(^{1}PROIMI – CONICET, Av. Belgrano y Pje. Caseros, 4000, Tucumán, Argentina; ^{2}Microbiología General, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina; ^{3}Microbiología Superior, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina)

Copper tolerant yeasts isolated from polluted area of Argentina

Liliana B. Villegas[1], María J. Amoroso[1, 2] and Lucía I. C. de Figueroa [1, 3, *]

Eleven yeasts were isolated from wastewater sediment samples collected from a copper filter mine plant, located in the province of Tucumán, Argentina, and tested for their heavy metal tolerance. Two isolates were selected based on their multiple tolerance to different heavy metals and their copper biosorption capacity was studied. Analysis of the 26S rDNA D1/D2 domain sequences indicates that isolate RCL-3 showed similarity with *Candida* sp. and RCL-11 with *Rhodotorula mucilaginosa*. Growth performance and copper toxicity of both yeasts were evaluated using YNB-glucose medium supplemented with 0.1, 0.2, 0.5 and 1 mM of Cu^{2+} solutions. *Candida* sp. RCL-3 was able to grow up to 7 mg ml^{-1} biomass in the presence of either 0.1 or 0.2 mM Cu^{2+}, and at 0.5 mM Cu^{2+} growth reached 5.5 mg ml^{-1}. *R. mucilaginosa* RCL-11 reached 8 mg ml^{-1} in the presence of 0.1 mM Cu^{2+}, and values of 6.5 and 5.5 mg ml^{-1} biomass were obtained at 0.2 and 0.5 mM Cu^{2+}, respectively. Copper accumulation profiles were different: the metal was librated from the intact cells by *Candida* sp. whereas *R. mucilaginosa* did not show release from the cells indicating intracellular storage. Specific biosorption of copper by both isolated yeasts showed increase with the initial copper supplied with the medium (up to 11.5 and 8.0 mg Cu g^{-1} biomass for *Candida* sp. and *R. mucilaginosa*, respectively). However, specific biosorption decreased with time.

Industrialization in many regions has increased the discharge of wastes into natural aquatic reservoirs or into soils, which is also true for waste waters containing heavy metals. The main sources for heavy metal contamination are mining activities and industrial wastewaters, discharging a variety of toxic metals such as Cd, Cu, Ni, Co, Zn and Pb into the environment (Soares *et al.* 2003, Malik 2004).

Cu (II) is known to be one of the most widespread heavy metal contaminants in the environment, and it is very toxic to living organisms (Dönmez and Aksu 2001). Traditional technologies for copper removal such as chemical precipitation, ion exchange or reverse osmosis processes can be very expensive and may have several disadvantages, such as unpredictable metal ion removal, high reagent requirements and the generation of toxic sludge, which are often difficult to dewater and require extreme caution when disposing of them (Silóniz *et al.* 2002a). New techniques, like biosorption, are required to reduce heavy metal concentrations to acceptable environmental levels at low costs.

Microorganisms may be used to remediate wastewaters or soils contaminated with heavy metals. The metal processing capacity of microorganisms can be used to concentrate, remove and recover metals from aqueous streams and enhance the efficiency of wastewater treatment processes (Amoroso *et al.* 1998). The types of microbial biomass of interest can be pragmatically those that can be easily obtained in large quantities. One of the most ubiquitous biomass types utilized on a large scale by man is yeasts. Earlier observations noting

* Corresponding author: Dr. L. I. C. de Figueroa;
 e-mail: proimiunt@arnet.com.ar or lfiguero@proimi.org.ar

the capacity of yeast strains to sequester heavy metals have been largely scattered and inconsistent, focusing mainly on the nutritional requirements or toxicology of the yeast cell (Volesky and May-Phillips 1995). However Abe *et al.* (2001) isolated a highly copper-tolerant yeast from the sediment of the Japan Trench; Silóniz *et al.* (2002b) described the ability of a yeast, isolated from sewage sludge, to take up copper in response to increasing concentrations of this metal in the culture medium. Moreover Balsalobre *et al.* (2003) indicated that both the tolerance to metals and the capacity of the uptake are dependent on ionic metal and yeast species. Yeasts can contribute to the understanding of metal uptake and toxicity, and how metal resistance may be achieved in eukaryotic cells. In a biotechnological context, yeasts may be useful in the metal-containing effluents treatment (Blackwell *et al.* 1995). Metal accumulation bioprocesses generally fall into one of two categories, biosorptive uptake by non-living or non-growing biomass and bioaccumulation by living cells (Akzu and Dönmez 2001).

The aim of this work was to describe procedures for isolating and selecting copper tolerant and/or resistant yeasts from polluted areas, and to investigate the ability of those isolates to remove cooper from synthetic culture medium.

Materials and methods

Sampling and isolation of yeast strain: Sediment samples were collected from wastewater sediment of a copper filter mine plant located in an agriculture area of Tucumán, Argentina (Fig. 1). Samples of $15-25$ g were obtained and transported in sterile plastic bags and kept at 4 °C until use.

