. An increase in CHT or CGC content as compared to the normal level could indicate that the zone where the mushrooms were gathered is contaminated with such metals. This fact allows one to use the aforementioned criterion as a biological indicator of the environmental pollution.

CONCLUSION

1. As judged by the structure and composition, the CGC isolated from fruit bodies of mushrooms belonging to the genus *Russula* are similar to those of the mold fungus *A. niger*.

2. For the fruit bodies of mushrooms belonging to *Russula* genus growing within the zones contaminated by heavy metals a tendency towards increasing the content CGC and CHT is observed. Thus, changing the cell wall structure represents one of the adaptation mechanisms inherent in mushrooms of the genus *Russula* related to man-caused contamination with heavy metals.

3. The greatest sorption capacity is exhibited by CGC of these mushrooms with respect to the main metal pollutants such as copper and lead. The ability for binding heavy metals at the expense of cell walls CGC is observed to increase mainly due to an overall increase in the content of CGC in the biomass of fruit bodies within contaminated zones as compared to non-contaminated ones.

4. A possible practical application of the results obtained from the studies performed could be represented by the use of widespread fungi with the limited food-and-taste value belonging to *Russula* genus (mushrooms such as russules) – in order to obtain nature-adapted highly selective sorption materials.

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