Full Paper

Sulphate metabolism of selenate-resistant *Schizosaccharomyces pombe* mutants

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Selenate-resistant mutants were obtained from several strains of *Schizosaccharomyces pombe*. The obtained mutants all belonged to the same genetic complementation group. They were low in sulphate uptake activity and in ATP sulphurylase activity. They grew on medium containing sulphite, thiosulphate, cysteine or glutathione but not methionine as the sole source of sulphur. From these results, the mutants were concluded to carry mutations in the ATP sulphurylase gene. Inability of the mutants to utilize methionine as a sulphur source is rationalized by the absence of the reverse transsulphurylation pathway in this organism; wild type strains must utilize methionine as a sulphur source after it is degraded to give rise to sulphate.

Key Words—ATP sulphurylase; methionine degradation; *Schizosaccharomyces pombe*; selenate resistance; sulphate utilization

Introduction

A source of assimilable sulphur is essential for growth. Almost all fungal species are able to utilize sulphate and most of them grow when other inorganic compounds such as sulphite, sulphide and thiosulphate replace sulphate (Suomalainen and Oura, 1971). Sulphate metabolism has been well studied in the yeast *Saccharomyces cerevisiae* (Thomas and Surdin-Kerjan, 1997) and in certain filamentous fungi, such as *Neurospora crassa* and *Aspergillus nidulans* (Marzluf, 1997). The first steps of sulphate metabolism by *S. cerevisiae* are illustrated in Fig. 1. Selenate is a toxic analogue of sulphate; its transport into the cell is intimately connected with that of the sulphate, as proved in several bacterial and fungal species. Metab-

olism of selenate also appears to proceed along the sulphate pathway since formation of stable 5'-adenylyl selenate catalyzed by the ATP sulphurylase has been reported (Heider and Böck, 1993; Wilson and Bandurski, 1958). It is highly probable that selenium is incorporated into the same metabolic intermediates as sulphur but direct evidence is lacking in most cases (Nyberg-Swenson, 1999).

Arst (1968) found that certain sulphate non-utilizing mutants of *Aspergillus nidulans* showed strong resistance to selenate simultaneously. These mutants belonged to two genetic complementation groups, *sB* and *sC*, which were further distinguished and characterized according to their sensitivity to chromate. *sB*[−] mutants have also gained chromate resistant phenotype, while *sC*[−] mutants retained the same degree of sensitivity as the wild type. The author concluded that the *sB* gene was coding for the sulphate permease and the *sC* gene for the ATP sulphurylase enzymes. Two other types of sulphite auxotrophic *A. nidulans* mutants had quite different phenotypes. PAPS-reduc-

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Fig. 1. Reduction steps of sulphate metabolism by *Saccharomyces cerevisiae* (from Thomas and Surdin-Kerjan, 1997).

Abbreviations: APS, 5'-adenylyl sulphate; PAPS, 3'-phospho-5'-adenylyl sulphate.

tase-deficient (*sA*⁻) mutants had only a weak selenate tolerance, while deficiency in APS-kinase caused hypersensitivity to selenate. Buxton and co-workers (1989) successfully complemented *sC*⁻ mutants of both *A. nidulans* and *A. niger* with the cloned ATP sulphurylase gene of *A. nidulans*.

Selenate and chromate-resistant mutants of *S. cerevisiae* were isolated and studied by Breton and Surdin-Kerjan (1977). Mutations in any of the first three genes of the sulphate reduction pathway resulted in resistance to selenate but the inhibitory concentrations were different: ATP sulphurylase-deficient mutants (*met3*) tolerated twenty to fifty times more selenate than the mutants lacking APS kinase or PAPS reduc-

