World Journal of Microbiology & Biotechnology **16**: 683–686, 2000.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Physiological and morphological characteristics of yeasts isolated from waste oil effluents

M. Cristina Romero[1,*], Mercedes E. Gatti[2], Susana Córdoba[2], M. Cecilia Cazau[1] and Angélica M. Arambarri[1]
[1]*"Instituto Spegazzini", Facultad de Ciencias Naturales y Museo, La Plata, Argentina*
[2]*Cát. Micología, Facultad de Ciencias Veterinarias, UNLP, Calle 53 no 477, 1900 La Plata, Argentina*
**Author for correspondence: calle 528 bis no 1632, 1900 La Plata, Argentina. Fax: +54-221-422-2904, E-mail: anmabarr@museo.fcnym.unlp.edu.ar*

Received in revised form 4 April 2000; accepted 16 October 2000

Keywords: *C. krusei* strains, chitin synthase activity, morphological differences, physiological types, polluted samples

Summary

Pichia membranaefaciens, *Cryptococcus laurentii*, *Rhodotorula glutinis* and *Candida krusei* were isolated from contaminated sites. A significant variability in cell forms and in assimilation profiles was observed in the *C. krusei* strains. The chitin synthase activity and chitin content allowed us to differentiate three strain types. The variability of the phenotypic traits was higher in *C. krusei* strains isolated from heavily polluted sites.

Introduction

The potential of yeast populations to adapt to widespread habitats is attributed to their lower susceptibility to pH changes, salinity increase and metabolic capacities to biotransform pollutants (Berdicevsky *et al.* 1993). Moreover, the occurrence of different colony forms, dimorphic growth, hyphal development, and other taxonomic traits are correlated with environmental conditions (Yaar *et al.* 1997). Therefore, yeasts may have an advantage to dominate in fluctuating conditions caused by oil spills or sewage discharge, as they are able to develop different physiological types (Cox *et al.* 1998).

The recent trends in mycological research point out the increasing yeast populations associated with urban and industrial runoff and the occurrence of different morphologies, correlated with stress factors (Rustchenko *et al.* 1997). Our objective was to characterize the yeasts presents in the sediments of the Rio Santiago channels, with different levels of pollution.

Materials and Methods

Samples of the surface sediments were taken from four channels that drain to the Rio Santiago, Argentina. The organic carbon concentrations were determined by CHN-analysis (Perkin-Elmer) and the total nitrogen by Macro–Kjeldahl method (APHA 1992). The total hydrocarbon concentrations were analysed by a FTIR-Perkin Elmer; ultrasonic extraction was realized with Cl_4C. A cell with BrK window, 0.35 mm thick, was employed.

Sediment samples were assayed for the presence of hydrocarbon-degrading yeasts by spread-plate methods, a mineral basal medium with phenanthrene as carbon source was employed (Romero *et al.* 1998). The yeasts were identified by morphological and physiological differences (Kurtzman & Fell 1998), and the G + C content by buoyant density in CsCl gradients generated by ultracentrifugation (Kurtzman *et al.* 1983).

The *Candida krusei* strains were subjected to chitin content measurements; they were grown in the yeast phase in YPD (1% yeast extract; 2% peptone; 2% dextrose) at 30 °C. Hyphal growth was induced by resuspending YPD-yeast cells in 10 vols 5% newborn calf serum at 35 °C, for 6 h. Then, cells and hyphae were freeze-dried to constant weight and the technique were performed on dried forms. The colorimetric measurement of chitosan treated with nitrous acid (Ride & Drysdale 1972), and the acid hydrolysis of whole cells to break down chitin to glucosamine were employed (Yabe *et al.* 1996).

To measure chitin synthase activity, 2.0×10^7 cells ml^{-1} grown on YPD were inoculated to induce germ tube formation into 20% calf serum. After 6 h incubation at 120 rev min^{-1}, 35 °C, cells with germ tubes were harvested. Mixed membrane fractions (MMF) were employed to resuspend the pellets in 50 mM Tris–HCl (pH 7.5), with 30% glycerol. The protein concentration was determined using the Coomassie Protein Assay kit.

Table 1. Traits of the yeast species.

