Effects of Metal-depleted Media on the Growth and Morphology of *Saccharomyces rouxii* and on the Status of Periplasmic Acid Phosphatase

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Saccharomyces rouxii cells were maintained on yeast extract/neopectone/glucose medium (YNG). Experimental medium was depleted of endogenous metal ions by repeated passage of YNG over a chelating ion-exchange resin. This treatment did not compromise the glucose or vitamin content but provided a basal medium to which essential salts could be added in defined concentrations. Accordingly it was possible to demonstrate growth requirements by this yeast species for K^+ , Mg^{2+} , Zn^{2+} and Fe^{3+} . Na^+ would not replace K^+ . Additions of $1.0\ \mu M$ - Ca^{2+} , NH_4^+ and Na^+ , or $0.1\ \mu M$ - Ni^{2+} , Sn^{2+} , Al^{3+} , Co^{2+} or Cr^{3+} to a minimal, reconstituted medium were without effect. Cu^{2+} and Mn^{2+} additions were not required for growth but, like Mg^{2+} , Zn^{2+} and Fe^{3+} , they positively affected the biosynthesis of periplasmic acid phosphatase. Cells from iron-depleted medium had only 1 unit (g dry wt) $^{-1}$ of native acid phosphatase activity although this could be stimulated 10-fold by addition of $FeCl_3$ to washed cell suspensions before enzyme assay. Additions of 10 – $60\ \mu M$ - $FeCl_3$ to untreated YNG medium progressively increased the concentration of acid phosphatase after 3 d of culture to an upper value of 75 units (g dry wt) $^{-1}$, and also increased the amount secreted into the growth medium. While unfortified YNG supported cells with only 28% of their acid phosphatase expressed (the bulk being subject to activation by Fe^{3+} in the assay medium) the cells from YNG fortified with $60\ \mu M$ - $FeCl_3$ displayed fully activated acid phosphatase. Cells from Mg^{2+} -depleted medium exhibited bizarre, elongated cell shapes and those from Zn^{2+} -deficient medium appeared somewhat angular by light microscopy. Fe^{3+} -deficient cells had apparently normal morphology.

INTRODUCTION

Saccharomyces rouxii Boutroux is an osmotolerant yeast of industrial significance and experimental interest (Onishi, 1963). Unusual features of this species include stabilization in nature as a haploid (Van der Walt, 1970), delayed fermentation of sucrose (Pappagianis & Phaff, 1956) which is attributable to a physically cryptic β -fructofuranosidase (EC 3.2.1.26) in young cells (Arnold, 1974), order of sensitivity to different polyene antimycotics (Arnold & Johnson, 1982), and unique periplasmic ultrastructure (Arnold *et al.*, 1974). Apart from ploidy, all of the above phenomena suggest that the cell envelope of *S. rouxii* is unusual when compared with other *Saccharomyces* species.

There have been studies (see for example Arnold & Johnson, 1982) on the tolerance of high concentrations of alkali metal ions by *S. rouxii* cells. However, we are not aware of any reports on the more general requirements of metals for growth. Heavy-metal nutrition takes on special interest here because *S. rouxii* has an unusual periplasmic acid phosphatase (EC 3.1.3.2) which requires ferric ions for enzymic activity, and is influenced by other metal cations (Arnold & Garrison, 1979). Accordingly, manipulation of the normal concentrations of metals in culture medium was undertaken with the initial expectation of eliciting effects on cellular growth and on the relative activity of acid phosphatase.

The approach was to start with the complex medium used previously in this laboratory, to remove metal cations with a chelation resin, and to supplement with defined concentrations of metal salts. We now report on the effects of specific concentrations of iron and other metals on cell yields of *S. rouxii*, on the native and potential activities of acid phosphatase in these cells, and on some morphological aberrances that can be attributed to individual metal deficiencies.

METHODS

Yeast. Saccharomyces rouxii (strain no. 48-28) was maintained on agar slants and handled as previously described (Arnold & Garrison, 1979). Standard YNG medium for liquid cultures contained yeast extract (Difco) (0.3%, w/v), neopeptone (Difco) (0.5%) and glucose (1%). Trials designed to demonstrate trace-element deficiencies were conducted in plastic (polymethylpentene) flasks (250 ml capacity, containing 40 ml medium) with polypropylene screw closures (Nalge Co., Rochester, N.Y., U.S.A.) on a gyrotary shaker at 28–30 °C. Cell yields were estimated turbidimetrically (Bestic & Arnold, 1976).

