Effect of Copper on Acid Phosphatase Activity in Yeast *Yarrowia lipolytica*

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Acid phosphatase (APase) activity of the yeast *Yarrowia lipolytica* increased with increasing Cu^{2+} concentrations in the medium. Furthermore, the enzyme in soluble form was stimulated *in vitro* by Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+} and Mg^{2+} and inhibited by Ag^{+} and Cd^{2+}. The most effective ion was Cu^{2+}, especially for the enzyme from cultures in medium containing Cu^{2+}, whereas APase activity in wall-bound fragments was only slightly activated by Cu^{2+}. The content of cellular phosphate involving polyphosphate was decreased by adding Cu^{2+}, regardless of whether or not the medium was rich in inorganic phosphate. Overproduction of the enzyme stimulated by Cu^{2+} might depend on derepression of the gene encoding the APase isozyme.

Key words: Acid Phosphatase, Copper, *Yarrowia lipolytica*

Introduction

Acid phosphatases (APases) are non-specific enzymes with a pH optimum below 7.0 that catalyze the hydrolysis of monoesters resulting in the release of inorganic phosphate. APases are typically located near the cell walls and organelles of fungi (González *et al.*, 1993). APases play important roles in the biosynthesis of yeast cell walls (Field and Schekman, 1980) and the enzyme is derepressed upon inorganic phosphate starvation (Moran *et al.*, 1989; Galabova *et al.*, 1993). The activity of APases is also increased by Cu^{2+} in *Aspergillus niger*, regardless of whether the medium is rich in inorganic phosphate or not (Tsekova *et al.*, 2002). The bacterium *Citrobacter* sp. accumulates heavy metals via the activity of an APase that produces inorganic phosphate (Jeong and Macaskie, 1999). These findings suggest that enhanced APase activity participates in Cu^{2+} resistance, causing precipitation as a phosphate-metal complex. Both Cu^{2+} and Al^{3+} induce a phosphorus deficiency in some plants and thereby enhance the activity of APases (Huttová *et al.*, 2002; Lee *et al.*, 2005). Cultured cells of tobacco also acquired Al^{3+} tolerance during phosphate starvation (Yamamoto *et al.*, 1996). Enzymatic APase activity is also influenced by various metal ions *in vitro*, being activated by Cu^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}, but inhibited by Al^{3+} and Hg^{2+} in potato tuber cells (Tu *et al.*, 1988). In phosphate-starved tomato cell culture, the enzyme APase is activated by Mg^{2+} and Mn^{2+}, but inhibited by Cu^{2+} and Zn^{2+} (Bozzo *et al.*, 2002). The enzymatic responses of APases to metal ions differ among kinds of cell types and culture conditions.

Yarrowia lipolytica is a strictly aerobic yeast. This dimorphic fungus has traditionally been used to produce amino or other organic acids due to its formidable excretory capacity (Antonucci *et al.*, 2001; Barth and Gaillardin, 1997; Fickers *et al.*, 2004). Furthermore, *Y. lipolytica* can survive in extreme environments containing high concentrations of NaCl or heavy metals (Andreishcheva *et al.*, 1999; Butinar *et al.*, 2005; Strouhal *et al.*, 2003; Zvyagilskaya *et al.*, 2001). In preliminary studies we found that Cu^{2+} increases the APase activity in the yeast *Y. lipolytica*, which can grow in high $CuSO_4$ concentrations. The increased activity of APase that occurs during culture under such conditions might reflect differences in the enzymatic activation by metal ions and/or in its increased production. However, little is understood about the response of APase to metal ions in *Y. lipolytica*. The present study examines the effect of metal ions on the activity of APase in *Y. lipolytica*, cultured *in vitro* in the presence or absence of Cu^{2+}.

Abbreviations: APase, acid phosphatase; pNPP, *p*-nitrophenylphosphate; PMSF, phenylmethylsulphonyl fluoride; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid).

