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Short Communication

Uptake of free and complexed silver ions by yeasts isolated from a gold mining industry in Brazil

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Damaging effects caused by toxic concentrations of metals in fungi can be avoided by several mechanisms such as accumulation of the metal by cell wall components and extracellular materials, chelation, or precipitation by secreted metabolites, and by intracellular compartmentalization or complexing by low molecular weight proteins (Gadd, 1990). Microbial cells usually have two distinct phases of metal uptake. The first is a metabolism-independent process (biosorption), which involves metal adsorption around the cell envelope (Volesky, 1990). The second is exclusively dependent on cell metabolism and involves active translocation of metals into the cell (bioaccumulation) (Blackwell et al., 1995). However, scant information is available about the toxicity and uptake of metal complexes. Under some conditions the concentration of free metal ions can be reduced in natural or polluted environments by complexing agents. This has been demonstrated for wastewaters containing cyanide that forms metal cyanide complexes [eg. $Ag(CN)_2^-$, $Cu(CN)_4^-$ and $Fe(CN)_6^-$]. In the present investigation, we studied the occurrence of yeasts during a gold extraction process by cyanidation and the resistance of the isolates to silver dicyanide [$Ag(CN)_2^-$], a metal cyanide complex usually present during the gold ore process. The $Ag(CN)_2^-$-resistant yeast strains were evaluated for the ability to accumulate free and complexed silver ions by metabolism-dependent and -independent processes.

Fifty-six yeast strains belonging to 21 species were isolated from different steps of the gold extraction process by cyanidation in a gold mining plant (Table 1). The yeasts were isolated by the enrichment technique and characterized by the standard methods of Van der Walt and Yarrow (1984). Identifications were done according to the keys of Barnett et al. (1990) and Kurtzman and Fell (1998). Yeasts were isolated from all collection sites, including those presenting high cyanide concentrations. The $Ag(CN)_2^-$ complex was toxic for 89% of the total yeasts isolated (data not shown). Six yeast isolates were clearly resistant to $Ag(CN)_2^-$, showing ability to grow in the presence of this compound. Viable cells of resistant strains were increased at least 40 times after three days of incubation in the presence of $Ag(CN)_2^-$ (Table 2). The minimum inhibitory concentrations (MIC) of $Ag(CN)_2^-$ for the resistant yeasts were 4.5 to 5 mg L^{-1}, with the exception of *Rhodotorula mucilaginosa* (UFMG-Y27), which presented the highest MIC, almost 50 mg L^{-1} (Fig. 1a, b). The MIC of $Ag(CN)_2^-$ were determined in Yeast Carbon Base (YCB; Difco, Detroit, MI, USA) supplemented with 0.1% $(NH_4)_2SO_4$ adjusted to pH 6.5, in the presence of 5 to 50 mg L^{-1} of $Ag(CN)_2^-$. The MIC was defined as the lowest concentration of inhibitor, above which no growth was observed.

The experiments for silver [Ag^+ or $Ag(CN)_2^-$] uptake by metabolism-dependent and -independent processes were done as described by Avery and Tobin (1992), using different periods of metal and microorganism contact. The medium used for yeast

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growth was YCB supplemented with 0.1% $(NH_4)_2SO_4$. Metal analysis was performed by using an atomic absorption spectrophotometer with an air-acetylene flame (Varian model AA-475) by reference to appropriate standard metal solutions according to APHA (1992). Batch culture studies in the presence of $Ag(CN)_2^-$ showed that the highest resistant yeast strain (*R. mucilaginosa* UFMG-Y27) also presented higher $Ag(CN)_2^-$ uptake levels than the other yeasts tested (Table 2). None of the yeast strains tested presented the capacity for $Ag(CN)_2^-$ uptake when meta-

Table 1. Frequency of yeast species isolated from the Mineração Morro Velho (Nova Lima, MG) mining site.