tase activity (*met14* and *met16*, respectively). *Met14* and *met16* mutants also gained chromate-resistant phenotype. It has also been shown that mutation in one of the two sulphate permease genes (*SEL*) conferred resistance to selenate, while mutants in the other permease gene (*CHR*) became resistant to chromate. Only a double mutant of these genes was resistant to both chromate and selenate. The existence of two (high and low affinity) independent sulphate transporter proteins in *S. cerevisiae* was first described by Smith et al. (1995) who induced resistance to selenate and chromate by disrupting the *SUL1* gene-encoded sulphate transporter protein. Systematic analysis of selenate and chromate resistant mutants identified three independent genes (*SUL1*, *SUL2* and *SUL3*) whose products were specifically involved in sulphate uptake (Cherest et al., 1997). *SUL1* and *SUL2* are encoding high affinity sulphate permeases, while the *SUL3* product participates in the transcriptional regulation of *SUL2*. It is not known, however, if *SUL1* and *SUL2* genes are identical to the formerly identified *CHR* and *SEL* genes.

In this study, selenate-resistant/sulphate-non-utilizing *S. pombe* mutants were isolated and characterized.

Materials and Methods

Culture media and strains. Strains used in this study are listed in Table 1.

Yeast strains were cultivated in YEPD medium (1% glucose, 0.5% yeast extract, 0.5% peptone) supplemented with 0.4 mM Na₂SeO₄ when required, or in a minimal medium (1% glucose, 0.5% (NH₄)₂SO₄, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 1 ml/L Wickerham vitamin solution), supplemented with the required growth components (40 µg/ml adenine, 100 µg/ml uracil, leucine, cysteine, tyrosine, lysine, methionine or arginine). We also used sulphur-free minimal medium (1% glucose, 0.5% NH₄Cl, 0.1% KH₂PO₄, 0.05% MgCl₂·H₂O); YED agar medium (1% glucose, 0.5% yeast extract, 2% agar) and SPA agar medium (0.1% KH₂PO₄, 1% glucose, 1 ml/L Wickerham vitamin solution, 2% agar) during the experiments.

Mutagenic treatments. UV irradiation: Strains were cultivated in YEPD medium until the late logarithmic phase of growth was reached. Two milliliter cell suspensions of 10⁷ cells/ml were irradiated in Petri dishes with a germicidal lamp. Mutants were isolated

Table 1. *S. pombe* strains used in this work and selenate-resistant mutants isolated from different wild type and auxotrophic strains.

Strain	Genotype	Source	Selenate-resistant isolates
<i>S. pombe</i> L972	h ⁻	CBS 7264	from Se ^R -1 to Se ^R -10
<i>S. pombe</i> L975	h ⁺	CBS 7265	from Se ^R -1 to Se ^R -10
<i>S. pombe</i> 0-82	h ⁻ <i>ade5</i>	GDU, auxotrophic mutant of CBS 7264	from Se ^R -1 to Se ^R -10
<i>S. pombe</i>	h ⁺ <i>leu1</i>	GDU, auxotrophic mutant of CBS 7265	from Se ^R -1 to Se ^R -10
<i>S. pombe</i> NCYC 1945	h ⁺ <i>met3-1</i>	auxotrophic mutant of CBS 7265	from Se ^R -1 to Se ^R -10
<i>S. pombe</i> B579	h ⁻ prototrophic	EBCU 579 (isolated from sulphited must)	from Se ^R -1 to Se ^R -5
<i>S. pombe</i> RIVE 4-1-1	prototrophic	RIVE 4-1-1	from Se ^R -1 to Se ^R -7

Abbreviation of culture collections: CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; EBCU, Department of Enology, Budapest Corvinus University, Budapest, Hungary; NCYC, National Collection of Yeast Cultures, Norwich, UK; GDU, Department of Genetics, University of Debrecen, Debrecen, Hungary; RIVE, Research Institute for Viticulture and Enology, Bratislava, Slovakia.

at a 5–10% survival rate.

Ethyl-methane-sulfonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatments: Cells were suspended in 30 mg/ml EMS or 25 µg/ml MNNG solutions at a concentration of 2×10^7 cells/ml and incubated for 60 min. Samples were drawn every 10 min. At the end of treatment mutagens were inactivated by adding Na₂S₂O₃ at 5% concentration.