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The dimorphic fungus *Y. lipolytica* assumes mycelia-producing fungal and/or yeast-like forms, depending on the culture conditions (Ruiz-Herrera and Sentandreu, 2002). The metabolic activities of the two forms differ (Gadd and Mowll, 1985). The cell wall composition of the yeast and mycelial forms of *Y. lipolytica* are qualitatively similar, but quantitatively different (Vega and Domínguez, 1986). Some transcription factors involved in the morphogenesis of dimorphic fungi are also activated by Cu^{2+} (Osiewacz and Nuber, 1996). The coexistence of morphologically different cell types in culture considerably complicates the quantitation of growth as well as other physiological responses. Therefore, we used a *Y. lipolytica* mutant that can only grow in the yeast-like form.

Materials and Methods

Organisms and culture

A mutant strain of *Yarrowia lipolytica mhy 1-1* (*MAT A*, *ura3-302, leu2-207, lys8-11, mhy1-1*) used this work was donated by Dr. Rachubinski, University of Alberta, Edmonton, Alberta, Canada (Hurtado and Rachubinski, 1999). The organism was kept at 4 °C by periodic transfer on nutrient agar slants containing the following ingredients ($g\,l^{-1}$): glucose (20); polypeptone (5); yeast extract (5); KH_2PO_4 (5) and $MgSO_4 \cdot 7H_2O$ (2). Yeast cultures were grown at 30 °C in 500-ml flasks containing 100 ml nutrient medium in a reciprocal shaker at 120 strokes min^{-1}. Filter-sterilized $CuSO_4 \cdot 5H_2O$ was added to liquid media to yield the various final concentrations.

Cell permeabilization

Cells were permeabilized as described by Galabova *et al.* (1996). Cells were harvested, washed twice with distilled water and then 9 ml of cell suspension (containing about 1 mg DW ml^{-1}) were mixed with 1 ml of Triton X-100. The cells were incubated at room temperature with intermittent shaking for 30 min and centrifuged at 3,000 × *g* for 10 min. The pellet was resuspended in 0.1 M sodium acetate buffer (pH 4.2). Permeabilized cells were used for the determination of total enzyme activities.

Subcellular fractionation

The harvested cells were washed twice with distilled water. Protease activity was protected by adding phenylmethylsulphonyl fluoride (PMSF, final concentration 1 mM) and then cultures were disrupted by vigorous vortex mixing with glass beads in 50 mM piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 7.0) containing 1 mM EDTA at 4 °C. Intact cells were absent according to the observation by light microscopy. The disrupted cells were then centrifuged at 10,000 × *g* for 30 min. The pellet was washed twice with 50 mM PIPES buffer (pH 7.0) by centrifugation at 10,000 × *g* for 30 min. The supernatant (soluble) and pellet (mainly comprising cell wall) were used for the determination of enzyme activities and protein.

Assay of APase activity

We assayed APase activity in a mixture comprising 0.5 ml of enzyme (permeabilized cell suspension, soluble and wall fractions), 0.5 ml of 0.25 M sodium acetate buffer (pH 4.2), 0.5 ml *p*-nitrophenylphosphate (pNPP; final concentration, 1.9 mM) and 0.5 ml of metal ions or distilled water. The reaction was initiated by adding substrate and a 10 min incubation at 30 °C was terminated by adding 1 ml of 0.1 M NaOH. One unit of activity was defined as the amount of enzyme required to release 1 nmol of *p*-nitrophenol in 1 min at 30 °C.

Cell dry weight and protein measurement

The cells were dried for 48 h at 90 °C in glass centrifuge tubes and then weighed (dry weight, DW). Protein concentrations were determined using the Folin-Ciocalteau reagent with bovine serum albumin as the standard (Lowry *et al.*, 1951).