Samples were resuspended in sterile enrichment solution ($1:10$ w/v) containing (in g l^{-1}): glucose, 1; yeast extract, 0.5; supplemented with 0.1 of the following antibiotics: tetracycline, erythromycin and chloramphenicol (Villegas *et al.* 2004). After shaking (200 rpm) $24-72$ h at 30 °C, suspensions were poured into Petri dishes with YM agar medium containing (in g l^{-1}): malt extract, 3; yeast extract, 3; peptone, 5; glucose, 10 and agar, 20; also supplemented with antibiotics. Inoculated plates were incubated at 30 °C and after approximately $3-5$ days, well isolated colonies were transferred onto the same medium until growth was observed.

Pure cultures were maintained on YEPD agar slants containing (in g l^{-1}): glucose, 20; yeast extract, 10; peptone, 10 and agar, 20. They were incubated at 30 °C for 24 h, subsequently stored at 4 °C and sub-cultured at regular intervals.

Qualitative screening for heavy metal tolerance in solid medium: Heavy metal tolerance of the isolates was tested using the method of agar diffusion previously described for actinomycetes (Amoroso *et al.* 1998). The method was modified to the screening yeasts using YNB-glucose-agar medium containing (in g l^{-1}): yeast nitrogen base (YNB), 6.7; glucose, 20 and agar, 15. A ditch in the centre of the plate was made with a sterile spatula. The ditch was filled with 500 µl of the heavy metal solution, while fresh cell suspensions of the isolated yeast prepared in distilled water were inoculated in lines extending from the ditch at a right angle no longer than 0.5 cm wide. Experiments were performed with the following heavy metals separately: Cu^{2+} (as $CuSO_4$), Ni^{2+} (as $NiSO_4$), Cd^{2+} (as $CdCl_2$) and Cr^{6+} (as $K_2Cr_2O_7$). Solutions tested with these salts were prepared in the range from 1 to 10 mM (final concentration). As a control, distilled water was filled into the ditch. *Saccharomyces cerevisiae* ATCC 32051 was used as reference strain.

Characterization of isolates: In order to determine whether the selected isolated yeasts (RCL-3 and RCL-11) belong to a basidiomycetous or an ascomycetous genus, Diazonium Blue B staining (DBB) was employed. Yeasts with a cell wall of typical basidiomycetes give a dark-red colour reaction when a buffered solution of DBB is applied to the cultures. Ascomycetous yeasts do not give this reaction. The mechanism of the reaction has not yet been elucidated. Nevertheless it was speculated that the colour formation involves tryptophane degradation intermediates and ketones associated with the cell wall (Yarrow 1998).

The molecular characterization of isolates RCL-3 and RCL-11 were carried out by sequencing the 26S rDNA D1/D2 domain using primers NL1 and NL4 for amplification (López *et al.* 2004). DNA sequencing on both strands of each isolate was performed by the dideoxy chain termination method

with an ABI Prism 3730 DNA Analyser, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reactions kit (PE Biosystems) according to the manufacturer's protocol. The sequences were registered in the GenBank Data Library under accession numbers **AY743221** (RCL-3) and **AY437842** (RCL-11) for the 26S rDNA D1/D2 domain.

Analysis of copper tolerance: Sensitivity towards copper was tested by agar diffusion assays using disks of approximately 6 mm diameter in PETRI dishes containing 20 ml of YNB-glucose-agar previously inoculated with 100 µl of an overnight culture. Disks were saturated with 50 µl of Cu^{2+} (as $CuSO_4$) solutions in the range from 10 to 20 mM (final concentration). The diameter of the growth inhibition was measured after incubation at 30 °C during 3–4 days. Water-saturated disks were used as a control. An inhibition zone larger than 10 mm diameter was arbitrarily considered as a metal sensitive response (that means that the lawn yeast was sensitive), whereas zones of inhibition of tolerant yeasts were smaller than 10 mm.

Quantitative copper tolerance analysis was tested in 250 ml ERLENMEYER flasks containing 100 ml YNB-glucose medium supplemented with 0.1, 0.2, 0.5 and 1 mM Cu^{2+}. Flasks were inoculated to a final concentration of 10^7 cells ml^{-1} with an active overnight pre-inoculum and incubated at 30 °C on a rotary shaker at 250 rpm during 80 h. The pH was buffered at 5.0 with 50 mM Tris-succinate (ROSS and PARKIN 1989). As a control, one flask inoculated and incubated under the same conditions but without copper solution was used. Samples of 5 ml were withdrawn periodically. Growth was monitored by optical density measurements at 620 nm. Biomass dry weight was determined by drying the washed cells at 105 °C to constant weight.

Copper concentrations were measured by atomic absorption spectrophotometry. Supernatant samples were diluted and analyzed directly. For determining intracellular metal concentration, cells were harvested by centrifugation and washed twice with distilled water. After that, 100 µl of concentrated nitric acid was added to the cell pellet and the mixture was boiled until the cells were completely dissolved (ABE *et al*. 2001). Samples volume was adjusted to 5 ml with distilled water. Copper determination from soil samples was performed using the nitric acid digestion as described above.

The results obtained in the present work were expressed as mean value of at least triplicate determinations of independent cultures. The statistical significance of differences among values was assessed by using the STUDENT's *t*-test and ANOVA.