	C. krusei	*P. membranaefaciens*	*Cr. laurentii*	*Rh. glutinis*
Growth at 37 °C	+	+	–	+
Pellicle in broth	+	+	–	–
Pseudo/true hyphae	V	+	–	–
Chlamydospores	V	–	–	–
Germ tubes	V	–	–	–
Capsule	–	–	+	–
Ascospores	V	–	–	–

Enzyme assays were performed with and without trypsin pre-treatment; the preparation used 100 ng trypsin (μl MMF)$^{-1}$ incubated for 5 min at 30 °C; and trypsin activation was stopped by 150 ng inhibitor (μl MMF)$^{-1}$. The synthase assay was carried out in 50 μl volume containing: 50 μg MMF protein, 25 mM *N*-acetylglucosamine, 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$ and 1 mM UDP-*N*-acetylglucosamine, which included 25 nCi UDP-[U-^{14}C]*N*-acetylglucosamine. After 30 min, the reactions were stopped with 1 ml 66% (v/v) ethanol. The mixtures were filtered through GF/C, presoaked in 10% (v/v) TCA, and the tubes were rinsed out with 2 × 1 ml 1% (v/v) Triton X-100. Each filter was washed with 4 × 2 ml 66% (v/v) ethanol, dried at 80 °C and placed in a vial for liquid scintillation counting.

Data from sample sites, yeast densities, chitin content and enzyme activities were evaluated by ANOVA (Wayne 1996), and the significance levels were set at $P = 0.01$.

Results and Discussion

Pichia membranaefaciens (Hansen) Hansen, *Cryptococcus laurentii* (Kufferath) Skinner, *Rhodotorula glutinis* (Fresenius) Harrison and *C. krusei* (Castellani) Berkhout were the dominant yeasts in the sampled sediments.

While the colony and cell forms of the three former species were uniform, *C. krusei* showed a significant variability in the physiological and morphological characteristics, between the four sampled sites. In the hydrocarbon medium, changes in the cell size was observed, cells were elonged to cylindrical, and appeared singly or in pairs. The colony morphology was variable, with multipolar budding and different pseudomycellium forms; other traits are shown in Table 1. The G + C values obtained for the five strains (C1, C2, C3, C4 and C5) ranged between 39.9–40.2 mol.%, this result confirmed that the isolate belongs to *C. krusei*, and the standard deviation of the replicas was 0.30 mol.%.

The chemical composition of the cell wall and enzyme patterns, can be used to distinguish between strains, so the chitin synthase activity and chitin content were determined. The major measurement of synthase activity was obtained in hyphal membranes that had approximately two- to three-fold higher activity than yeast membranes. The higher values were found in trypsinized hyphae and yeast membranes of C3 strain, intermediate activities characterized to cell forms of C1 and C2, and lower values were obtained with trypsinized hyphae and yeast membranes for C4 and C5 (Figure 1).

The enzyme activation gave less difference in hyphae (10–30%) than in cell membranes (42–45%) of C4 and C5 with and without trypsin; but the activities of the trypsinized cell types of C1, C2 and C3 were significantly higher respect to the untreated ones. An increase of 64, 53 and 51% was obtained for trypsinized hyphae and 100, 83 and 78% for yeast forms, respectively.

The chitin content obtained with the nitrous acid method for hyphal forms of the C1, C2, C4 and C5

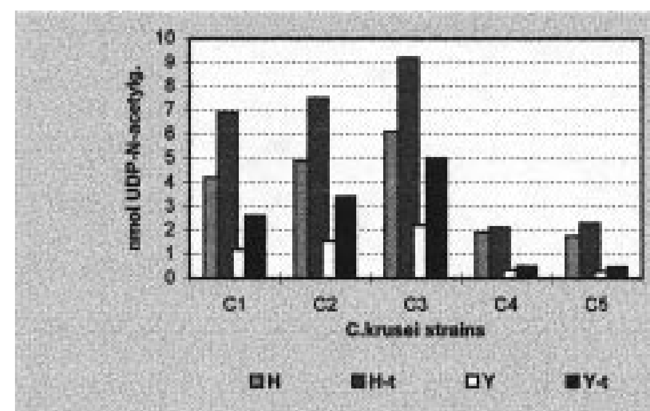

Figure 1. Measurement of chitin synthase activity in each *C. krusei* strain. (Units: nmol UDP-*N*-acetylglucosamine chitin min^{-1} (mg prot.)$^{-1}$; H: hyphae, H-t: hyphae plus trypsin, Y: yeast, Y-t: yeast plus trypsin).