Assay of inorganic phosphate

Phosphate compounds were extracted from cells using a slight modification of the method of Okorokov *et al.* (1983). Free inorganic phosphate was extracted from cells stirred for 15 min with 0.5 M $HClO_4$ at 4 °C. After centrifugation, phosphate compounds (mainly polyphosphates) were extracted from the pellet with 1 M $HClO_4$ for 10 min at 100 °C. Total phosphate was determined by hydrolyzing yeast cells with 50% H_2SO_4 for 15 min at about 300 °C. Inorganic phosphate was determined using a modification of the procedure of Ames and Dubin (1960). A 1:1 mixture of fresh 10% ascorbic acid and 2.5% ammonium molybdate containing 0.1% ammonium potassium tartrate in 1 N H_2SO_4 (ascorbic-molybdate; 0.2 ml) was mixed with 5 ml samples at room temperature and then inorganic phosphate levels were compared 15 min later by spectrophotometry at 883 nm against a blank containing only water (Hitachi U-3000 type).

Results

Effect of Cu^{2+} on cell growth and APase activity

Fig. 1 shows the effect of Cu^{2+} on the growth and the APase activity of *Y. lipolytica*. When cultured in medium containing Cu^{2+} for 24 h, 5 mM Cu^{2+} were required to inhibit cell growth by 50%. In contrast, APase activity was proportionally increased with increasing concentrations of Cu^{2+}. The enzyme activity of cells cultured in medium containing 6 mM Cu^{2+} was about 15-fold more than that of cells cultured without Cu^{2+}. Fig. 2 shows increasing APase activity during the growth of *Y. lipolytica*. In medium containing Cu^{2+} at 2 mM, APase activity slightly decreased until 9 h and then increased to about 1.6-fold of the initial enzyme activity for 24 h (Fig. 2A). In contrast, APase activity did not significantly increase in Cu^{2+}-free control medium for 24 h. The growth profiles in medium with or without 2 mM Cu^{2+} were similar (Fig. 2B). The APase activity was also immediately enhanced about 1.4-fold, when cells were transferred from control medium to medium containing 2 mM Cu^{2+}.

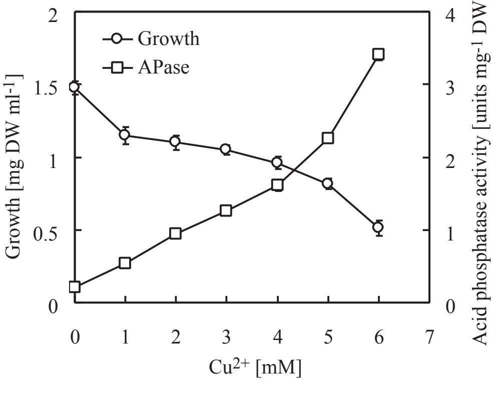

Fig. 1. Effect of Cu^{2+} on growth and acid phosphatase activity of *Y. lipolytica*. Cells were cultured in medium containing various concentrations of Cu^{2+} for 24 h at 30 °C. Results are shown as averages of three replicates with standard error.

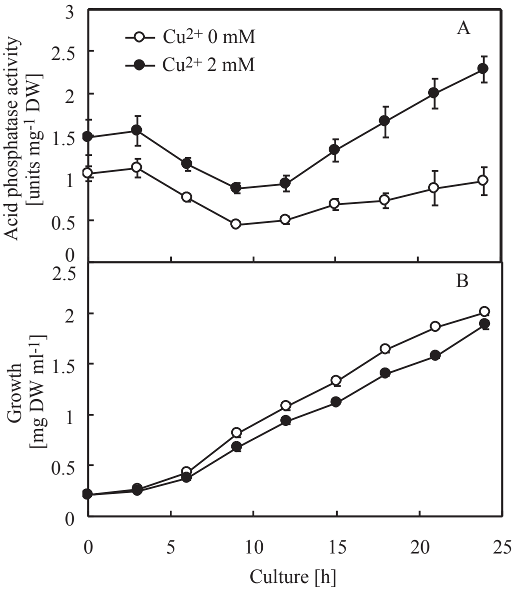

Fig. 2. (A) Acid phosphatase activity and (B) growth of *Y. lipolytica*. Cells were cultured in medium with or without Cu^{2+} at 2 mM for 24 h at 30 °C. Permeabilized cells as described in Materials and Methods were used for determination of enzyme activities. Results are shown as averages of three replicates with standard error.