Results

Isolation of heavy metal tolerant yeast strains

Eleven yeasts were isolated from wastewater sediment samples collected from a copper filter mine plant, located in the province of Tucumán, Argentina (Fig. 1). This polluted area allowed to isolate indigenous yeast strains able to live in these unfavourable environments and to evaluate their copper uptake capacity in relationship to their growth measured by biomass increase. Concentrations of Cu in the wastewater sediment samples were found to be in the range of 0.52 to 75.3 mg g^{-1}.

The 11 isolated yeasts were first studied, along with the reference strain *S. cerevisiae*, for evaluating the capacity to tolerate a variety of heavy metals by using the qualitative method of agar diffusion. Yeast growth was assumed as a qualitative parameter of heavy metal tolerance. Growth up to the ditch was considered tolerant to the concentration supplied, while growth inhibition was considered sensitive. The size of growth inhibition extending from the ditch gave an approximate idea about the level of tolerance in cases where no complete tolerance was observed. Qualitative analysis of growth in media containing 1, 5 and 10 mM of Cu^{2+} (as $CuSO_4$), Ni^{2+} (as $NiSO_4$), Cd^{2+} (as $CdCl_2$) and Cr^{6+} (as $K_2Cr_2O_7$), was systematized using 4 categories: from no growth (non-heavy-metal tolerant yeast) to abundant growth (highly heavy-metal tolerant yeast). The isolated yeasts have been designed "RCL" followed by a serial number. This assay was carried out in order to evaluate whether the isolates were capable to tolerate either one or more of the heavy metals studied (Table 1).
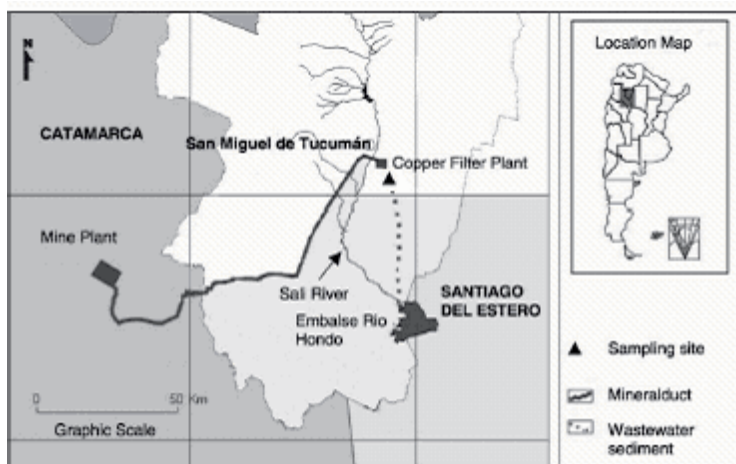
Fig. 1
Map showing location of study site in Tucumán, Argentina. Reproduced from BENIMELI *et al.* (2003) with permission

Two isolated yeasts out of the 11, RCL-3 and RCL-11, were selected for semiquantitative and quantitative tolerance to Cu^{2+}, based on their multiple tolerances to the metals tested. From Table 1 it can be observed that these two yeasts showed abundant growth in presence of Cu^{2+} up to 10 mM (Fig. 2). Coincidentally, RCL-3 presented abundant growth in the presence of all the concentrations tested of Cr^{6+} (as $K_2Cr_2O_7$), and RCL-11 displayed abundant growth in the presence of all the concentrations tested of Ni^{2+} (as $NiSO_4$) and Cd^{2+} (as $CdCl_2$), whereas the reference strain *S. cerevisiae*, together with the other ones, showed some inhibition at the highest metal concentrations tested.

Table 1
Qualitative screening of yeast isolates for heavy metal tolerance

Isolated yeasts	Heavy metal concentrations (mM)											
	Cu^{2+}			Ni^{2+}			Cr^{6+}			Cd^{2+}		
	1	5	10	1	5	10	1	5	10	1	5	10
RCL-1	+++	+++	++	+++	++	+	++	++	+	++	++	+
RCL-2	+++	++	++	+++	++	+	++	+	–	++	++	+
RCL-3	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	++	++
RCL-4	+++	++	++	+++	++	+	++	++	+	+++	++	+
RCL-5	+++	++	++	+++	++	++	++	++	++	++	+	–
RCL-6	+++	++	++	+++	++	+	++	+	–	+++	++	++
RCL-7	+++	+++	++	+++	++	+	+++	++	+	+++	++	++
RCL-8	+++	+++	++	+++	++	++	++	+	–	++	+	–
RCL-9	+++	++	++	+++	++	+	++	+	+	++	++	+
RCL-10	+++	++	++	++	+	–	++	+	–	+++	+++	++
RCL-11	+++	+++	+++	+++	+++	+++	++	++	++	+++	+++	+++
S. cerevisiae	+++	++	–	+++	++	+	++	+	–	+++	++	++