Species	Site 1[a]	Site 2	Site 3	Site 4	Site 5
Aureobasidium pullulans	1	2	—	—	1
Candida famata	1	1	4	—	—
Candida boidinii	—	—	—	—	1
Candida citrea	—	1	—	—	—
Candida dattila like[b]	2	—	—	—	—
Candida guilliermondii	—	2	—	—	—
Candida intermedia like	—	—	—	—	1
Candida kluyveri	1	—	—	—	—
Candida krusei	1	—	—	—	—
Candida pseudointermedia	—	1	—	—	—
Candida vinaria like	1	—	—	—	—
Candida sp.	—	6	1	—	—
Cryptococcus flavus	—	—	—	—	1
Cryptococcus sp.	—	1	—	—	1
Debaryomyces hansenii	—	—	2	—	—
Geotrichum sp.	—	3	1	—	—
Hanseniaspora guilliermondii	—	2	—	—	—
Hanseniaspora sp.	—	1	—	3	—
Pichia kluyveri	—	—	—	—	1
Rhodotorula glutinis	—	—	—	3	4
Rhodotorula mucilaginosa	—	3	1	—	—
Tremella sp.	—	—	—	1	—
Total	7	23	9	7	10

[a] Site 1: Ore washing tank, pH 7.3. Site 2: Thickener 1, the tank that receives the cyanide-containing solution after contact with gold ore, pH 10.0, 350 mg/L cyanide. Site 3: Clarification feeding tank, pH 10.5, 150 mg/L cyanide. This tank is used to prepare the solution for transfer to the precipitation tank. Site 4: Tank receiving the solution after gold removal by electroplating after the addition of zinc powder, pH 10, 100 to 150 mg/L cyanide. Site 5: Basin of wastewater contention, where the "sterile" solution obtained at the end of the gold removal process is kept for natural degradation.

[b] Species similar in characteristics to species indicated.

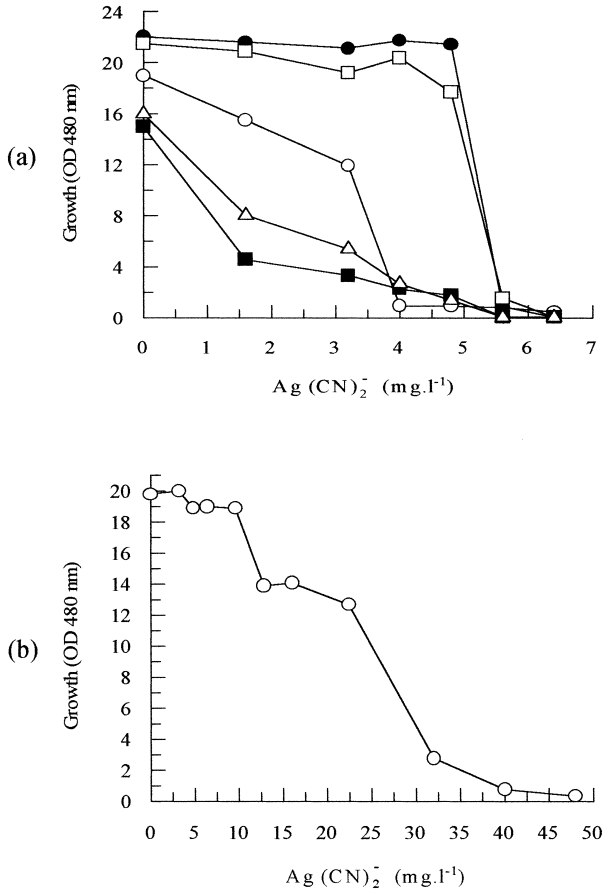

Fig. 1. Minimum inhibitory concentrations (MIC) of $Ag(CN)_2^-$ for *Tremella* sp. UFMG-Y07 (○), *Candida guilliermondii* UFMG-Y22 (●), *Candida guilliermondii* UFMG-Y23 (□), *Aureobasidium pullulans* UFMG-Y28 (■), *Geotrichum* sp. UFMG-Y33 (△)—(a), and *Rhodotorula mucilaginosa* UFMG-Y27 (○)—(b).

Table 2. Cell viability of $Ag(CN)_2^-$-resistant yeast strains after 3 days of incubation in Yeast Carbon Base (YCB) medium supplemented with $Ag(CN)_2^-$ (5 mg L^{-1}) and $(NH_4)_2SO_4$ (0.1%). The maximum uptake of free and complexed silver ions by live yeast biomass is also shown.