Experiments were also conducted in acid-washed borosilicate-glass flasks with colorimeter tubes attached as side arms (Bellco Glass Inc., Vineland, N.J., U.S.A.). These experiments were thought to be more susceptible to trace metal contamination (from the glass) than those conducted in plasticware, but they enabled facile generation of growth curves by non-invasive measurements of turbidities.

Inocula were obtained by centrifuging 3-d cultures (on standard YNG medium), washing the cells twice with sterile 0.1 M-sodium succinate buffer (pH 5.8), and resuspending them in the same buffer to a concentration equivalent to 3.2 mg dry wt ml⁻¹. The succinate buffer was pretreated with Chelex resin as described below. Each experimental flask was inoculated with 0.2 ml washed cell suspension and cultures were incubated for 3 d, after which time the cells were harvested, washed in Chelex-treated succinate buffer, and resuspended with 5.0 ml of the same buffer. A portion of this suspension was taken for microscopic examination; the remainder was used for assays. A sample of spent culture fluid was also retained for assays.

Removal of metal ions. The water used in these studies was distilled from a glass apparatus and then passed through successive columns of mixed-bed ion-exchange resins and activated charcoal (Barnstead Co., Boston, Mass., U.S.A.). Chelex 100 (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) was purchased in the Na⁺ form, washed briefly with distilled and deionized water, and deaerated under reduced pressure. Chromatography columns (1.6 cm diam.) and ancillary tubing and reservoirs were constructed of polyethylene or teflon. A 20 cm column was used to treat about 10 l of distilled and deionized water to further reduce metal ion contamination.

A similar column was used to treat 2-litre samples of 0.2 M-sodium succinate buffer (pH 5.8) and the buffer was passed through the column twice. The column was finally washed with water to give a total volume of 4 l and the pH value was adjusted to 5.8 by addition of double-distilled 6 M-HCl (G. Frederick Smith Chemical Co., Columbus, Ohio, U.S.A.). Resin was washed and regenerated according to the manufacturer's recommendation. Chelex 100 was converted to the K⁺ form (see below) by sequential treatment with 1 M-HCl, water, 1 M-KOH, and water, using several bed volumes for each treatment.

For the removal of metal ions from YNG medium, the initial solution was 250 ml of 4 × strength constituents which were dissolved by stirring and briefly raising the temperature to 90 °C. After cooling to room temperature this solution was filtered through a membrane filter (0.22 µm pore size; Millipore) and then passed through a 20 cm column of Chelex 100 in the K⁺ form. The pH value of the effluent, which was close to 11.0, was adjusted to 6.5 by addition of double-distilled 6 M-HCl. The medium was recycled through the same column of Chelex 100 and then the column was washed with about 200 ml water. The treated medium was made up to 500 ml by addition of Chelex-treated water and the pH value was adjusted again to 6.5. Samples (20 ml) were dispensed into culture flasks and appropriate volumes of salt solutions and of Chelex-treated water were added to give a final volume of 40 ml of 'Chelex-treated YNG' at a strength otherwise equivalent to normal YNG.

Yeast extract ash. Yeast extract (1 g) in a porcelain crucible was heated in a muffle furnace to 350 °C, held at that temperature for 3 h, then heated to 500 °C for a further 2 h. After cooling, the white ash was dissolved in 1 ml 6 M-HCl and 80 ml Chelex-treated water. The pH value was raised to 6.5 by addition of 10 M-NaOH and the final volume was adjusted to 83 ml. This solution was used at the rate of 10 ml per 40 ml final volume of medium (i.e. a final concentration equivalent to 0.3% yeast extract).