– non growth; + limited growth; ++ moderate growth; +++ abundant growth
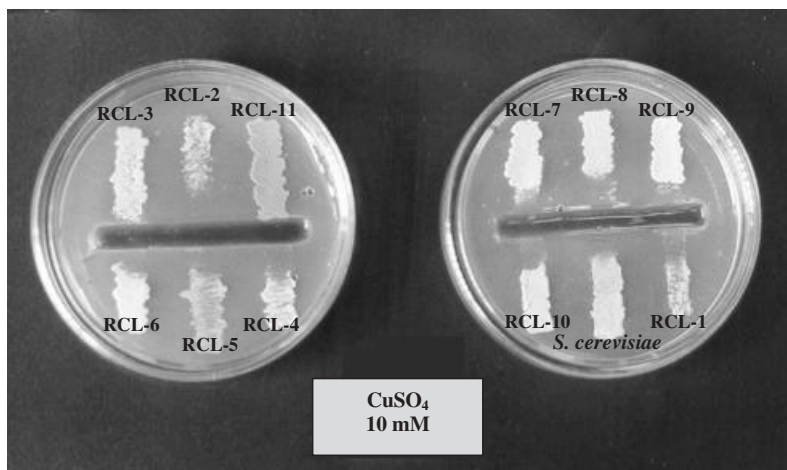
Fig. 2
Growth of isolated yeasts in the presence of 10 mM of CuSO$_4$

Identification of RCL-3 and RCL-11

The selected isolates, RCL-3 and RCL-11 were preliminary characterized. A variety of physiological and morphological characteristics distinguish the ascomycetous and basidiomycetous yeasts. The nature of the cell wall provides the initial dichotomy in classification. DBB reaction is the most practical test for establishing the basidiomycetous affinity of yeast. RCL-11 gave a dark-red colour reaction indicating that it belongs to a basidiomycetous genus, while RCL-3 did not show this reaction, meaning that it belongs to an ascomycetous genus.

The 26S rDNA D1/D2 domain sequence analysis (approximately 600 bp), revealed a highly conserved relationship at the species level, consistent with the molecular identification of different yeasts (KURTZMAN *et al*. 2001). According to the BLAST alignment, the RCL-3 D1/D2 domain showed a 98% similarity with available sequences from *Candida* sp. On the other hand, RCL-11 D1/D2 domain showed 100% similarity with available sequences from *Rhodotorula mucilaginosa*.

Copper tolerance

Growth determination for semiquantitative analyses test was useful for comparing the copper tolerance amongst the selected yeasts *Candida* sp. RCL-3, *R. mucilaginosa* RCL-11 and the reference strain *S. cerevisiae*. Increasing the metal concentration in plate diffusion experiments resulted in a marked inhibition of growth. An inhibition zone of 10 mm diameter was arbitrarily designated as a semiquantitative criterion to determine the copper tolerance of the tested yeasts. As the result, *Candida* sp. turned out to be sensitive to Cu^{2+} at 16 mM or higher; *R. mucilaginosa* at 14 mM and *S. cerevisiae* at 10 mM Cu^{2+} concentrations.

Growth performance and copper toxicity were evaluated in quantitative analyses. Fig. 3A and B show the effect of Cu^{2+} concentrations on growth of *Candida* sp. RCL-3 and *R. mucilaginosa* RCL-11. After 72 h of cultivation, *Candida* sp. was able to grow up to 7 mg ml^{-1} biomass (dry weight) in the presence of either 0.1 or 0.2 mM Cu^{2+}; however, at 0.5 mM Cu^{2+} the growth reached only 5.5 mg ml^{-1}. No growth was observed at 1.0 mM Cu^{2+} initial concentration, while growth without Cu^{2+} led to a maximum biomass value of 14.5 mg ml^{-1} (Fig. 3A). As can be observed, the growth was inhibited 52 and 62% in the presence of 0.1
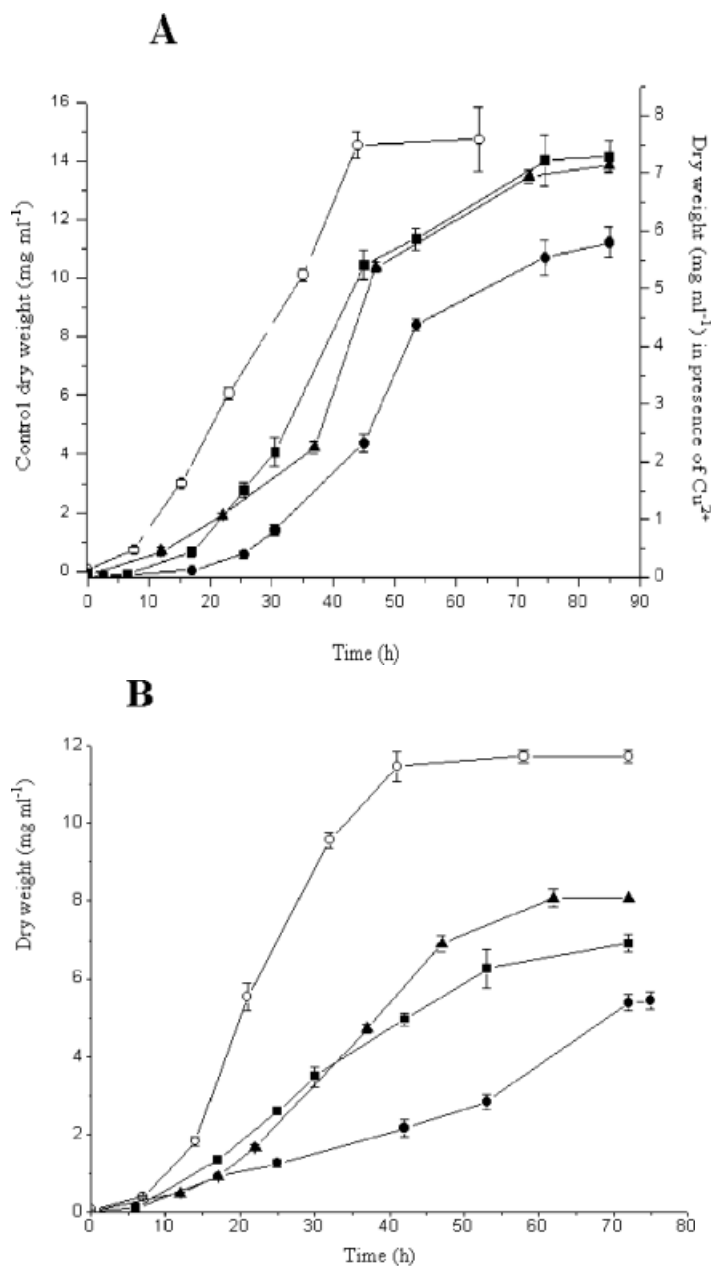
A