Strain	Initial viability (cfu ml^{-1})×10^5	Final viability (cfu ml^{-1})×10^7	Silver uptake[a] (mg g^{-1})	
			Ag^+	$Ag(CN)_2^-$
Tremella sp. UFMG-Y07	0.5	1.2	38.2	0.5
Candida guilliermondii UFMG-Y22	7.4	21.4	36.6	0.8
Candida guilliermondii UFMG-Y23	5.1	49.5	46.0	0.8
Rhodotorula mucilaginosa UFMG-Y27	1.3	9.6	19.3	1.8
Aureobasidium pullulans UFMG-Y28	1.1	0.4	18.3	1.4
Geotrichum sp. UFMG-Y33	0.2	2.0	35.7	1.3

[a] The biomass concentration was 1 mg ml^{-1} (dry weight), and the initial concentration of silver as Ag^+ and $Ag(CN)_2^-$ was 20 mg L^{-1}.

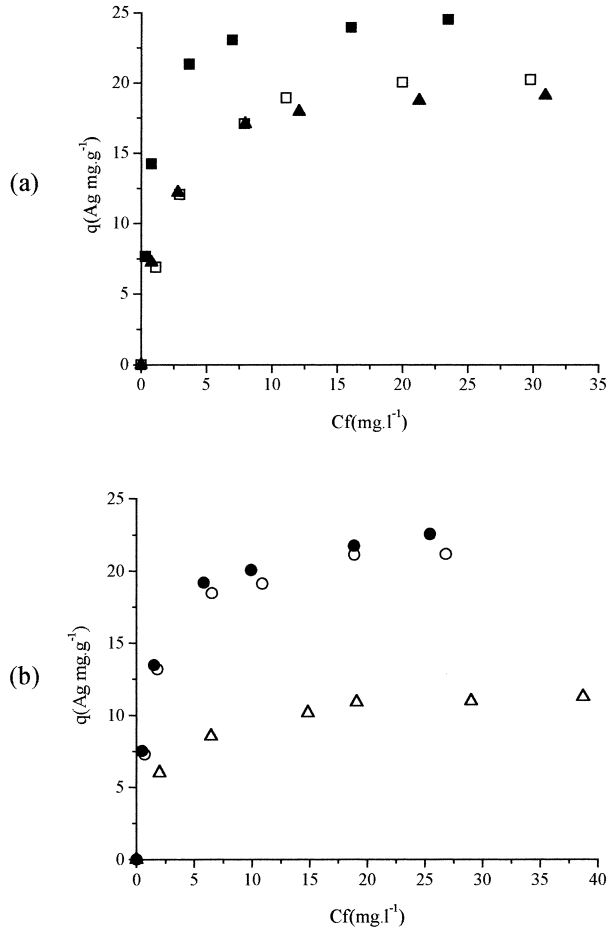

Fig. 2. Representative adsorption isotherms of free silver ions (Ag^+) by *Tremella* sp. UFMG-Y07 (■), *Candida guilliermondii* UFMG-Y22 (□), *Candida guilliermondii* UFMG-Y23 (▲)—(a), *Rhodotorula mucilaginosa* UFMG-Y27 (○), *Aureobasidium pullulans* UFMG-Y28 (△), and *Geotrichum* sp. UFMG-Y33 (●)—(b): 3 h incubation time over a range of initial Ag^+ concentrations of 1 to 50 mg L^{-1}.

bolically inactive (data not shown). The ability of living yeast to accumulate silver as a metal cyanide complex better than nonliving yeast is in agreement with results from our earlier studies (Gomes and Linardi, 1996). The accumulation of silver dicyanide anions by living yeast cells may be explained by the ability of living fungi to exhibit different mechanisms of resistance that can enable them to accumulate free or complexed metals.

