SMYTE 26

Expression profile of genes involved in hydrogen sulphide liberation by *Saccharomyces cerevisiae* under different nitrogen concentrations

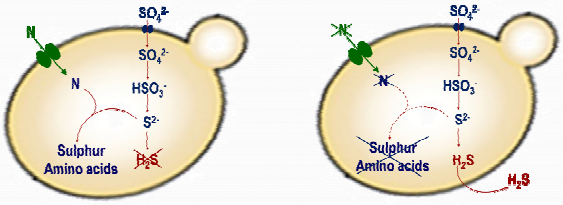
Mendes-Ferreira A.[1], Barbosa C. [1], del Olmo M. [2], Mendes-Faia A. [1] and Leão C.[3]

[1]IBB-Centro de Genética e Biotecnologia- Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal.
[2]Departament de Bioquímica i Biologia Molecular, Universitat de València, València, Spain.
[3]Instituto de Investigação em Ciências da Vida e Saúde (ICVS), Escola de Ciências da Saúde, Universidade do Minho, Braga, Portugal

BACKGROUND

Hydrogen sulphide (H_2S) is a secondary metabolite produced by yeasts during alcoholic fermentation, and its amounts largely depends on the yeast strain, media composition and fermentation conditions. In *Saccharomyces cerevisiae*, sulphide is the product of the Sulphate Reduction Sequence (SRS) pathway and acts as an intermediate in the biosynthesis of sulphur-containing amino acids. The biosynthesis of sulphur amino acids requires nitrogen-containing carbon precursors derived from the intracellular nitrogen pool and sulphide from the sulphate reduction pathway.

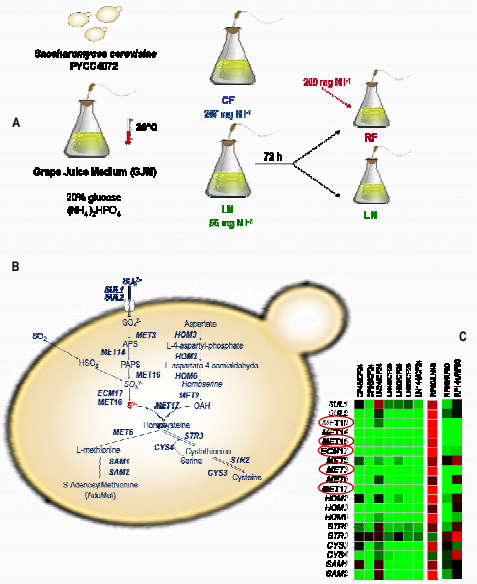
HOW IS NITROGEN LINKED TO H_2S PRODUCTION ?



AIMS

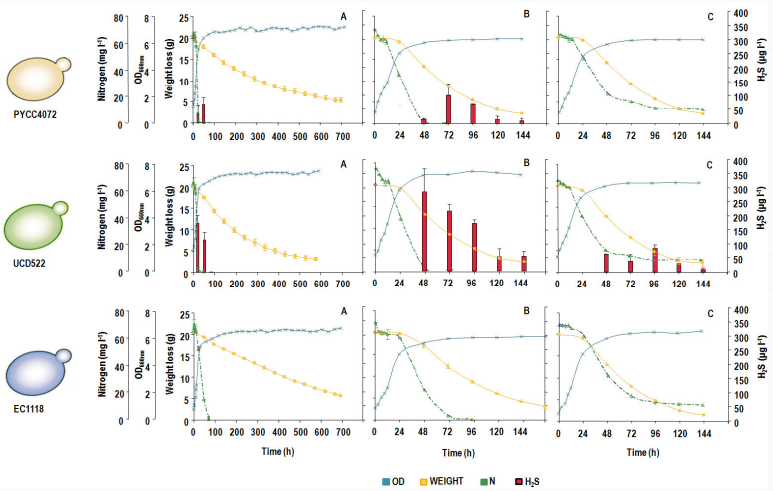
The present work intends to establish a relationship between nitrogen availability and H_2S liberation with expression levels of genes involved in the SRS pathway. Our ultimate goal is to elucidate molecular mechanisms underlying H_2S production in *Saccharomyces cerevisiae* associated to nitrogen deficiency, in order to minimize its incidence in alcoholic beverage production.