B

Fig. 3
Effect of Cu^{2+} concentration on the cell growth of: (**A**) *Candida* sp. RCL-3 and (**B**) *R. mucilaginosa* RCL-11. (○) control; (▲) 0.1; (■) 0.2 and (●) 0.5 mM Cu^{2+}

or 0.2 and 0.5 mM Cu^{2+}, respectively. *R. mucilaginosa* reached 8 mg ml^{-1} biomass after 72 h incubation in the presence of 0.1 mM Cu^{2+}. Values of 6.5 and 5.5 mg ml^{-1} biomass were obtained at 0.2 and 0.5 mM Cu^{2+}, respectively. No growth was observed at 1.0 mM Cu^{2+} initial concentration, and a maximum biomass value of 11 mg ml^{-1} was obtained without Cu^{2+} (Fig. 3B). In terms of growth inhibition the behaviour of *R. mucilaginosa* RCL-11 was also different to *Candida* sp. RCL-3 since *R. mucilaginosa* showed 28, 41 and 50% of biomass reduction in the presence of 0.1, 0.2 and 0.5 mM Cu^{2+}, respectively.

Biosorption kinetics of *Candida* sp. RCL-3 and *R. mucilaginosa* RCL-11 in media containing 0.1, 0.2, and 0.5 mM Cu^{2+} were tested. When both yeasts were incubated in the presence of 0.1 mM CuSO$_4$, the intracellular copper concentration increased to 0.09 and 0.06 mM for *Candida* sp. and *R. mucilaginosa* after 37 h of growth, respectively. However, the copper accumulation profiles were different: the metal was adsorbed to the outside the cells by *Candida* sp. whereas for *R. mucilaginosa* intracellular storage was seen up to the end of the experiment (Fig. 4A). Biosorption kinetics of copper removal when adding 0.2 mM CuSO$_4$ clearly indicated for both yeasts significant differences with respect to biosorption capacity. Maximum uptake of copper (0.14 mM) was achieved by *Candida* sp. after 30 h of cultivation; this value was maintained inside the cells during the first 15 h of growth, being librated later to the supernatant of the culture. However the behaviour of *R. mucilaginosa* in this assay was similar to that observed with 0.1 mM Cu^{2+} in the medium (Fig. 4B).
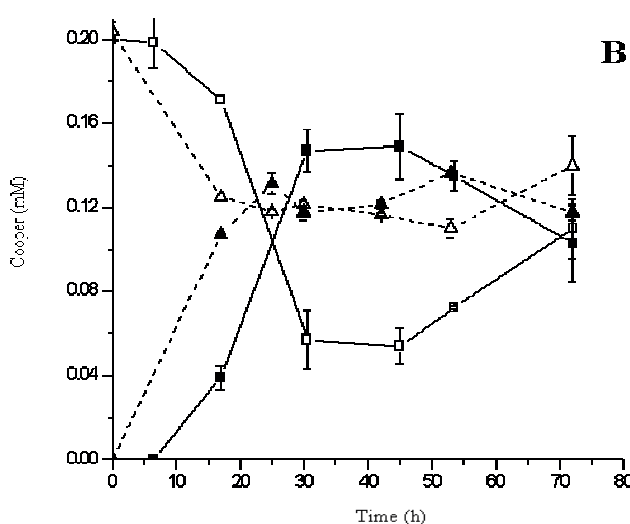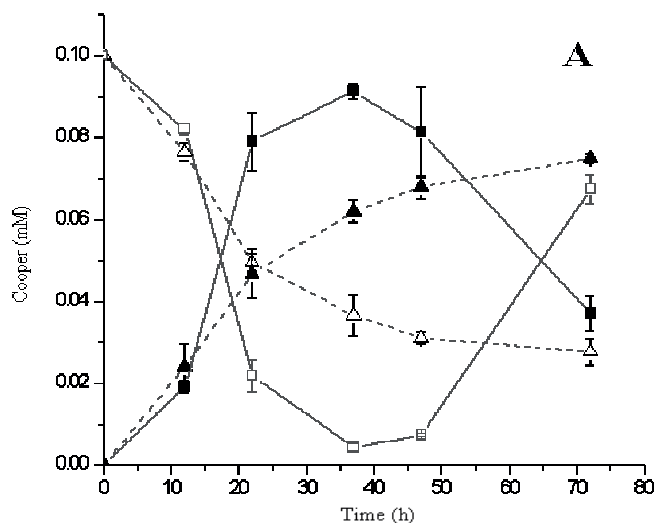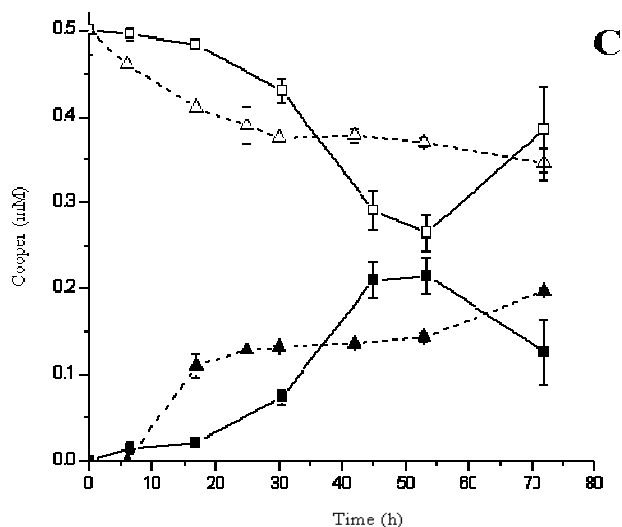
Fig. 4
Intra and extracellular copper concentration of *Candida* sp. RCL-3 and *R. mucilaginosa* RCL-11 cultures. (**A**) 0.1; (**B**) 0.2 and (**C**) 0.5 mM Cu^{2+} initial concentrations. (■) *Candida* sp. RCL-3 intracellular, (□) *Candida* sp. RCL-3 extracellular, (▲) *R. mucilaginosa* RCL-11 intracellular and (△) *R. mucilaginosa* RCL-11, extracellular copper concentration