Isolation of selenate-resistant mutants. Minimal inhibitory concentration (MIC) of sodium selenate was determined in the first step. We obtained 0.1 mM and 0.2 mM MIC values for the *S. pombe* strains used in the mutagenic treatments. For isolation of selenate-resistant mutants, 0.4 mM sodium selenate was applied. After mutagenic treatments, cells were plated on YEPD medium containing 0.4 mM sodium selenate and incubated at 30°C in the dark. After 4–7 days, growing colonies were isolated and re-inoculated into the same broth medium.

Determination of sulphate utilization of the selenate-resistant mutants. Sulphate utilization of the mutants was determined by their ability to grow on a minimal medium containing 0.5% ammonium sulphate as the sole sulphur source. A minimal medium containing 100 µg/ml cysteine as a generally utilizable sulphur source was used as a positive control.

Determination of chromate-resistance of selenate-resistant mutants. Similarly to the case of sodium selenate, MIC value of K₂CrO₄ was determined and proved to be 2.5 mM. Growth of selenate-resistant mutants was tested at 2.5, 3.0 and 3.5 mM K₂CrO₄ concentrations on YEPD media.

Complementation analysis of selenate-resistant mutants by sexual crossing. Strains were grown in YED agar plates for 3 days at 30°C. Dense suspensions (ca. 10^9 cells/ml) were prepared in distilled water and 15–15 µl suspensions of the crossed strains with complementing auxotrophic mutations and opposite mating types were mixed on the surface of SPA medium in a spot of ca. 1 cm diameter. After incubation for 3 days at 30°C, growing colonies were transferred to 3 different minimal media; 1) Minimal medium containing cysteine as a sulphur source—for testing the genetic complementation of auxotrophic mutations to determine if the conjugation was successful; 2) Minimal medium containing sulphate as the sole sulphur source—for testing the complementation of selenate-resistant/sulphate-non-utilization mutations; 3) Minimal media supplemented with the required amino acid(s) or nucleotide bases—for testing back mutations of the auxotrophic parental strains.

Measurement of sulphate transport. Cells were incubated at 30°C in a rotary shaker (180 rpm) for 15 h in YEPD medium, then harvested, suspended in a minimal medium supplemented with the desired nutrients and further incubated for 12 h. Cells were centrifuged, washed twice with distilled water and transferred into a sulphur-free medium containing the same nutrients as previously. After incubation for 1.5 h, 10 µl Na₂³⁵SO₄ (287 MBq/ml; Isotope Institute, Budapest, Hungary) was added to 10 ml culture (4×10^7 cells/ml). At intervals, 3 × 1 ml samples were removed and each mixed with 9 ml of 10 mM non-radioactive Na₂SO₄ solution, then incubated for at least 10 min. After being col-

lecting by filtration on nitrocellulose membranes (pore diameter 0.45 μm , Sartorius, Ltd., Göttingen, Germany), the cells were washed with 10 ml distilled water. The membranes were placed into a vial containing scintillation cocktail (Ultima Gold LSC cocktail, Packard-Canberra Co., Boston, MA, USA) and the radioactivity was measured with a scintillation counter (Tri-Carb 1600 TR, Packard, Canhera Co.).

Inactivation of yeast cells. The fungistatic compound 2-(ethyl-mercury-thio)-sodium benzoate (commercial name is Merthiolate or Thiomersal) was used for the metabolic inactivation of yeast cells. Merthiolate (50 $\mu\text{g/ml}$) was added to the samples 10 min before the radioactive sulphate.