Live and nonlive biomass from resistant yeasts were tested for the ability to accumulate free silver ions (Ag^+), and both presented the ability of Ag^+ uptake (Fig. 2 and Table 2). With the exception of *R. mucilaginosa* (UFMG-Y27), more Ag^+ was accumulated by living cells than by nonliving cells. This was expected since living cells can present all types of nonmetabolic interactions with metals as well as other interactions that would require active metabolism (Gadd and White, 1993). The *Candida guilliermondii* (UFMG-Y23) strain presented the highest capacity for Ag^+ uptake during metabolism-dependent experiments [46 mg g (dry wt)$^{-1}$]. During metabolism-independent experiments, the sorption isotherms for the yeast biomass types established the maximum uptake values and the affinity of the reactive sites on the cell surface for silver. It can be seen that *Tremella* sp. (UFMG-Y07) was the best sorbent for free silver ions. Not only was the maximum uptake highest for *Tremella* sp. (25 mg g^{-1}), but this strain also presented the highest affinity for silver (*b*) (Fig. 2 and Table 3). Isotherms, which are steep from the origin at low residual metal concentrations, are highly desirable because they indicate high affinity of the sorbents for the metals (Volesky, 1990).

Table 3. Biosorption parameters for silver uptake resulting from equilibrium uptake studies of six yeast strains.

Strain	q_{max}	*b*
Tremella sp. UFMG-Y07	25.1±0.29	1.5±0.09
Candida guilliermondii UFMG-Y22	22.4±0.31	0.4±0.02
Candida guilliermondii UFMG-Y23	20.1±0.32	0.6±0.05
Rhodotorula mucilaginosa UFMG-Y27	22.2±0.32	0.7±0.05
Aureobasidium pullulans UFMG-Y28	11.7±0.18	0.4±0.04
Geotrichum sp. UFMG-Y33	22.6±0.32	0.9±0.17

q_{max}, the maximum metal accumulated given in mg metal g^{-1}.
b, Langmuir constant related to adsorption energy.

Table 3 shows the values for *b* and q_{max} for the different biomass types obtained by fitting the experimental data with the Langmuir model (Volesky, 1990). The sequence of the calculated maxima uptakes (q_{max}) observed was UFMG-Y07>22, 27, 33>23>28, whereby the sequence of relative affinity given by the values of *b*, was UFMG-Y07>33>27>23>22, 28. The Langmuir parameter *b*, the ratio of the adsorption rate constant to the desorption rate constant, is an indication of the "apparent affinity" of the biosorbent toward the metal. Thus the higher the value of *b*, the higher the affinity of the biomass toward the metal. By comparing the sequence of affinity to the maximum uptake, it was observed as an inversion between the yeasts UFMG-Y22 and 23, and though the values of q_{max} were 22.4 and 20.1 mg g^{-1}, respectively, their relative affinities were 0.40 and 0.63, respectively. This can be explained because the total number of sites in both strains may be about the same, but the composition of these sites is different, even though both are from the same species. The maximum accumulation of free silver ions by *R. mucilaginosa* (UFMG-Y27) was almost the same for both active and inactive cells (Table 2 and Fig. 2). This is an indication that the main mechanism of silver ion uptake by *R. mucilaginosa* (UFMG-Y27) is basically a metabolism-independent

process.

The results described here indicate that the mechanisms of free and complexed silver uptake differ considerably. Silver uptake as $Ag(CN)_2^-$ by living cells was much lower than the uptake of free silver ions, with the ligand cyanide causing approximately 90 to 98% inhibition of silver uptake by living yeast cells (Table 2) in a strictly metabolism-dependent process. However, a high interaction of free silver ions with the yeast cell surface (biosorption) was observed, with accumulation occurring in both living and nonliving yeast cells.

The studies on silver dicyanide toxicity and uptake were of special relevance in the present investigations. Solutions originating from industrial activities commonly have various organic and inorganic metal ligands forming metal complexes of difficult removal by conventional physicochemical processes. As demonstrated in the present study, the $Ag(CN)_2^-$ complex is toxic for almost all yeast strains isolated, and only living cells presented the ability to take up silver as $Ag(CN)_2^-$. Therefore living resistant microorganisms should be considered for metal cyanide complex removal from industrial effluents. Because of the capacity for silver uptake and high resistance against $Ag(CN)_2^-$ of *R. mucilaginosa* (UFMG-Y27), studies of its application in bioprocesses for silver-bearing effluent treatment should be carried out.

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