Effect of metal ions on APase activities in vitro

To assess the effect of metal ions on APase activity, soluble enzyme isolated from cells cultured in medium with or without 2 mM Cu^{2+} was incubated with various metal ions for 10 min at 30 °C (Fig. 3). The enzymatic activities of cells cultured with or without 2 mM Cu^{2+} were 8.4 and 5.2 units mg^{-1} protein, respectively (Fig. 3A). The specific activity of APase obtained after culture in medium containing 2 mM Cu^{2+} was about 1.6-fold higher that in Cu^{2+}-free medium. Furthermore, in the presence of 0.05 mM Cu^{2+} APase activities of cells cultured in medium with or without Cu^{2+} were increased to 12.1 and 39.2 units mg^{-1} protein, respectively. The APase was also significantly activated by adding Mn^{2+} and Co^{2+}, but inhibited by Ag^{+} and Cd^{2+}. Other cations such as Mg^{2+} and Ni^{2+} had no appreciable effect.

Furthermore, Co^{2+} (1 mM) was the most effective metal ion among those added to the reaction mixture at the same concentration, stimulating APase activity 3.4- and 5.5-fold in cells cultured without and with Cu^{2+}, respectively (Fig. 3B). The enzymatic activities were also significantly in-

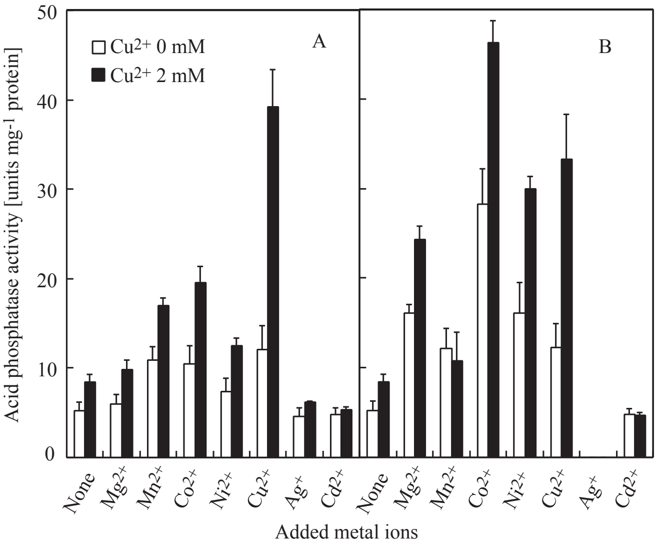

Fig. 3. Effect of metal ions on acid phosphatase activity from *Y. lipolytica*. Cells were cultured in medium with or without 2 mᴍ Cu^{2+} for 24 h at 30 °C and then enzyme activities were estimated without and with added metal ions at concentrations of 0.05 mᴍ (A) or 1 mᴍ (B). Permeabilized cells as described in Materials and Methods were used for determination of enzyme activities. Results are shown as averages of three replicates with standard error.