Utilization patterns of organic and inorganic sulphur sources. Growth of strains was tested in sulphur-free minimal medium supplemented with one of the following compounds: sulphate (250 $\mu\text{g/ml}$), thiosulphate (250 $\mu\text{g/ml}$), sulphite (250 $\mu\text{g/ml}$), glutathione (100 $\mu\text{g/ml}$), cysteine (100 $\mu\text{g/ml}$) or methionine (100 $\mu\text{g/ml}$). The pH of the media was adjusted to 5.5. Strains were inoculated to the surface of solid media and into the same liquid media (10^6 cells/ml), to detect background growth without utilization of the added sulphur source supported by impurities of the agar and by sulphur derived from decomposed cells. The toxic effect of certain sulphur-sources (e.g. sulphite, thiosulphate) can also be decreased by using liquid media.

ATP sulphurylase assay. Strains were cultivated in 250 $\mu\text{g/ml}$ sulphite-containing minimal medium at 30°C in a rotary shaker (180 rpm) for 14–16 h. Ca. 10^9 cells were collected by centrifugation, washed in 40 mM Tris buffer (pH=8.0) and resuspended in the same buffer. Half volume of glass beads (diameter=0.5 mm) was added to the suspension and Vortexed 20-times in 1 min pulses, cooling the suspension in ice for 1 min in the breaks. Supernatant was removed and centrifuged for 20 min at 4°C (14,000 rpm, Sigma 225/99 rotor). The upper phase was separated and used for ATP sulphurylase assay. Activity of ATP sulphurylase was measured by colorimetric molybdolysis (P_i formation) assay (Wilson and Bandurski, 1958) as described by Segel et al. (1987). Reaction was stopped after 30 min incubation. Protein content of the cell lysate was determined by the Bradford protein assay kit (Bio-Rad Ltd., Hercules, CA, USA). Measurements were run in triplicates and were repeated twice.

Results and Discussion

Isolation of selenate-resistant mutants

By treating *S. pombe* strains with UV, MNNG and EMS, mutants that grew on a medium containing 0.4 mM selenate were induced (Table 1). The obtained mutants had MIC values of 50 times higher than the original strains did. All the mutagenized strains—excluding the *met3-1* mutant—were, however, wild type concerning their sulphur metabolism. Selenate resistance of the mutants was transmitted stably through mitosis. None of the obtained selenate-resistant mutants grew on medium containing sulphate as the sole sulphur source. This is an indication that the selenate-resistant mutants of *S. pombe* have defect(s) in the sulphate assimilation pathway like those of *S. cerevisiae* (Breton and Surdin-Kerjan, 1977), *A. nidulans* (Arst, 1968), and *A. fumigatus* (De Lucas et al., 2001). We therefore examined the mutants in this regard as described later in this report. It maybe worth mentioning here that a strain bearing the *met3-1* mutation (Kohli et al., 1977) gave rise to selenate-resistant mutants like the other strains (Table 1). This result strongly suggests that the *S. pombe* *MET3* gene is not a homologue of *S. cerevisiae* *MET3*, note that mutations in *S. cerevisiae* *MET3*, encoding ATP sulphurylase, confer selenate resistance.

Complementation analysis of selenate-resistant mutants by sexual crossing

Complementation analysis was performed by sexual hybridization of 72 selenate-resistant mutants of opposite mating types (h^+ and h^-) having complementing auxotrophic markers. On the basis of genetic complementation, prototrophic hybrids were formed in every crossing on minimal medium supplemented with cysteine as the sole sulphur source. Sulphate utilization capability of the hybrids, however, was not restored, so that the tested mutations were concluded to be in the same complementation group.

Chromate resistance of the selenate-resistant mutants

Selenate resistance of the mutants did not influence their sensitivity to chromate. It can be explained thus: 1) Mutation did not occur in the sulphate permease gene, similarly to the sC^- mutants of *A. nidulans* (Arst, 1968); 2) Mutation affected the sulphate permease gene but selenate resistance is not accompanied with chromate resistance, like SEL^- mutants of *S. cere-*

Fig. 2. ^{35}S -Sulphate uptake of *S. pombe* B579 and 0-82 wild type strains (Se^{S}) and their selenate-resistant mutants (Se^{R}).

d.w.: dry weight.