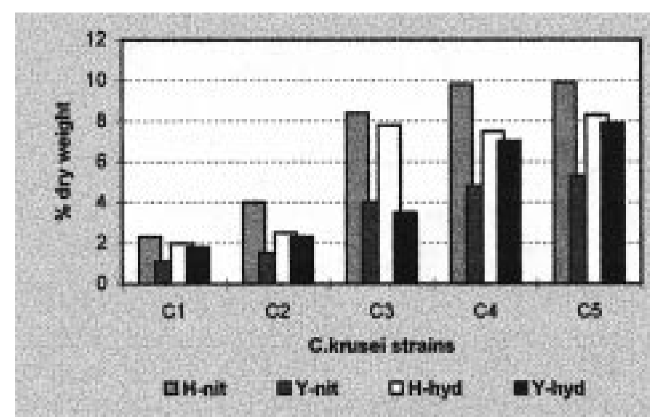

Figure 2. Chitin contents of *C. krusei* strains. (H-nit and Y-nit: nitrous acid method; H-hyd and Y-hyd: acid hydrolysis method.).

Table 2. Chemical characteristics of the sampled sites (carbon, nitrogen and organic matter expressed as percentage of 100 g dry weight of sediment).

	Este channel (1)	Oeste channel (2)	Regatas St. (3)	Zanjón St. (4)
pH	7.3	6.8	6.7	7.1
Organic carbon (%)	20.40	4.94	1.25	0.56
Total nitrogen (%)	0.553	0.336	0.037	0.046
Organic matter (%)	35.00	8.52	2.12	0.96
C/N relationship	37	15	34	12
Total hydrocarb. (ppm)	1773.2	898.7	702.3	460.8

strains were two- to three-fold higher than the ones observed in yeast cells; whereas the chitin content estimated by acid hydrolysis for each yeast form was slightly different. The chitin content of yeast and hyphal forms of the five *C. krusei* strains, reflected the previously mentioned resemblance between C1–C2 and C4–C5; and the nitrous acid method and the hydrolysis technique demonstrated difference for C3 (Figure 2).

The relationship between the chitin content and chitin synthase activity for hyphae and yeast of C1–C2 revealed a value of 3–2 nmol UDP min^{-1} (mg prot.)$^{-1}$; however, a significant minor ratio 0.1–0.2 was obtained for C4–C5 cell forms and 1 nmol UDP min^{-1} (mg prot.)$^{-1}$ was observed for both cell types of C3 strain. These relationships confirmed the similarity between C1–C2, C4–C5 and C3.

The sampled sites included areas subjected to diverse levels of pollution; Channel Este (1) receives discharge from the YPF-petroleum Refinery, Channel Oeste (2) is located in an urban area with industrial and domestic contamination. Regatas St. (3) is open to Rio Santiago and Zanjón St. (4) is a slight contaminated place (Table 2). According to the hydrocarbon levels, C/N and organic matter concentrations, the sites were different from each other ($P < 0.01$).

Candida krusei was the most frequently occurring yeast in the samples studied, and it made up about 62 and 52% in sites 1 and 2, as a significant difference in the physiological features of *C. krusei* was observed, together with a higher percentage of the C3, C4 and C5 (Figure 3). Regatas St. results resembled this data. Zanjón St. had half of the hydrocarbon level, a decrease in the *C. krusei* presence and a higher frequency of C1–C2 strains.

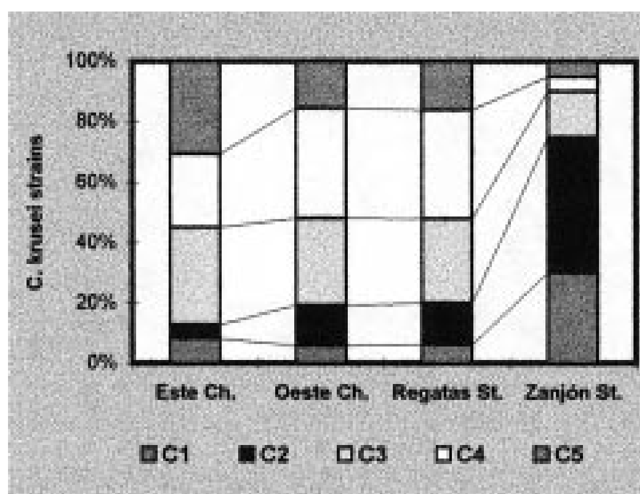

Figure 3. Frequency of *C. krusei* strains (C1, C2, C3, C4 and C5) in each sampled sites.