Analytical methods. Conditions for acid phosphatase assay followed previous descriptions (Arnold & Garrison, 1979) except that the substrate solution (20 mM-disodium *p*-nitrophenyl phosphate in 100 mM-sodium succinate buffer; pH 5.8) was pretreated with Chelex 100 in the Na⁺ form to minimize contamination by iron. Native acid phosphatase activity of cells was measured with cell suspensions that had been washed in iron-free succinate buffer. The full potential for acid phosphatase activity (i.e. after activation with Fe³⁺) was estimated on samples of cells that were preincubated for 3 h with equal volumes of 1 mM-FeCl₃ in succinate buffer.

The concentrations of *glucose* in YNG and in Chelex-treated YNG media were measured with 3,5-dinitrosalicylic acid reagent (Sumner, 1921). *Iron* was assayed by reduction with hydroxylamine and measure-

ment as the ferrous complex of 2,4,6-tripyridyl-s-triazine, which has a molar extinction coefficient of $24100 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 595 nm (Collins *et al.*, 1959). Typically 100 ml growth medium was assayed, and the chromogen was concentrated by extraction into nitrobenzene. Considerable difficulty was encountered in obtaining clean phase separations, so the organic layer was centrifuged, and any remaining traces of interface material disappeared when the final volume of the organic phase was adjusted with a few ml of absolute alcohol. Zinc was extracted as the dithiozonate from aqueous solution at pH 8.5 into chloroform. The organic layer was then treated in another separatory funnel with 10 ml 0.1 M-HCl to release the zinc into the aqueous phase, which was subsequently assayed by flame atomic absorption spectroscopy. The same apparatus was used for magnesium except that media could be monitored directly without need for partial purification or concentration. Manganese concentration in yeast extract was determined by flame atomic absorption spectroscopy on an appropriate amount of ashed sample dissolved in 3 M-HNO₃.

Salts. Analytical reagent (A.R.) grade magnesium sulphate was recrystallized three times from 50% (v/v) ethanol in plasticware. All other metals were added as A.R. grade sulphate or chloride salts without further purification.

Microscopy. Equal volumes of 1% (w/v) agar (at 50 °C) and yeast suspension were mixed, held briefly at 50 °C, and then a drop of the mixture was applied to a glass microscope slide and covered with a glass slip. Light micrographs were taken at 1180 × magnification and a green filter was employed to improve contrast in the unstained specimens.

RESULTS AND DISCUSSION

Essentiality of K⁺ for growth

One experiment was conducted with YNG medium that had been pretreated with Chelex-100 resin in the Na⁺ form. We compared growth rates for *S. rouxii* on YNG, treated-YNG, and treated-YNG to which MgSO₄ and a mixture of heavy metal salts was added. After 32 h all of the flasks containing treated-YNG, with or without additions, showed negligible growth, whereas growth ($1.3 \text{ mg dry wt ml}^{-1}$) was achieved in control flasks with untreated YNG. At this time 1 ml of sterile 40 mM-KCl (in Chelex-treated succinate buffer) was added to one of the duplicate flasks in each medium to give a final K⁺ concentration of approximately 4 mM. In the succeeding 40 h, yeast concentrations of 2.7 mg ml^{-1} were achieved in the controls (with or without added K⁺) and of 1.5 mg ml^{-1} in the treated YNG media to which heavy metals and K⁺ were added. Little growth (0.05 mg ml^{-1}) was observed on media without the K⁺ supplement.

Potassium is essential for the growth of *S. cerevisiae* and *S. uvarum* (see for example Suomalainen & Oura, 1971), and the present data support this conclusion for *S. rouxii*. Even without adaptation this species will endure up to 1.5 M-NaCl in otherwise normal medium (Arnold & Johnson, 1982) but it is now clear that Na⁺ will not substitute for K⁺. Potassium nutrition was not studied further and indeed all subsequent pretreatments of YNG medium employed Chelex-100 resin in the K⁺ form. Accordingly K⁺ was the replacement ion in all basal media. As indicated above, YNG medium contains ample K⁺ to support growth and extra K⁺ is well tolerated by *S. rouxii*, even at two orders of magnitude higher concentration (Arnold & Johnson, 1982) than that resulting in Chelex-treated medium.