Fig. 3. Inhibition of ^{35}S -sulphate uptake of *S. pombe* 0-82 wild type (Se^{S}) strain and selenate-resistant mutant ($\text{Se}^{\text{R-2}}$) by merthiolate (50 $\mu\text{g/ml}$).

d.w.: dry weight.

visiae (Breton and Surdin-Kerjan, 1977).

Sulphate transport and ATP sulphurylase assay

Sulphate uptake of two wild type strains and their descendent selenate-resistant mutants was investigated using a minimal medium containing radioactively labeled sulphate ($^{35}\text{SO}_4^{2-}$). Results obtained with the

strains B579 and 0-82 and their selenate-resistant mutants are shown in Fig. 2. After 1-h incubation there was a significant difference between sulphate uptake of the resistant mutants and the wild type strains: the mutants were able to take up 54 and 20% of the sulphate taken up by the B579 and the 0-82 wild type strains, respectively. To exclude the possibility that sul-

phate accumulation measured in the resistant mutants was the consequence of sulphate adsorption by the cell wall, we treated the cells before adding the sulphate with the non-specific metabolic inhibitor merthiolate. This compound is routinely used for killing pathogenic fungi without disintegration of the cellular structure (Deighton et al., 1979). Merthiolate caused complete inhibition of $^{35}\text{SO}_4^{2-}$ uptake in 50 $\mu\text{g/ml}$ concentration, both in wild type and selenate-resistant mutant strains studied (Fig. 3). This result proves that decreased sulphate accumulation of selenate-resistant mutants is the consequence of decreased intracellular accumulation and indicates the existence of a permease-mediated sulphate transport in *S. pombe*.

The limited decrease of sulphate uptake by the selenate-resistant mutants had to allow considerable growth on sulphate containing medium, provided that the activities of enzymes catalyzing the reduction of sulphate to sulphite did not change. Because sulphate did not allow growth of mutants at all, we may conclude that the decreased sulphate uptake is neither the consequence of a "leaky" mutation of the sulphate permease gene, nor led the mutation to inactivation of only one of the presumed sulphate permeases. It is more probable that the mutation inactivated one of the genes encoding for the sulphate activation (ATP sulphurylase), phosphorylation (APS kinase) or reduction

(PAPS reductase) and consequently the sulphate accumulated intracellularly inhibited its own transport as was already demonstrated in *S. cerevisiae* (Logan et al., 1996). Because all the obtained mutants tolerated very high concentration of selenate, we could assume that the inactivated gene was coding for the ATP sulphurylase enzyme.

ATP sulphurylase activity was measured in B579 and 0-82 strains and in their selenate-resistant mutants. Results shown in Table 2 support our hypothesis that the mutants lost their sulphate utilization ability consequently to inactivation of the ATP sulphurylase enzyme.

Table 2. ATP sulphurylase activity of strains B579 and 0-82 and their selenate-resistant (Se^R) mutants.

Strains	Specific enzyme activity (U*/mg protein)
B579	43.82 ± 10.7
B579 Se^R -2	2.62 ± 2.29
0-82	52.07 ± 5.95
0-82 Se^R -2	5.03 ± 4.53

U* represents activity unit (nmol P_i produced per min).

Table 3. Assimilation of different sulphur sources by *S. pombe* wild type (selenate-sensitive) and selenate-resistant mutant strains.