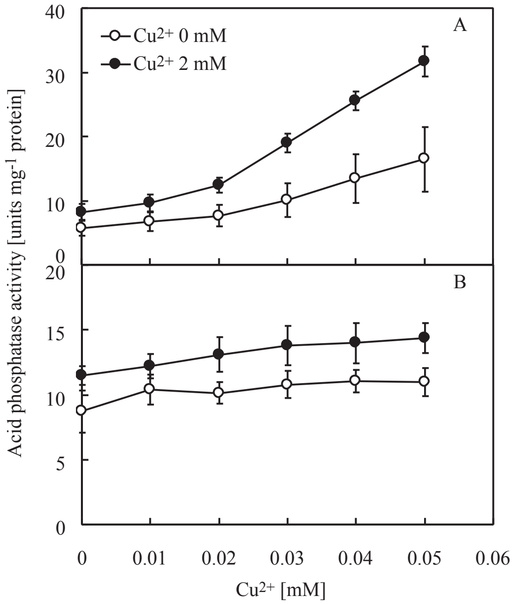

Fig. 4. Effect of Cu^{2+} on acid phosphatase from *Y. lipolytica*. Cells were cultured in medium with or without Cu^{2+} at 2 mᴍ for 24 h at 30 °C and then disrupted with glass beads. The homogenate was centrifuged and separated into soluble and pellet fractions as described in Materials and Methods. Enzyme activities in soluble (A) and pellet (B) fractions were estimated at various concentrations of Cu^{2+}. Results are shown as averages of three replicates with standard error.

creased by Mg^{2+} and Ni^{2+}, but not by concentrations of Cu^{2+} above 1 mᴍ. The enzyme activity was obviously increased in cells grown in Cu^{2+} solution. The most toxic metal ion tested was Ag^{+}, which at 1 mᴍ completely inhibited the APase activity. Fig. 4 shows the effect of Cu^{2+} on the soluble and wall-bound APase activities. The enzymatic activities were measured at a concentration range between 0.01 and 0.05 mᴍ Cu^{2+}. The enzyme activity in the soluble form was proportionally increased by Cu^{2+} concentrations above 0.01 mᴍ, reaching 2.8- and 3.8-fold in the presence of 0.05 mᴍ Cu^{2+} in cells cultured both with and without 2 mᴍ Cu^{2+} in the media, respectively (Fig. 4A). The APase activity in the wall-bound fragments of cells cultured with or without Cu^{2+} increased by about 1.2-fold in 0.05 mᴍ Cu^{2+} solution (Fig. 4B). The amount of APase was almost equally distributed between the soluble and the wall-bound forms (data not shown) and the presence of 2 mᴍ Cu^{2+} in the medium did not affect this distribution.

Phosphate content

To evaluate the effect of Cu^{2+} on cellular phosphate levels, cells were cultured for 24 h at 30 °C in the presence of various concentrations of Cu^{2+}. Fig. 5 shows that the cellular phosphate content in yeast cell extracts gradually decreased with increasing Cu^{2+} concentrations in the medium. The

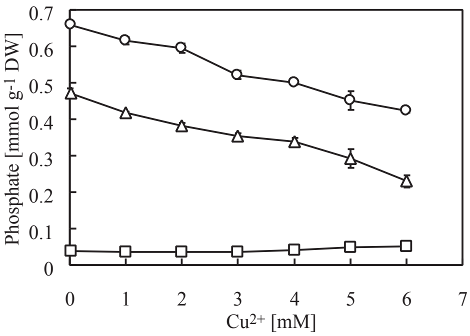

Fig. 5. Content of orthophosphate, polyphosphate and total phosphate in *Y. lipolytica*. Cells were cultured in medium containing various concentrations of Cu^{2+} for 24 h at 30 °C and then fractionated as described in Materials and Methods. (○) Total phosphate; (△) polyphosphate; (□) orthophosphate. Results are shown as averages of three replicates with standard error.

total phosphate content of cells from culture in 6 mM Cu^{2+} medium was about 60% of cells from Cu^{2+}-free medium. The amount of low molecular weight phosphate compounds in culture with or without Cu^{2+} comprised about 0.4 μmol mg^{-1} DW, which was about 6% of the total. The polyphosphate content significantly decreased with increasing concentration of Cu^{2+} in the medium. Polyphosphate accounted for about 70% of the total phosphate content of cells cultured with or without Cu^{2+}.