The characterization of yeasts isolated from polluted sites is difficult, since the organisms increase their morphological and physiological variability (MacGillivray & Sharis 1994). The chitin content and synthase activity underlined the differences between C1–C2, C3, C4–C5 *C. krusei* strains, and confirmed the variability in the isolated phenotypes (Munro *et al.* 1998). According to our results, the phenotypic variation of *C. krusei* strains may be attributed to the contaminated levels of the environment, controlling this factor the appearance of different phenotypes in the heavily polluted samples, as the C3, C4 and C5 strains (Elliott & Futcher 1993; Radcliffe *et al.* 1997).

Acknowledgements

This work was supported by grants from CONICET, National University of La Plata (Fac. Cs. Naturales y Museo) and from CIC (Prov. Bs.As), Argentina.

References

APHA. 1992 *Standard Methods for the Examination of Water and Wastewater*. 18th edn. Washington, D.C.: American Public Health Association. ISBN 0-87553-207-1.

Berdicevsky, I., Duek, L., Merzbach, D. & Yannai, S. 1993 Susceptibility of different yeast species to environmental toxic metals. *Environmental Pollution* **80**, 41–44.

Cox, P.W., Paul, G.C. & Thomas, C.R. 1998 Image analysis of the morphology of filamentous microorganisms. *Microbiology* **144**, 817–827.

Elliot, B. & Futcher, B. 1993 Stress resistance of yeast cells in largely independent of cell cycle phase. *Yeast* **9**, 33–42.

Kurtzmann, C.P. & Fell, J.W. 1998 *The Yeasts*, *A Taxonomic Study*. 4th edn. Amsterdam, The Netherlands: Elsevier Science B.V. 1SBN 0-44481312-8.

Kurtzman, C.P., Phaff, H.J. & Meyer, S.A. 1983 Nucleic acid relatedness among yeast. In: *Yeast genetics*, *fundamental and applied aspects*, eds Spencer, J.F.T., Spencer, D.M. & Smith, A.R.W. pp 140–166 Berlin-Heidelberg-New York: Springer. ISBN 0-38790793-9.

MacGillivray, A.R. & Shiaris, M.P. 1994 Relative role of eukaryotic and prokaryotic microorganisms in phenanthrene transformation

in coastal sediments. *Applied and Environmental Microbiology* **60**, 1154–1159.

Munro, C.A., Schofield, D.A., Gooday, G.W. & Gow, N.A.R. 1998 Regulation of chitin synthesis during dimorphic growth of *Candida albicans*. *Microbiology* **144**, 391–395.

Radcliffe, P.A., Binley, K.M., Trevethick, J., Hall, M. & Sudbery, P.E. 1997 Filamentous growth of the budding yeast *Saccharomyces cerevisiae* induced by overexpression of the *WHI*2 gene. *Microbiology* **143**, 1867–1876.

Ride, J.P. & Drysdale, R.B. 1972 A rapid method for the chemical estimation of filamentous fungi in plant tissue. *Physiological Plant Pathology* **2**, 7–15.

Romero, M.C., Cazau, M.C., Giorgieri, S. Arambarri, A.M. 1998 Phenanthrene degradation by microorganisms isolates from a contaminated stream. *Environmental Pollution* **101**, 1–5.

Rustchenko, E.P., Howard, D.H. & Sherman, F. 1997 Variation in assimilating functions occurs in spontaneous *Candida albicans* mutants having chromosomal alterations. *Microbiology* **143**, 1765–1778.

Wayne, W.D. 1996 *Bioestadística*. 2nd edn, p. 149, UTEHA, Noruega ed., México.

Yaar, L., Mevarech, M. & Koltin, Y. 1997 A *Candida albicans* RAS-elated gene (CaR SR1) is involved in budding, cell morphogenesis and hypha development. *Microbiology* **143**, 3033–3044.

Yabe, T., Yamada-, T., Kasahara, S., Furuichi, Y., Nakajima, T., Ichishima, E., Arisawa, M. & Yamada, H. 1996 *HKR* 1 encodes a cell surface protein that regulates both cell wall β-glucan synthesis and budding pattern in the yeast *Saccharomyces cerevisiae*. *Journal of Bacteriology* **178**, 477–483.