In the experiment performed with 0.5 mM Cu^{2+}, *Candida* sp. showed comparable performance of biosorption kinetics to that observed with 0.2 mM Cu^{2+}, whereas *R. mucilaginosa* showed similar behaviour to those presented with both previous Cu^{2+} concentrations tested (Fig. 4C).

Specific biosorption analysis of copper by both yeasts showed that the biosorption, defined as metal uptake per gram of biomass, increased with the initial copper fed in the medium. After 17 h of incubation in medium containing 0.5 mM Cu^{2+}, values of 11.5 and 8.0 mg Cu g biomass^{-1} for *Candida* sp. and *R. mucilaginosa*, respectively, were obtained. However, under the experimental conditions used in the present study, lower specific biosorption values were observed with time (Fig. 5A and B).

Discussion

Isolation of copper tolerant yeasts from polluted areas was performed in order to investigate the ability of those strains to remove copper from synthetic medium. The results obtained by using the qualitative method of agar diffusion suggest that the copper tolerance of both *Candida* sp. and *R. mucilaginosa* might allow additional tolerance towards others metals. ABE *et al*. (2001) studied the tolerance of *Cryptococcus* N6 and found high tolerance only against Cu^{2+} and moderate tolerance towards others metals tested. BALSALOBRE *et al*. (2003) demonstrated that copper was less toxic than cadmium to yeasts isolated from sewage sludge.

It is important to point out that SILÓNIZ *et al*. (2002b) and BALSALOBRE *et al*. (2003) have also identified *R. mucilaginosa* strains isolated from a leachate of a uranium mineral heap as well as from municipal wastes. This yeast has a world-wide distribution in terrestrial, aquatic and marine habitats and can growth on a wide variety of substrates. As expected, the yeasts isolated from wastewater sediment of a copper filter mine plant exhibited a higher tolerance than *S. cerevisiae*. This result is similar to that obtained by SILÓNIZ *et al*. (2002b).