Discussion

We found here that APase activity in the Cu^{2+}-tolerant yeast *Y. lipolytica* increased dose-dependently according the amount of Cu^{2+} in the culture medium. Derepression of the gene encoding the enzyme under inorganic phosphate deficient conditions results in APase synthesis (Moran *et al.*, 1989; Galabova *et al.*, 1993). Even when the medium is rich in inorganic phosphate, Cu^{2+} elicits increased APase biosynthesis in *Aspergillus niger* and *Rhizopus delemar* (Tsekova *et al.*, 2000, 2002). Gabbrielli *et al.* (1989) also reported that Ni^{2+} increases APase activity in plants of the metal-tolerant *Alyssum* species, but not in metal-sensitive plants. The growth curve of *Y. lipolytica* in medium containing Cu^{2+} positively correlated with the increasing activity of APase (Fig. 2), indicating that increased enzyme activity is required for the growth in such medium.

When *Acidithiobacillus ferrooxidans* is cultured in medium containing high concentrations of heavy metal ions, Cu^{2+} stimulates polyphosphate degradation and phosphate efflux (Alvarez and Jerez, 2004). We also found that the total phosphate content comprising mainly polyphosphate decreased after culture in medium containing Cu^{2+} (Fig. 5). Therefore, the increased activity of APase elicited by Cu^{2+} probably resulted from a decrease in intracellular phosphate, which caused derepression of the APase gene (Galabova *et al.*, 1993). In addition, Al^{3+} also causes a phosphate deficiency in barely roots, by increasing APase activity (Huttová *et al.,* 2002). An inorganic phosphate deficiency renders Al^{3+} tolerance of cultured tobacco cells through the decreased accumulation of metal ions (Yamamoto *et al.,* 1996). In contrast, some microorganisms can accumulate heavy metals *via* enzymically-mediated precipitation as insoluble metal phosphates and the APase activity varies according to the growth conditions (Roig *et al.*, 1995; Turnau and Dexheimer, 1995). We found that the total cellular phosphate content was decreased by culture in the presence of Cu^{2+}, indicating that excess accumulated Cu^{2+} was probably not sequestered as an intracellular metal-phosphate complex in *Y. lipolytica*. We supposed that decreased cellular phosphate and/or a subsequent increase in APase activity participates in the efflux of Cu^{2+} as metal-phosphate complexes in a Cu^{2+} tolerance mechanism of the yeast *Y. lipolytica*.

We also demonstrated that the increased activity of *Y. lipolytica* APase was due not only to production during growth in the presence of Cu^{2+}, but also to stimulation by metal ions. The increase of APase activity induced by Al^{3+} was accompanied by an increase in the amount of APase isoform(s) (Huttová *et al.*, 2002). The yeast *Y. lipolytica* also has some APase isozymes (Galabova *et al.*, 1993; Tréton *et al.*, 1992). These results indicate that the repressible enzyme(s) in *Y. lipolytica* has a metal activating nature like the metalloenzymes synthesized in the presence of metals in *Zea mays* (Tu *et al.*, 1988) and *Aspergillus niger* (Mullaney and Ullah, 1998).

The responses of soluble and bound APase to Cu^{2+} differed, since metal ions slightly activated the enzyme bound to the wall (Fig. 4). Moran *et al.* (1989) reported that the kinetic behavior of *Y. lipolytica* APase is non-Michaelian, that is, the enzyme has multiple binding sites for its substrate. The purified *Y. lipolytica* enzyme shows size heterogeneity, indicating an apparent molecular weight in the range of 90,000–200,000 according to SDS-polyacrylamide gel electrophoresis (López and Domínguez, 1988). The activity of *Y. lipolytica* APase is also activated by increasing the ionic strength of the reaction mixture (González *et al.*, 1993), whereas that of purified APase from sycamore cell walls is not similarly activated. However, the enzyme is activated when bound to small cell wall fragments of *Acer pseudoplatanus* (Noat *et al.*, 1980). Therefore, the enzyme APase apparently hydrolyses its substrate in different cell types via a more complex mechanism. The present study found that APase activation by some metal ions might be caused by a slight modification of its molecular structure during growth in medium containing Cu^{2+} as well through the synthesis of its isoform. Further studies are required to determine whether metal-activating APase is a repressible enzyme and/or whether it plays a role in the Cu^{2+} tolerance of *Y. lipolytica*.

