Biotransformation Mechanism of Inorganic Selenium into Selenomethionine and Selenocysteine by Saccharomyces Boulardii: In Silico Study

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Abstract

The biosynthesis of inorganic selenium into seleno amino acids has been studied in recent years. Thus, it has been reported that *Saccharomyces cerevisiae* bioaccumulates selenium from the metabolism of inorganic selenium. Based on the studies conducted, several authors have proposed a biotransformation metabolism of selenate into selenomethionine or selenocysteine. However, the pathway in different yeast is unknown. Therefore, and given the relevance of *Saccharomyces boulardii* as probiotic yeast, this study aims to propose the pathway used by *S. boulardii* to biosynthesize inorganic selenium into organic species. A comparative *in silico* study was performed for *Saccharomyces boulardii* ASM141397V1 with the genome-scale metabolic model of *Saccharomyces cerevisiae* S288C. Orthologous genes were identified using BLASTp of NCBI. In addition, a circular representation was done using CIRCOS software. The metabolic pathway for the assimilation of selenium was proposed based on the results obtained.

Introduction

Yeasts can permanently incorporate ions from the environment into their cell structure (Kieliszek and Błażejak 2013). In recent years, yeasts enriched with selenium and species *Saccharomyces boulardii*, *Candida utilis*, *Yarrowia lipolytica*, *Kluyveromyces marxianus*, *Rhodotorula glutinis* have been widely studied (Pérez-Corona et al. 2011; Prange et al. 2019; Rajashree and Muthukumar 2013; Martiniano et al. 2020; Suhajda et al. 2000; Kieliszek et al. 2017; Kieliszek and Dourou 2020; Wang et al. 2019). Research is mostly based on the metabolism of yeasts and their capability to biotransform inorganic selenium and bind it to proteins, mainly enzymes (Kieliszek and Błażejak 2013). Selenium concentrations bioaccumulated by yeasts are variable; still, concentrations in *Saccharomyces cerevisiae* can reach up to 5.64 mg of selenium/g dw of yeast (Kieliszek et al. 2016).

On the other hand, *Saccharomyces boulardii* is a yeast originally isolated from lychee and is the only probiotic one approved for human consumption by the Food and Drug Administration (FDA) (McCullough et al. 1998; Moon et al. 2020). The regular use of *Saccharomyces boulardii* prevents and treats acute diarrhea associated to bacterial infections and other gastrointestinal disorders (Moon et al. 2020). In addition to studies on its probiotic properties (Czerucka et al. 2007), *Saccharomyces boulardii* has been reported to produce selenium nanoparticles (Bartosiak et al. 2019). Therefore, the production process of organic selenium by yeasts is considered a green technology (Bartosiak et al. 2019; Patel et al. 2013).

Despite the number of studies available, only a few explain the use of *Saccharomyces boulardii* to obtain selenomethionine (SeMet) and/or selenocysteine (SeCys). The capacity of this yeast to produce seleno amino acids represents an opportunity in biotechnology to obtain more bioavailable and less toxic selenium (Schrauzer 2000; Kitajima and Chiba 2013).

Studies have proven that the biosynthesis of selenium takes place through a pathway similar to that of sulfur. Selenium replaces sulfur and is incorporated into the cell as SeMet and/or SeCys (Kieliszek and Błażejak 2013; Bierla et al. 2013). Another mechanism proposed is transsulfuration through a non-
specific enzyme pathway yet to be documented (Ouerdane and Mester 2008). Even though these mechanisms exist, not all yeasts synthesize both seleno amino acids, such as the case of *Saccharomyces cerevisiae* BY4741 which does not synthesize methionine using inorganic sulfur (Ouerdane and Mester 2008). Therefore, some genes involved in enzyme coding for selenium synthesis in *Saccharomyces cerevisiae* are likely orthologous to those found in *Saccharomyces boulardii*. So, given that the genome of *Saccharomyces cerevisiae* is the most studied and best characterized among eukaryotes (Fisk et al. 2006), in this work we performed an *in silico* study to identify the probable orthologous genes involved in selenium biosynthesis by *Saccharomyces boulardii* (nom. inval.) ASM141397v1 to propose a pathway for sodium selenate biotransformation into SeMet and SeCys.