Efficacy of Chelex-100 treatment

Ion-exchange column treatments, as described in Methods, were effective in depleting metal ions from the complex medium. Thus the concentration of Mg²⁺ was lowered from $247 \text{ } \mu\text{M}$ to $1 \text{ } \mu\text{M}$ and that of Zn²⁺ from $2.8 \text{ } \mu\text{M}$ to $0.05 \text{ } \mu\text{M}$. The iron content of YNG medium was initially $1.8 \text{ } \mu\text{M}$, decreased to $1.4 \text{ } \mu\text{M}$ after ultrafiltration, and was further reduced to $0.3 \pm 0.1 \text{ } \mu\text{M}$ after Chelex-resin treatment.

The major source of metal ions was the yeast extract component of the medium (Grant & Pramer, 1962). Analysis of ashed yeast extract showed the Mn²⁺ concentration of YNG medium to be less than $0.04 \text{ } \mu\text{M}$. Mn²⁺, Cu²⁺ and Ca²⁺ can also be removed by Chelex resin treatment (Bio-Rad Laboratories, Product Bulletin 2020) although our media were not analysed for these ions.

Table 1. *Effects of metal depletion and restoration on growth of Saccharomyces rouxii*

The inoculation rate was 0.018 mg dry wt ml⁻¹ and control cultures achieved a growth of 2.72 mg dry wt ml⁻¹ after 72 h. Concentrated stock solutions of additions were prepared in Chelex-treated water.

Pretreatment of YNG medium	Additions	Relative growth after 72 h (%)	
		Glassware	Plasticware
None	None	100	100
	Ash*	103	102
	Vitamins†	98	96
Chelex-100	None	4	3
	Metals‡	79	74
	Metals, vitamins	79	79
	Metals except Mn ²⁺	79	79
	Metals except Cu ²⁺	78	76
	Metals except Fe ³⁺	46	43
	Metals except Zn ²⁺	35	33
	Metals except Mg ²⁺	4	4
	Metals, ash	105	102
	Metals, extra Zn ²⁺ §	100	99

* Yeast extract ash, as described under Methods, was added to a final concentration equivalent to 0.3% (w/v) yeast extract.

† Final concentrations (µg ml⁻¹) were: inositol, 10; thiamin, 4; pyridoxine, 1; pantothenic acid, 0.5; and biotin, 0.02.

‡ Final concentrations (µM) were: Mg²⁺, 100; Fe³⁺, 10; Zn²⁺, Mn²⁺ and Cu²⁺, each 0.1, all as sulphate or chloride salts.

§ Final concentration of Zn²⁺ was 1.0 µM.

Growth in metal-deficient media

Chelex-treated YNG supported a very small amount of growth (Table 1). Several preliminary trials were conducted with this basal medium in order to establish metal requirements for the species. Because trace-metal contamination is inevitable, and cumulates with multiple additions of essential salts, it was desirable to seek minimum concentrations in each case so that requirements for trace elements might be revealed when their nominal sources were individually withheld.

The relatively large requirement for magnesium prompted recrystallization of MgSO₄ as described in Methods but we elected not to further purify the other salts. Within this operational definition it was possible to demonstrate requirements for K⁺ (see above), Mg²⁺, Fe³⁺ and Zn²⁺ (Table 1). The addition of a defined mixture of metal salts to treated-YNG elicited about 79% of the growth achieved on untreated YNG. This particular mixture was apparently deficient in some mineral; this was rectified with yeast extract ash or, specifically and completely, with extra ZnCl₂. No requirement for added Mn²⁺ or Cu²⁺ was demonstrable in these experiments. Untreated YNG medium was not limited by mineral content or by vitamins, and vitamin concentrations were apparently not compromised by the Chelex-100 treatment. Further increases in the amounts of Mg²⁺, Fe³⁺, Cu²⁺ or Mn²⁺ compared with those given in Table 1 did not increase growth; and additions of 1 µM-Ca²⁺, NH₄⁺ or Na⁺ ions, or of 0.1 µM-Ni²⁺, Sn²⁺, Al³⁺, Co²⁺ or Cr³⁺ ions had no effect on growth (data not shown). The reducing sugar content of Chelex-treated YNG was within 3% of the untreated YNG medium. After addition of minerals, growth on treated YNG was limited only by the amount of glucose (data not shown).