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Alvarez S. and Jerez C. A. (2004), Copper ions stimulate polyphosphate degradation and phosphate efflux in *Acidithiobacillus ferrooxidans*. Appl. Environ. Microbiol. **70**, 5177–5182.

Ames B. N. and Dubin D. T. (1960), The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**, 769–775.

Andreishcheva E. N., Isakova E. P., Sidorov N. N., Abramova N. B., Ushakova N. A., Shaposhnikov G. L., Soares M. I. M., and Zvyagilskaya R. A. (1999), Adaptation to salt stress in a salt-tolerant strain of the *Yarrowia lipolytica*. Biochemistry Mosc. **64**, 1061–1067.

Antonucci S., Bravi M., Bubbico R., Di Michele A., and Verdone N. (2001), Selectivity in citric acid production by *Yarrowia lipolytica*. Enz. Microb. Technol. **28**, 189–195.

Barth G. and Gaillardin C. (1997), Physiology and genetics of the dimorphic fungus *Yarrowia lipolytica*. FEMS Microbiol. Rev. **19**, 219–237.

Bozzo G. G., Raghothama K. G., and Plaxton W. C. (2002), Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. Eur. J. Biochem. **269**, 6278–6286.

Butinar L., Santos S., Spencer-Martins I., Oren A., and Gunde-Cimerman N. (2005), Yeast diversity in hypersaline habitats. FEMS Microbiol. Lett. **244**, 229–234.

Fickers P., Nicaud J. M., Gaillardin C., Destain J., and Thonart P. (2004), Carbon and nitrogen sources modulate lipase production in the *Yarrowia lipolytica*. J. Appl. Microbiol. **96**, 742–749.

Field C. and Schekman R. (1980), Localized secretion of acid phosphatase reflects the pattern of cell surface growth in *Saccharomyces cerevisiae*. J. Cell Biol. **86**, 123–128.

Gabbrielli R., Grossi L., and Vergnano O. (1989), The effects of nickel, calcium and magnesium on the acid phosphatase activity of two *Alyssum* species. New Phytol. **111**, 631–636.

Gadd G. M. and Mowll J. L. (1985), Copper uptake by yeast-like cells, hyphae, and chlamydospores of *Aureobasidium pullulans*. Exp. Mycol. **9**, 230–240.

Galabova D., Tuleva B., and Balasheva M. (1993), Phosphatase activity during growth of *Yarrowia lipolytica*. FEMS Microbiol. Lett. **109**, 45–48.

Galabova D., Tuleva B., and Spasova D. (1996), Permeabilization of *Yarrowia lipolytica* cells by Triton X-100. Enzyme Microb. Technol. **18**, 18–22.

González F. J., Fauste C., Burguillo F. J., and Domínguez A. (1993), Kinetic behaviour of a repressible acid phosphatase from the yeast *Yarrowia lipolytica*: A comparative study between the solubilized enzyme, the enzyme bound to cell-wall fragments and the enzyme bound to intact cells. Biochim. Biophys. Acta **1162**, 17–27.

Hurtado C. A. R. and Rachubinski R. A. (1999), *MHY1* encodes a C_2H_2-type zinc finger protein that promotes dimorphic transition in the yeast *Yarrowia lipolytica*. J. Bacteriol. **181**, 3051–3057.

Huttová J., Tamás L., and Mistrík I. (2002), Aluminium induced acid phosphatase activity in roots of Al-sensitive and Al-tolerant barley varieties. Rostlinná Výroba **48**, 556–559.

Jeong B. C. and Macaskie L. E. (1999), Production of two phosphatases by a *Citrobacter* sp. grown in batch and continuous culture. Enzyme Microb. Technol. **24**, 218–224.

Lee T.-M., Huang Y.-L., and Chen M.-H. (2005), Copper induction of phosphorous deficiency in *Ulva fasciata* (Ulvales, Chlorophyta). Phycologia **44**, 620–628.

