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GENETICS OF SULPHATE ASSIMILATION IN *SCHIZOSACCHAROMYCES POMBE*

(A SHORT REVIEW)

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Sulphur plays an important role in yeasts, especially in the biosynthesis of methionine and cysteine. The inorganic sulphur source, sulphate, is taken up by the cells via the sulphate-permease(s). After its transport, it is activated and subsequently reduced to sulphide or serves as a donor for sulphurylation reactions. Selenate anion (SeO_4^{2-}), which has the same metabolic pathway as sulphate, is toxic for the cells of *Schizosaccharomyces pombe*. We isolated selenate resistant mutants which cannot utilize sulphate, therefore they need organic sulphur source for growth. One of the selenate resistant mutants was successively transformed with *S. pombe* genomic libraries and the gene complementing the selenate resistance was identified as that of coding for the ATP-sulphurylase enzyme.

Keywords: *S. pombe*, sulphate utilization, selenate resistant mutants, ATP sulphurylase gene

It has long been known that certain species of the fission yeasts belonging to the *Schizosaccharomyces* genus are able to metabolize high amounts of L-malic acid in wine via the malo-alcoholic fermentation pathway. *Schizosaccharomyces pombe* (*S. pombe*) was reported to be capable of complete decomposition of malic acid in must by converting it to ethanol and CO_2. The high malo-alcoholic activity of *S. pombe* is attributed to the high substrate affinity of its malic enzyme in one hand [1] and to its active transport system for malate [2] on the other hand. This wine deacidification method has not been used in the practice till now, mainly because of the production of undesirable sulphur compounds, including hydrogen sulphide causing off-flavours. This compound is the normal product of yeast sulphur metabolism and imparts a

distinctive “rotten egg” character to the wine. H_2S and other sulphur containing off-flavours in must and wine originates from organic (e.g. cysteine and methionine) and inorganic (e.g. sulphate and sulphite) compounds.

To avoid these negative effects of *S. pombe* metabolism during deacidification of wine different methods can be used. Snow and Gallander [3] inoculated the must with *S. pombe*, reduced the malic acid content, then removed the cells and reinoculated the must with *Saccharomyces cerevisiae* (*S. cerevisiae*) wine yeast strain. However, removal of *S. pombe* and reinoculation with *S. cerevisiae* is a very troublesome process. Application of immobilized *S. pombe* cells can solve this problem. Immobilized cells were used for malo-alcoholic fermentation of must [4], red wine [5] or white wine [6].

Production of hydrogen sulphide is a serious problem not only in wine-making but in beer fermentation, too. In a brewer's yeast, hydrogen sulphide production was controlled by cloning some genes that play a role in sulphur metabolism. Tezuka et al. [7] cloned the NHS5 gene, coding for the suppressor of hydrogen sulphide formation. Similar results were achieved by cloning the MET2 gene, coding for the homoserine O-acetyl transferase, which catalyzes the conversion of homoserine to O-acetyl homoserine [8] and by cloning the MET25 gene, which catalyzes the reaction of O-acetyl homoserine with hydrogen sulphide to form homocysteine [9].

A source of assimilable sulphur is essential for yeast growth. Almost all yeast species are able to utilize sulphate and most of them grow when sulphate is replaced by sulphite or thiosulphate. The ability of yeast species to utilize organic sulphur sources such as methionine and cysteine, however, varies consistently. The majority of *Saccharomyces* spp. cannot build the sulphur of cysteine and cystine into their cellular components effectively and the utilization of the sulphur present in methionine is only a little more effective, in contrast to other yeasts, which can grow on a big number of different S-sources [10]. Utilization of sulphite as the sole sulphur source is very much pH dependent. The reason is the toxic effect of sulphur dioxide, which is formed from sulphite and enters the cell at low pH. Other forms of sulphite, generated at higher pH values (HSO_3^-, SO_3^{2-}), cannot be taken up by the yeast cells [11].

Sulphur metabolism has been well studied in the yeast *S. cerevisiae* [12] and in some filamentous fungi, such as *Neurospora crassa* and *Aspergillus nidulans* (*A. nidulans*) [13]. The existence of two (high and low affinity) independent sulphate transporter proteins in *S. cerevisiae* was described by Smith et al. [14]. Cherest et al. [15] identified two different genes, SUL1 and SUL2 in this yeast. Both were encoding, however, for high affinity sulphate permeases.

After the sulphate has been transported into the cell, it undergoes an activation reaction with the help of ATP yielding adenylyl sulphate (APS), which is subsequently

phosphorylated to yield phosphoadenylyl phosphate (PAPS). The first step in the activation is catalyzed by the enzyme ATP-sulphurylase, which is encoded by the MET3 gene of *S. cerevisiae* [16].

Although *S. pombe* is a physiologically and genetically well-studied microorganism, its sulphate metabolism has not been studied at all. A detailed analysis of the physiology and genetics of sulphur metabolism is a prerequisite for developing an efficient strain improvement strategy for ecological purposes at all.

Selenate resistant mutants of *A. nidulans* are unable to utilize sulphate as a sole sulphur source. They were found to belong to two complementary groups (sB^- and sC^-), which can be clearly differentiated by their resistance to chromate. The chromate resistant mutants (sB^-) have a defect in sulphate permease, while the sensitive ones (sC^-) have a defect in the sulphate reductase enzyme complex [17]. Different *Aspergillus* mutants that are unable to use sulphate as a sulphur source and are also resistant to selenate can be complemented with the cloned gene of ATP-sulphurylase [18, 19]. Resistance for selenate and chromate in the yeast *S. cerevisiae* can be induced by disrupting the SUL1 gene encoded sulphate transporter protein, which is responsible for the transfer of sulphate and its toxic analogues across the plasma membrane [14].

Schizosaccharomyces pombe mutants that were unable to utilize sulphate and simultaneously showed the selenate resistant phenotype were induced and selected. These mutants grew on complex medium supplemented with 0.4 mM sodium selenate, which was lethal for the wild-type strains and were not able to grow on minimal medium containing sulphate as sole sulphur source. Their ability to grow on media containing different kinds of sulphur sources has been tested. These experiments showed that, generally, wild-type strains were able to utilize inorganic and organic sulphur sources while mutant strains could not grow on sulphate and thiosulphate but were still able to grow on organic sulphur compounds [20]. To study the genetic background of these selenate resistant/sulphate non-utilizing strains, the S 18-82 Se^R mutant strain was transformed with genomic libraries of selenate sensitive/sulphate utilizing *S. pombe* strains prepared as described in Benkő et al. [21]. Each positive clone tested had recombinant plasmids containing approximately 4.5 kb long chromosomal fragments and showed selenate sensitive/sulphate utilizing characteristics. One positive clone was selected for further analysis, which had been sequenced. We identified a 1473 bp long gene within the cloned fragment showing high homology with ATP-sulphurylase genes of other microorganisms (details are in publication). This enzyme catalyses the first step in the activation of sulphate entering the yeast cells [22].

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