**Materials And Methods**

**Identification of the aim**

The genetic comparison was carried out through an *in silico* study. *Saccharomyces boulardii* (nom. inval.) ASM141397v1 was used as study object. It was compared against the sequence of *Saccharomyces cerevisiae* s288c due to the genetic similarity between both yeasts. The process was carried out according to the models MM904 (King et al. 2016) and Kegg (Ogata et al. 1999) for *S. cerevisiae*. The information on the genomes of the yeasts was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/genome).

**Bioinformatic search**

To perform the search of the genes involved in the production of SeMet and SeCys, we used the Entrez in the NCBI database (https://www.ncbi.nlm.nih.gov/genome). We searched sequences of the genes reported in selenium metabolism for *Saccharomyces cerevisiae* s288c. The same procedure was carried out for *Saccharomyces boulardii* (nom. inval.) ASM141397v1. Sequences for the identification of orthologous genes were prepared.

**Orthologous genes**

With the information gathered, we searched the orthologous genes using the BLAST server at NCBI (Stephen et al. 1997). BLASTp program (protein-protein BLAST) was used to blast a protein sequence which became the input in FASTA format. In addition, we chose to collate the data exclusively with *S. cerevisiae* s288c to compare sequences and find regions of local similarity between them (Bhagwat and Aravind 2007). A total number of 9361 amino acids were analyzed, and a circular scheme was created with Circos software (http://circos.ca/).
According to the analysis of homologous genes found, a metabolic pathway was proposed for selenium assimilation and the subsequent production of SeMet and SeCys by *Saccharomyces boulardii*. The construction considered the proposals by Kieliszek et al. (2015) and Lazard et al. (2018) for *S. cerevisiae*.

**Results And Discussion**

**Bioinformatic search**

According to Lazard et al. (2018), the genes involved in the biotransformation of selenate into SeMet and SeCys for *Saccharomyces cerevisiae* are those shown in Table 1. These genes reported were searched in the gene sequence for *S. boulardii*. Although the genes identified for *S. boulardii* are putatively named, the enzyme they encode for is the same in both yeasts.
| Gene   | Chromosome | locus_tag Saccharomyces cerevisiae s288c | locus_tag Saccharomyces boulardii (nom. inval.) ASM141397v1 | Enzyme produced                                                                 |
|--------|------------|------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------|
| SUL1   | II         | YBR294W                                  | AB282_00450                                               | High affinity sulfate permease of the SulP anion transporter family             |
| SUL2   | XII        | YLR092W                                  | AB282_03394                                               | High affinity sulfate permease                                                |
| MET3   | X          | YJR010W                                  | AB282_02749                                               | ATP sulfurylase                                                               |
| MET14  | XI         | YKL001C                                  | AB282_03058                                               | Adenylylsulfate kinase                                                        |
| MET16  | XVI        | YPR167C                                  | AB282_05395                                               | 3'-phosphoadenylsulfate reductase                                             |
| MET10  | VI         | YFR030W                                  | AB282_01793                                               | Subunit alpha of assimilatory sulfate reductase                               |
| MET17  | XII        | YLR303W                                  | AB282_03569                                               | O-acetyl homoserine-O-acetyl serine sulfhydrylase                             |
| MET6   | Va         | YER091C                                  | AB282_01662                                               | Cobalamin-independent methionine synthase                                     |
| SAM1   | XII        | YLR180W“                                 | AB282_03468                                               | S-adenosylmethionine synthetase                                               |
| SAM2   | IV         | YDR502C                                  | AB282_00999                                               | S-adenosylmethionine synthetase                                               |
| SAH1   | Va         | YER043C                                  | AB282_01610                                               | S-adenosyl-L-homocysteine hydrolase                                           |
| CYS4   | VII        | YGR155W                                  | AB282_01996                                               | Cystathionine betasynthase                                                    |
| STR3   | VII        | YGL184C                                  | AB282_02293                                               | Peroxisomal cystathionine betal-lyase                                         |
| CYS3   | I          | YAL012W                                  | AB282_00053                                               | Cystathionine gammalyase                                                      |
| STR2   | X          | YJR130C                                  | AB282_02643                                               | Cystathionine gammasythase, converts cysteine into cystathionine              |
| GSH1   | X          | YJL101C                                  | AB282_02849                                               | Gamma glutamylcysteine synthetase                                             |
Orthologous genes