López M. C. and Domínguez A. (1988), Purification and properties of a glycoprotein acid phosphatase from the yeast form of *Yarrowia lipolytica*. J. Basic Microbiol. **28**, 249–263.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951), Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275.

Moran A., Burguillo F. J., López M. C., and Domínguez A. (1989), Kinetic properties of derepressible acid phosphatase from the yeast form of *Yarrowia lipolytica*. Biochim. Biophys. Acta **990**, 288–296.

Mullaney E. J. and Ullah A. H. J. (1998), Conservation of the active site motif in *Aspergillus niger* (ficuum) pH 6.0 optimum acid phosphatase and kidney bean purple acid phosphatase. Biochem. Biophys. Res. Commun. **243**, 471–473.

Noat G., Crasnier M., and Ricard J. (1980), Ionic control of acid phosphatase activity in plant cell walls. Plant Cell Environ. **3**, 225–229.

Okorokov L. A., Lichko L. P., and Andreeva N. A. (1983), Changes of ATP, polyphosphate and K^+ contents in *Saccharomyces carlsbergensis* during uptake of Mn^{2+} and glucose. Biochem. Int. **6**, 481–488.

Osiewacz H. D. and Nuber U. (1996), GRISEA, a putative copper-activated transcription factor from *Podospora anserina* involved in differentiation and senescence. Mol. Gen. Genet. **252**, 115–124.

Roig M. G., Manzano T., Diaz M., Pascual M. J., Paterson M., and Kennedy J. F. (1995), Enzymically-enhanced extraction of uranium from biologically leached solutions. Int. Biodeterior. Biodegradation **35**, 93–127.

Ruiz-Herrera J. and Sentandreu R. (2002), Different effectors of dimorphism in *Yarrowia lipolytica*. Arch. Microbiol. **178**, 477–483.

Strouhal M., Kizek R., Vacek J., Trnková L., and Némec M. (2003), Electrochemical study of heavy metals and metallothionein in yeast *Yarrowia lipolytica*. Bioelectrochemistry **60**, 29–36.

Tréton B. Y., Le Dall M.-T., and Gaillardin C. M. (1992), Complementation of *Saccharomyces cerevisiae* acid phosphatase mutation by a genomic sequence from the yeast *Yarrowia lipolytica* identifies a new phosphatase. Curr. Genet. **22**, 345–355.

Tsekova K., Galabova D., and Todorova K. (2000), Copper accumulation and phosphatase activities of *Aspergillus* and *Rhizopus*. Z. Naturforsch. **55c**, 708–712.

Tsekova K., Galabova D., Todorova K., and Ilieva S. (2002), Phosphatase activity and copper uptake during growth of *Aspergillus niger*. Process Biochem. **37**, 753–758.

Tu S.-I., Brouillette J. N., Nagahashi G., and Kumosinski T. F. (1988), Effects of multivalent cations on cell wall-associated acid phosphatase activity. Plant Physiol. **88**, 61–68.

Turnau K. and Dexheimer J. (1995), Acid phosphatase activity in *Pisolithus arrhizus* mycelium treated with cadmium dust. Mycorrhiza **5**, 205–211.

Vega R. and Domínguez A. (1986), Cell wall composition of the yeast and mycelial forms of *Yarrowia lipolytica*. Arch. Microbiol. **144**, 124–130.

Yamamoto Y., Masamoto K., Rikiishi S., Hachiya A., Yamaguchi Y., and Matsumoto H. (1996), Aluminium tolerance acquired during phosphate starvation in cultured tobacco cells. Plant Physiol. **112**, 217–227.

Zvyagilskaya R., Andreishcheva E., Soares M. I. M., Khozin I., Berhe A., and Persson B. L. (2001), Isolation and characterization of a novel leaf-inhabiting osmo-, salt-, and alkali-tolerant *Yarrowia lipolytica* yeast strain. J. Basic Microbiol. **41**, 289–303.