Strain	Sulphur source					
	Sulphate (250 $\mu\text{g/ml}$)	Thiosulphate (250 $\mu\text{g/ml}$)	Sulphite (250 $\mu\text{g/ml}$)	Glutathione (100 $\mu\text{g/ml}$)	Cysteine (100 $\mu\text{g/ml}$)	Methionine (100 $\mu\text{g/ml}$)
L975	++	++	+	++	++	+
L975 Se^R -1	—	++	+	++	++	—
L972	++	++	+	++	++	+
L972 Se^R -1	—	++	+	++	++	—
0-82	++	++	+	++	++	+
0-82 Se^R -2	—	++	+	++	++	—
B579	++	++	++	++	++	+
B579 Se^R -2	—	++	++	++	++	—
RIVE 4-1-1	++	++	+	++	++	++
RIVE 4-1-1 Se^R -1	—	++	+	++	++	—
NCYC1945 <i>met3-1</i>	—	—	—	—	—	+
NCYC 1945 <i>met3-1</i> Se^R -1	—	—	—	—	—	—

++, good growth (late exponential phase has been reached within 24 h); +, weak growth (late exponential phase has been reached after 48 h); —, no growth.

Utilization patterns of different inorganic and organic sulphur sources of selenate-resistant mutants

As shown in Table 3, wild type strains could grow efficiently in culture media containing 250 µg/ml sulphate as a sole sulphur source. As mentioned before, selenate-resistant mutants were unable to propagate on sulphate as the sole sulphur-source. Sulphite proved to be a moderately assimilable sulphur-source for both the wild type strains and the selenate-resistant mutants. At pH 3.8, however, sulphite showed an inhibitory effect on the strains at the applied concentration as was earlier shown in *S. cerevisiae* (Rose, 1987). This can be attributed to the increased uptake of sulphur dioxide generated at this pH (Macris and Markakis, 1974). Thiosulphate proved to be an easily utilizable sulphur-source for both the wild type strains and their selenate-resistant mutants at 1 mg/ml or lower concentrations, but at higher concentrations its nutritive effect was masked by its inhibitory activity.

Organic sulphur sources such as glutathione, cysteine and methionine supported growth of wild type strains, but glutathione and cysteine proved generally better than methionine. This is in accordance with the findings of Brzywczy and Paszewski (1994) who attributed this difference to the lack of reverse transsulphurylation pathway from methionine to cysteine. Brzywczy and co-workers (2002) proved that this is due to the lack of two enzyme activities, cystathione β-synthase and cystathione γ-lyase. Surprisingly, all the selenate-resistant mutants lost the ability to utilize methionine as the sole sulphur-source, indicating that the main route for incorporation of the sulphur atom from methionine is the sulphate assimilation pathway. It is highly probable that the S²⁻-group of methionine is oxidized to sulphate via a degradation route in *S. pombe*, similarly as it was shown for glutathione in *S. cerevisiae* (Miyake et al., 1999). This sulphate pool is the only sulphur-source when methionine is the sulphur supply alone for the cells. Inability of the selenate-resistant mutants to utilize methionine as the sole sulphur-source is the consequence of the lack of ATP sulphurylase activity.

Sulphur-source assimilation properties of wild type and selenate-resistant mutants were identical not only in the isogenic strains (i.e. L972, L975 and their auxotrophic mutants) but also in strains from other sources. This indicates that sulphate and methionine assimilation pathways are identical within *S. pombe*.