The ability of the yeast strains to growth in the presence of different concentrations of Cu^{2+} (Fig. 3A and B) are different to those obtained by ABE *et al*. (2001). These authors
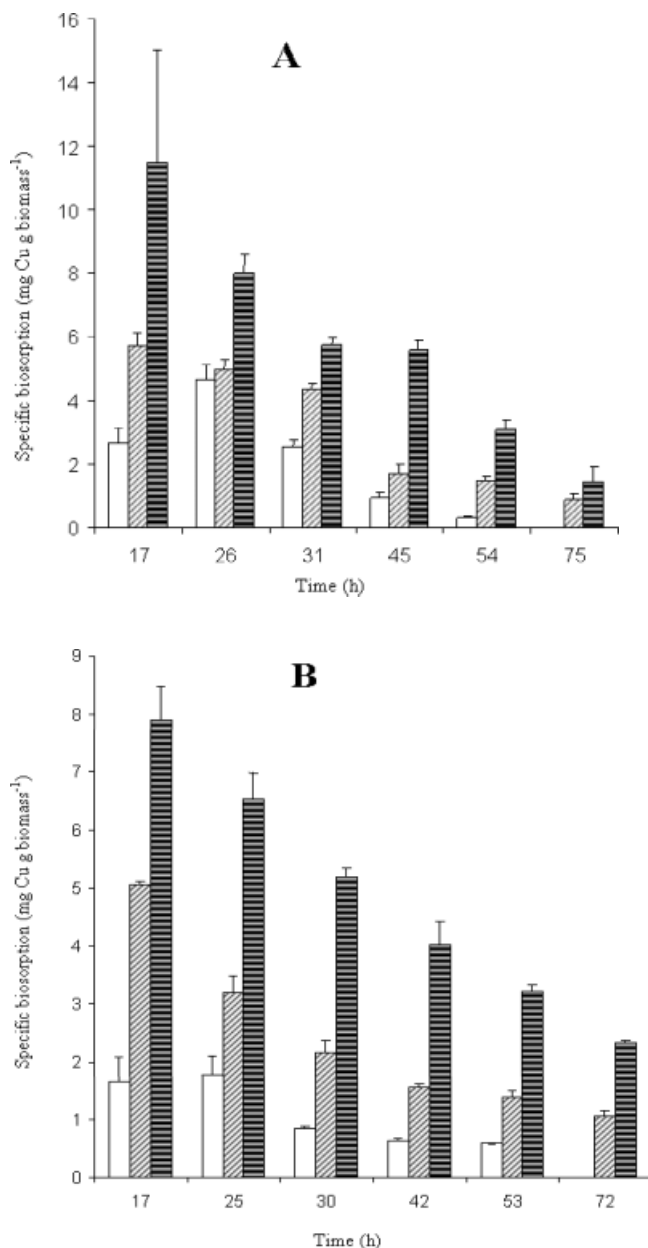
Fig. 5
Specific biosorption (mg Cu g biomass^{-1}) at different Cu^{2+} concentrations of: (**A**) *Candida* sp. RCL-3 and (**B**) *R. mucilaginosa* RCL-11.
(▯) 0.1; (▨) 0.2 and (▤) 0.5 mM Cu^{2+}

isolated highly copper-tolerant *Cryptococcus* sp. yeast, with the ability to grow in YEPD broth containing 1 and 10 mM Cu^{2+}. However, the tolerance towards high copper concentrations could be attributed to the low availability of Cu^{2+} ions because of the complex organic compounds in the supplemented medium used in the assays (VOLESKY and HOLAN 1995). In tolerance assays minimal medium would be used instead of YEPD, due to organic components of complex media absorb the metal ions. Moreover, YNB also minimize the complexation of the heavy metal ions (VILLEGAS *et al.* 2004). As expected, the growth rate of

both yeasts decreased when the concentration of Cu^{2+} were increased in the medium which is in accordance with the results obtained by SILÓNIZ *et al.* (2002a).

Earlier studies on heavy metals biosorption have shown that pH is the single most important parameter affecting the biosorption process (BRADY and DUNCAN 1994, AKSU and DÖNMEZ 2001). Furthermore, ROSS and PARKIN (1989) reported that in yeast copper uptake is markedly reduced by a low pH and they could show a clear optimum between pH 5.0 and 5.5. In the present work, pH 5.0 was established by use of a buffered medium. The pH was controlled during the experiments.

Data from Fig. 4A, B and C might be explained considering that *Candida* sp. RCL-3 belongs to an ascomycetous genus while *R. mucilaginosa* RCL-11 to a basidiomycetous one. The difference in cell wall structure, cytology, physiology, protein and enzyme contents, etc., between ascomycetous and basidiomycetous yeasts are well established (MOORE 1998). For this reason it can be assumed that the feature of *Candida* sp. RCL-3 to liberate copper to the extracellular space and of *R. mucilaginosa* to keep the metal inside the cell are related to their phylogenetic differences.

Decreased specific biosorption was observed with time indicating that both yeasts possess a high capacity to take up copper from the medium initially, which was eventually limited by Cu^{2+} concentration and culture age. SOARES *et al.* (2002) reported that the age of the culture has been shown to effect metal uptake. ABE *et al.* (2001) isolated yeasts from deep-sea sediments and studied the mechanisms of the cells for growing in the presence of different concentrations of $CuSO_4$. These authors concluded that the decrease of cell viability presumably was due to the considerable increase of copper in the cells.

In the present report, two copper resistant yeasts were isolated, characterized and described which will be useful for further investigations on the fundamental basis of their copper tolerance mechanisms and the practical applications for bioremediation in contaminated environments.

Acknowledgements

References

ABE, F., MIURA, T., NAGAHMA, T., INOUE, A., USAMI, R. and HOROKOSHI, K., 2001. Isolation a highly copper-tolerant yeast, *Cryptococcus* sp., form the Japan Trench and the induction of superoxide dismutase activity by Cu^{2+}. Biotechnol. Lett., **23**, 2027–2034.