GENOME-WIDE ANALYSIS



Overview of the experimental design used for genome wide expression studies with *S. cerevisiae* PYCC4072 in synthetic grape-juice medium (*panel A*) , under different nitrogen concentrations (CF-control fermentation, 267 mg of N l^{-1}); LN-low nitrogen fermentation, 66 mg of N l^{-1}) and RF- re-fed fermentation, 66 + 200mg of N l^{-1}). Sulphate Reduction pathway (*panel B*) and transcript profiles of genes involved in SRS pathway, obtained by macroarray analysis in *S. cerevisiae* PYCC4072 (*panel C*) grown under different nitrogen conditions. The expression diagram, show, from top to bottom, relative expression levels of each gene and, from left to right, comparisons between time points. CF24, sample from the control fermentation carried out with 267 mg N l^{-1} at 24h after inoculation when the amount of assimilable nitrogen remaining in the medium was 178 mg l^{-1}; RF80, sample from the re-fed fermentation (66+200 mg N l^{-1} at 72h supplied as diammonium phosphate) collected 8h after addition, at 80h, when the amount of assimilable nitrogen present in the medium was 154 mg l^{-1}; CF48, CF96, LN24, LN48, LN80, LN96, LN144, RF96 and RF144 are samples from the control (CF), the low nitrogen fermentation (LN) conducted with 66 mg N l^{-1} and from the re-fed fermentation (RF) collected throughout the experiments. In all these last time points the nitrogen was low or even absent.

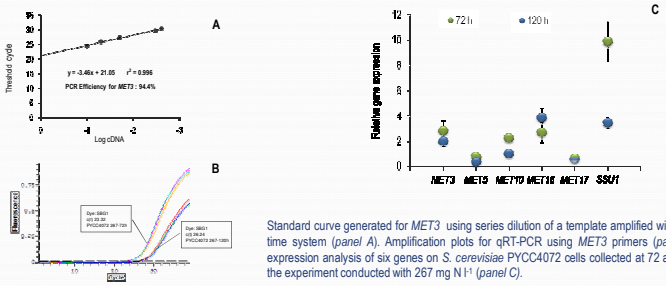
PHENOTYPING ANALYSIS OF H_2S FORMATION IN THREE *S. cerevisiae* STRAINS



Fermentation profiles and hydrogen sulphide liberation (histograms) by the yeast strains PYCC4072, UCD522, EC1118 grown in synthetic grape juice medium at 20ºC, containing an initial nitrogen concentration of 66 (A), 267 (B) and 402 mg N l-1 (C). Data points are the mean from triplicate fermentations ± SD (smaller error bars are hidden behind the data symbols).

ON GOING WORK...

From the above experiments, cells were collected for RNA extraction and qRT-PCR assays are being conducted using five *MET* genes as well as *SSU1*, encoding a plasma membrane sulphite pump involved in sulphite metabolism and required for efficient sulphite efflux.



Standard curve generated for *MET3* using series dilution of a template amplified with iCycler real time system (*panel A*). Amplification plots for qRT-PCR using *MET3* primers (*panel B*). Gene expression analysis of six genes on *S. cerevisiae* PYCC4072 cells collected at 72 and 120h from the experiment conducted with 267 mg N l^{-1} (*panel C*).

FINAL REMARKS

Definitive conclusions cannot be made at this time regarding the relationship between H_2S liberation and the expression levels of the genes under study. However this preliminary work does suggest that mRNA abundance of these genes were higher at 72h than at 120h. Additional assays are required to elucidate this event.

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