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References

- Arst, H. N. (1968) Genetic analysis of the first step of sulphate metabolism in *Aspergillus nidulans*. *Nature*, **219**, 268–270.
- Breton, A. and Surdin-Kerjan, Y. (1977) Sulphate uptake in *Saccharomyces cerevisiae*: Biochemical and genetic study. *J. Bacteriol.*, **132**, 224–232.
- Brzywczy, J. and Paszewski, A. (1994) Sulphur amino acid metabolism in *Schizosaccharomyces pombe*: Occurrence of two O-acetylhomoserine sulphydrolases and the lack of the reverse transsulphurylation pathway. *FEMS Microbiol. Lett.*, **121**, 171–174.
- Brzywczy, J., Sienko, M., Kucharska, A., and Paszewski, A. (2002) Sulphur amino acid synthesis in *Schizosaccharomyces pombe* represents a specific variant of sulphur metabolism in fungi. *Yeast*, **19**, 29–35.
- Buxton, F. P., Gwynne, D. I., and Davies, R. W. (1989) Cloning of a new bidirectionally selectable marker for *Aspergillus* strains. *Gene*, **84**, 329–334.
- Cherest, H., Davidian, J.-C., Thomas, D., Benes, V., Ansorge, W., and Surdin-Kerjan, Y. (1997) Molecular characterization of two high affinity sulphate transporters in *Saccharomyces cerevisiae*. *Genetics*, **145**, 627–635.
- Deighton, F. J., Hall, N. K., and Larsh, H. W. (1979) Merthiolate treatment of pathogenic fungi. *J. Clin. Microbiol.*, **10**, 144–146.
- De Lucas, J. R., Dominguez, A. I., Higuero, Y., Martinez, O., Romero, B., Mendoza, A., Garcia-Bustos, J. F., and Laborda, F. (2001) Development of a homologous transformation system for the opportunistic human pathogen *Aspergillus fumigatus* based on the *sC* gene encoding ATP sulphurylase. *Arch. Microbiol.*, **176**, 106–113.
- Heider, J. and Böck, A. (1993) Selenium metabolism in microorganisms. *Adv. Microb. Physiol.*, **35**, 71–133.
- Kohli, J., Hottinger, H., Munz, P., Strauss, A., and Thuriaux, P. (1977) Genetic mapping of *Schizosaccharomyces pombe* by mitotic and meiotic analysis and induced haploidization. *Genetics*, **87**, 471–489.
- Logan, H. M., Cathala, N., Grignon, C., and Davidian, J. C. (1996) Cloning of a cDNA encoded by a member of the *Arabidopsis thaliana* ATP sulphurylase multigene family. *J. Biol. Chem.*, **271**, 12227–12233.
- Macris, B. J. and Markakis, P. (1974) Transport and toxicity of sulphur dioxide in *Saccharomyces cerevisiae* var. *ellipsoidus*. *J. Sci. Food Agric.*, **25**, 21–29.
- Marzluf, G. A. (1997) Molecular genetics of sulphur assimilation in filamentous fungi and yeast. *Annu. Rev. Microbiol.*, **51**, 73–96.
- Miyake, T., Sammoto, H., Kanayama, M., Tomochika, K., Shinoda, S., and Ono, B. (1999) Role of the sulphate assimila-

- tion pathway in utilisation of glutathione as a sulphur source by *Saccharomyces cerevisiae*. *Yeast*, **15**, 1449–1457.
- Nyberg-Swenson, B. E. (1999) The selenium link: The missing link in our understanding of biochemical triggering reactions? *Med. Hypoth.*, **52**, 125–131.
- Rose, A. H. (1987) Responses to the chemical environment. *In* The Yeasts: Yeasts and the Environment, Vol. 2, ed. by Rose, A. H. and Harrison, J. S., Academic Press, London, pp. 5–40.
- Segel, I. H., Renosto, F., and Seubert, P. A. (1987) Sulphate-activating enzymes. *Methods Enzymol.*, **143**, 334–339.
- Smith, F. W., Hawkesford, M. J., Prosser, I. M., and Clarkson, D. T. (1995) Isolation of cDNA from *S. cerevisiae* that encodes a high-affinity sulphate transporter at the plasma membrane. *Mol. Gen. Genet.*, **247**, 709–715.
- Suomalainen, H. and Oura, E. (1971) Yeast nutrition and solute uptake. *In* The Yeasts: Physiology and Biochemistry of Yeasts, Vol. 2, ed. by Rose, A. H. and Harrison, J. S., Academic Press, London, pp. 3–74.
- Thomas, D. and Surdin-Kerjan, Y. (1997) Metabolism of sulphur amino acids in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.*, **61**, 503–532.
- Wilson, L. G. and Bandurski, R. S. (1958) Enzymatic reactions involving sulphate, sulfite, selenate and molybdate. *J. Biol. Chem.*, **233**, 975–981.