AKSU, Z. and DÖNMEZ, G., 2001. Comparison of copper(II) biosorptive properties of live and treated *Candida* sp. J. Env. Sci. Health Part A Tox. Hazard Subst. Environ. Eng., **36**, 367–81.

AMOROSO, M. J., CASTRO, R. G., CARLINO, F. J., ROMERO, N. C., HILL, R. T. and OLIVER, G., 1998. Screening of heavy metal-tolerant actinomycetes isolated from the Salí River. J. Gen. Appl. Microbiol.*,* **44**, 129–132.

BALSALOBRE, L., DE SILÓNIZ, M. I., VALDERRAMA, M. J., BENITO, T., LARREA, M. T. and PEINADO, J. M., 2003. Occurrence of yeasts in municipal wastes and their behaviour in presence of cadmium copper and zinc. J. Basic Microbiol., **43**, 185–193.

BLACKWELL, K. J., SINGLENTON, I. and TOBIN, J. M., 1995. Metal cation uptake by yeast: a review. Appl. Microbiol. Biotechnol., **43**, 579–584.

BRADY, D. and DUNCAN, J. R., 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol., **41**, 149–154.

BENIMELI, C. S., AMOROSO, M. J., CHAILE, A. P. and CASTRO, G. R., 2003. Isolation of four aquatic streptomycetes strains capable of growth on organochlorine pesticides. Biores. Technol., **89**, 133–138.

DÖNMEZ, G. and AKSU, Z., 2001. Bioaccumulation of copper (II) and nickel (II) by the non-adapted and adapted growing *Candida* sp. Wat. Res*.*, **35**, 1425–1434.

KURTZMAN, C. P., ROBNETT, C. J. and YARROW, D., 2001. Three new species of *Candida* from apple cider: *C. anglica, C. cidri* and *C. pomicola*. Antonie van Leeuwenhoek, **80**, 237–244.

LÓPEZ, F., DELGADO, O. D., MARTÍNEZ, A., SPENCER, J. F. T. and DE FIGUEROA, L. I. C., 2004. Characterization of a new xylitol-producer *Candida tropicalis* strain. Antonie van Leeuwenhoek, **85**, 281–286.

MALIK, A., 2004. Metal bioremediation through growing cells. Environ. Int., **30**, 261–278.

MOORE, R. T., 1998. Cytology and ultrastructure of yeasts and yeast-like fungi. In: The Yeasts, a Taxonomic Study (4th edn; KURTZMANN, C. P. and FELL, J. W., Eds.), pp. 33–44. Elsevier Science BV, Amsterdam.

ROSS, I. S. and PARKIN, M. J., 1989. Uptake of copper by *Candida utilis*. Mycol. Res., **93**, 33–37.

SILÓNIZ, M., BALSOLOBRE, C., VALDERRAMA, M. and PEINADO, J., 2002a. Feasibility of copper uptake by the yeast *Pichia guilliermondii* isolated form sewage sludge. Res. Microbiol., **153**, 173–180.

SILÓNIZ, M., PAYO, E. M., CALLEJO, M. A., MARQUINA, D. and PEINADO, M. J., 2002b. Environmental adaptation factors of two yeasts isolated from the leachate of a uranium mineral heap. FEMS Microbiol. Lett., **210**, 233–237.

SOARES, E. V., DUARTE, A. P. R. S. and BOAVENTURE, R. A., 2002. Viability and release of complexing compounds during accumulation of heavy metals by a brewer's yeast. Appl. Microbiol. Biotechnol., **58**, 836–841.

SOARES, E. V., HEBBELINCK, K. and SOARES, H. M. V. M., 2003. Toxic effects caused by heavy metals in the yeast *Saccharomyces cerevisiae*: a comparative study. Can. J. Microbiol., **49**, 336–343.

VILLEGAS, L., AMOROSO, M. J. and DE FIGUEROA, L. I. C., 2004. Selection of tolerant heavy metal yeasts from different polluted sites. In: Methods in Biotechnology, Vol 16: Environmental Biology, Methods in Protocols (SPENCER, J. F. T. and SPENCER, A. L. R., Eds.), pp. 249–256. Humana Press Inc., Totowa, New Jersey.

VOLESKY, B. and HOLAN, Z. R., 1995. Biosorption of heavy metals. Biotechnol. Prog., **11**, 235–250.

VOLESKY, B. and MAY-PHILLIPS, H. A., 1995. Biosorption of heavy metals by *Saccharomyces cerevisiae.* Appl. Microbiol. Biotechnol., **42**, 797–806.

YARROW, D. 1998. Methods for the isolation, maintenance and identification of yeasts. In: The Yeasts, a Taxonomic Study (4th edn.; KURTZMANN, C. P. and FELL, J. W., Eds.), pp. 77–100. Elsevier Science BV, Amsterdam.

Mailing address. Dr. LUCÍA I. C. DE FIGUEROA, PROIMI – CONICET, Av. Belgrano y Pje. Caseros, 4000 – Tucumán – Argentina
E-mail: proimiunt@arnet.com.ar or lfiguero@proimi.org.ar