Differences in growth obtained in plastic compared with glass vessels were insignificant or non-existent (Table 1). Furthermore, growth curves of cultures in glass flasks did not indicate any unusual patterns (such as secondary growth) when metal-limited media were used. These findings suggest that elution of metals from acid-washed glassware was not a significant source of contamination.

Table 2. *Effects of iron supplementation on growth and acid phosphatase activity of Saccharomyces rouxii*

The basal medium was YNG. The results are shown as means $\pm$ S.E.M.; *n* indicates the number of separate experiments.

Addition or modification	Yield after 72 h (mg ml ⁻¹)	Acid phosphatase [Units (g dry wt cells) ⁻¹]			
		Cells		Medium	
		Native	Potential*	Native	Potential*
None (<i>n</i> = 5)	2.74 $\pm$ 0.02	18 $\pm$ 2	47 $\pm$ 5	2 $\pm$ 1	10 $\pm$ 4
10 μ M-Fe (<i>n</i> = 2)	2.78	77	90	9	11
30 μ M-Fe (<i>n</i> = 3)	2.81 $\pm$ 0.05	51 $\pm$ 6	54 $\pm$ 7	64 $\pm$ 3	73 $\pm$ 3
60 μ M-Fe (<i>n</i> = 1)	2.85	74	75	73	80
Filtered† (<i>n</i> = 3)	2.63 $\pm$ 0.04	5 $\pm$ 1	37 $\pm$ 7	1 $\pm$ 1	16 $\pm$ 4
Filtered† + 30 μ M-Fe (<i>n</i> = 3)	2.78 $\pm$ 0.07	47 $\pm$ 10	48 $\pm$ 11	59 $\pm$ 3	68 $\pm$ 5

* After treatment of harvested samples with FeCl₃ as described in Methods.

† As described in Methods.

Status of acid phosphatase

Arnold & Garrison (1979) used yeast extract that had been pretreated with magnesia to lower the endogenous concentration of orthophosphate in YNG medium and thus achieved derepression of acid phosphatase synthesis in *S. rouxii* during the first day of growth. The magnesia pretreatment was deemed an avoidable complication to the present nutritional study. However, cultures were routinely carried for 3 d so that reasonable titres of acid phosphatase were attained. Nonetheless, total acid phosphatase yields were somewhat lower than those obtained when magnesia-treated medium was used.

The acid phosphatase of *S. rouxii* can be activated by addition of Fe³⁺ ions to the assay medium, while Hg²⁺, Cu²⁺, Sn²⁺ and Cd²⁺ ions are inhibitory at 1 mM (Arnold & Garrison, 1979). Furthermore, using purified enzyme preparations, an inactive apoenzyme can be generated by reduction with ascorbic acid and subsequent gel-exclusion chromatography. Enzyme activity is returned in full by incubation of the protein fraction with ferric chloride (W. N. Arnold, unpublished).

Supplementation of YNG medium with 10, 30 or 60 μ M-FeCl₃ had no significant effect on the cell yield but increased the total amount of acid phosphatase by 77%, 123% and 171% respectively (Table 2). The degree of enzyme expression (in cells plus medium) increased from 35% in unfortified medium to 95% of potential in the medium fortified with 60 μ M-FeCl₃. The two larger supplements of iron also increased the proportion of acid phosphatase secreted into the growth medium (cf. Arnold & Garrison, 1979). These effects were also observed with iron addition to filtered medium from which some iron had apparently been removed as a colloidal or particulate complex (analyses given above).

A number of experiments (with separate batches of Chelex-treated YNG) investigated the effects of individual cation deficiencies on the status of acid phosphatase in resultant cells (Table 3). In all cultures the amount of enzyme secreted into the growth medium was very low. This was also true of media that were supplemented with excess iron, (about 33 μ M; data not shown) and suggests that secretion of the enzyme is not simply controlled by iron availability as the data in Table 2 might indicate.

Table 3. *Effects of metal deprivation on the activity of acid phosphatase in Saccharomyces rouxii cultures*

The basal medium was Chelex-treated YNG supplemented with 100 μM - Mg^{2+} , 3–10 μM - Fe^{3+} , 0.75 μM - Zn^{2+} , 0.1 μM - Cu^{2+} and 0.1 μM - Mn^{2+} . The results are shown as means $\pm$ s.e.m.; n indicates the number of separate experiments.

Variations from basal medium	Acid phosphatase [Units (g dry wt cells) ⁻¹]			
	Cells		Medium	
	Native	Potential*	Native	Potential*
None ($n = 8$)	13 $\pm$ 2	32 $\pm$ 6	0.3 $\pm$ 0.2	1.5 $\pm$ 0.4
Minus Cu^{2+} ($n = 4$)	8 $\pm$ 1	21 $\pm$ 3	0.5 $\pm$ 0.3	2.5 $\pm$ 0.3
Minus Mn^{2+} ($n = 4$)	7 $\pm$ 1	13 $\pm$ 1	0.5 $\pm$ 0.3	1.0 $\pm$ 0.6
Low Zn^{2+} (0.075–0.375 μM) ($n = 4$)	8 $\pm$ 1	14 $\pm$ 1	0	0
Minus Zn^{2+} ($n = 5$)	5 $\pm$ 1	7 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1
Minus Fe^{3+} ($n = 7$)	1 $\pm$ 0.3	11 $\pm$ 2	0.3 $\pm$ 0.2	0.7 $\pm$ 0.5
Low Mg^{2+} (10–50 μM) ($n = 4$)	1 $\pm$ 0.5	2 $\pm$ 0.6	0	0

* After treatment with FeCl_3 as described in Methods.

When the initial iron concentration was of the order of 0.3 μM (based on analysis of Chelex-treated YNG) the cell yield was approximately 45% of normal (Table 1) while the amount of acid phosphatase produced was about 20% and the expressed activity (no added iron in the assay) was only 5% of normal cells (Tables 2 and 3). When iron was incorporated into Chelex-treated medium at 3–10 μM , which is sufficient for growth (Table 1), the total amount of acid phosphatase synthesized was about 60% of that in untreated YNG cultures but the proportion of the total enzyme expressed was similar (Tables 2 and 3).

Although a medium based on Chelex-treated YNG without intentional addition of Cu^{2+} or Mn^{2+} ions supported full growth (Table 1), depletion of these elements seemingly affected the amount of acid phosphatase produced (Table 3). Olson & Johnson (1949) incorporated 0.1 μM - CuSO_4 into their defined medium for yeasts, but no requirement for Mn^{2+} was demonstrated. In many organisms copper is essential for cytochrome oxidase function as well as for certain other enzymes, and manganese is at least an essential component of enolase. Notwithstanding manganese and copper contamination of other essential salts added to our media, and also carry-over in the inoculum derived from normal medium, we conclude that the requirements for these elements by *S. rouxii* are exceedingly small.

Zinc depletion affected growth (Table 1) as well as acid phosphatase synthesis (Table 2). The requirement for Mg^{2+} ions was met by an initial concentration of 100 μM ; decreasing the concentration to 50 μM lowered the cell yield by 25% but reduced the amount of acid phosphatase by 94%. The amount of growth obtained with no added Mg^{2+} was so low (only five or six times the dry weight of the inoculum) that assessment of acid phosphatase activities was not meaningful.

Morphological changes

Cells grown on mineral-sufficient medium were spheroidal, and mature cells were typically 5 $\mu\text{m} \times 3 \mu\text{m}$ (Fig. 1a). Magnesium-deficient medium supported little growth but the resultant cells were bizarre in shape and variable in size (Fig. 1b). Several of the complex cell clusters displayed units reminiscent of short pseudohyphae. We have never observed this morphology in *S. rouxii* cultures grown on normal medium. On the other hand, *S. rouxii* cells are occasionally

Fig. 1. Micrographs of *Saccharomyces rouxii* grown on Chelex-treated YNG medium supplemented with yeast extract ash (a); or on a defined salt mixture but lacking Mg^{2+} (b), Zn^{2+} (c), or Fe^{3+} (d). All the micrographs were taken at the same magnification; the scale bar represents $10\text{ }\mu\text{m}$.

elongated to the extent of being two to three times longer in one dimension than the other (e.g. see the line drawings of Van der Walt, 1970). We had previously noted elongation in *S. rouxii* cells as a sporadic phenomenon, restricted to the late stages of growth, amounting to less than 10% of the population, and without explanation even though a nutritional cause seems likely. In any event, that phenomenon is qualitatively different from the one reported here for magnesium-deficient cells.

Cells derived from zinc-depleted medium (Fig. 1c) were close to normal size, but they displayed a propensity for angularity. This may be due to irregular wall thickening. Iron-deficient cells exhibited apparently normal morphology to the extent revealed by light microscopy (Fig. 1d).

Preliminary examinations by transmission electron microscopy have indicated abnormal cell walls in zinc- and magnesium-deficient cells. In the zinc-deficient organisms there was aberrant cell wall development in the region of bud scars. Magnesium-deficient cells have unusual thickenings in what would normally be abscission zones between adjacent mother and daughter cells. A more extensive ultrastructural study is under way but has been hampered by idiopathic problems in fixation and sectioning of metal-deficient cells, once again suggesting unusual cell walls.

Concluding comments

The choice of YNG medium (rather than a completely synthetic medium) for these studies was based upon two considerations. First, the extant literature on *S. rouxii* was generated from cells grown on YNG, or a complex medium close to that composition, and the present nutritional results are thus more easily related to existing biochemical and morphological data. Secondly, we have been impressed with the buffering capacity of YNG medium, which is a property of the neopeptone component. Thus the 3-d trials reported here had final pH values of 5.0–5.5. On the other hand considerable difficulty has been encountered in avoiding low pH values in cultures on glucose, inorganic salts and vitamins. Low pH affects yields, as well as the status of acid phosphatase (Arnold & Garrison, 1979), which would have been a complication to the present studies.

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REFERENCES

- ARNOLD, W. N. (1974). Expression of cryptic β -fructofuranosidase in *Saccharomyces rouxii*. *Journal of Bacteriology* **120**, 886–894.
- ARNOLD, W. N. & GARRISON, R. G. (1979). An Fe^{3+} -activated acid phosphatase in *Saccharomyces rouxii*. *Journal of Biological Chemistry* **254**, 4919–4924.
- ARNOLD, W. N. & JOHNSON, B. P. (1982). Effects of polyenes, detergents, and other potential membrane perturbants on an osmotolerant yeast, *Saccharomyces rouxii*. *Applied and Environmental Microbiology* **43**, 311–318.
- ARNOLD, W. N., GARRISON, R. G. & BOYD, K. S. (1974). Periplasmic structure in *Saccharomyces rouxii* (Boutroux) an osmophil. *Applied Microbiology* **28**, 1047–1054.
- BESTIC, P. B. & ARNOLD, W. N. (1976). Linear transformation of standard curves for yeast turbidity. *Applied and Environmental Microbiology* **32**, 640–641.
- COLLINS, P. F., DIEHL, H. & SMITH, G. F. (1959). 2,4,6-tripyridyl-s-triazine as a reagent for iron. *Analytical Chemistry* **31**, 1862–1867.
- GRANT, C. L. & PRAMER, D. (1962). Minor element composition of yeast extract. *Journal of Bacteriology* **84**, 869–870.
- OLSON, B. H. & JOHNSON, M. J. (1949). Factors producing high yeast yields in synthetic media. *Journal of Bacteriology* **57**, 235–246.
- ONISHI, H. (1963). Osmophilic yeasts. *Advances in Food Research* **12**, 53–94.
- PAPPAGIANIS, D. & PHAFF, H. J. (1956). Delayed fermentation of sucrose by certain haploid species of *Saccharomyces*. *Antonie van Leeuwenhoek* **22**, 353–370.
- SUMNER, J. B. (1921). Dinitrosalicylic acid: a reagent for estimation of sugar in normal and diabetic urine. *Journal of Biological Chemistry* **47**, 4–9.
- SUOMALAINEN, H. & OURA, E. (1971). Yeast nutrition and solute uptake. In *The Yeasts*, vol. II, pp. 3–74. Edited by A. H. Rose & J. S. Harrison. London and New York: Academic Press.
- VAN DER WALT, J. P. (1970). *Saccharomyces rouxii* Boutroux. In *The Yeasts, a Taxonomic Study*, pp. 682–690. Edited by J. Lodder. Amsterdam: North Holland Publishing Co.