Once the genes in *S. boulardii* were identified, a homology analysis was conducted to verify whether the genes between strains were orthologous (Stephen et al. 1997). Figure 1 shows the results of the analysis in a circular plot to identify orthologous genes in *S. boulardii* and *S. cerevisiae*. Seventeen genes are arranged in ascending order to the left, according to the number of amino acids analyzed. To the right are yeasts *S. boulardii* and *S. cerevisiae*. There are homologous links between each gene and *S. cerevisiae* (blue) and *S. boulardii* (green). According to the BLASTp results, they encode the same enzymes and have the same functions. Then, *S. boulardii* can carry out selenium biosynthesis as *S. cerevisiae* does. The main difference was observed in genes SAH1 and MET6, which are found in the V chromosome of *S. cerevisiae* and the Va chromosome in *S. boulardii*, respectively.

Biosynthesis pathway of SeMet and SeCys in *S. boulardii* *(nom. inval.)* ASM141397v1

According to the results obtained in the search of genes involved in the biotransformation of selenium and following the KEGG PATHWAY database (Ogata et al. 1999) as well as the information available on selenium biotransformation by *S. cerevisiae* (Kieliszek et al. 2015; Lazard et al. 2018), we proposed a pathway of seleno amino acid bio synthesis by *S. boulardii*.

Discussion

The biotransformation of selenate into organic selenium starts with yeast detoxification by an excess of sodium selenate. The pathway through which this takes place is similar to that of sulfur metabolism for the production of sulfur-containing amino acids. In this pathway, selenium substitutes sulfur and is incorporated into the chemical structure of methionine and cysteine (Bierla et al. 2013).

Despite the homology between the gene sequences of these yeasts, *S. boulardii* has unique physiological and metabolic properties, such as resistance to temperature and acid stress (Fietto et al. 2004; Khatri et al. 2013). Similarly, hexose transporter genes (*HXT*11, *HXT*9), genes involved in asparagine catabolism (*ASP*3-1, *ASP*3-2, *ASP*3-3, *ASP*3-4), transporter gene ARN2, genes involved in thiamine or pyridoxin biosynthesis (*SNZ*2, *SNZ*3), and CUP1 metallothionein gene are absent from *Saccharomyces boulardii* *(nom. inval.)* ASM141397v1 (Khatri et al. 2017). However, none of these differences affects the capability of *S. boulardii* to biotransform inorganic selenium into organic selenium and its subsequent insertion in

| Gene          | Chromosome | locus_tag | Enzyme produced         |
|---------------|------------|-----------|-------------------------|
| *GSH2*        | XV         | YOL049W   | Glutathione synthetase  |

| locus_tag | Enzyme produced         |
|-----------|-------------------------|
| *saccharomyces boulardii (nom. inval.)* ASM141397v1 | |
seleno proteins, seleno-nanoparticle production, and reduction to elemental selenium by detoxification processes.

Noting that biosynthesis starts with selenium detoxification and is carried out through a pathway similar to that of sulfur, we propose the beginning of absorption. Selenium could be absorbed in two different ways. The first one is through sulfur ABC membrane transporters, which are encoded by operon cysAWTP and where transport for selenium ions uses energy from hydrolysis of bound ATP. The second system is through the transport of selenium using sulfate permeases (Kieliszek et al. 2015) encoded by AB282_00450 and AB282_03394. These enzymes transfer selenate through the plasma membrane from the exterior.

Once the selenate is in the interior, the biotransformation process starts with the activation of selenate. This process is carried out through a sequence of two reactions. In the first one, the rest of the adenosyl-phosphoryl is transferred from ATP to selenate by the action of enzyme ATP sulfurylase encoded by AB282_02749. This produces adenylyl selenate, which is in turn phosphorylated to produce 3’phosphoadenyllyl selenate through enzyme adenylyl-sulfate kinase (AB282_03058). Activated selenate is reduced to sulfite to carry out SeMet and SeCys biosynthesis. First, enzyme 3’-phosphoadenylsulfate reductase (AB282_05395) reduces it to adenosine 3’,5’-bisphosphate and free selenite, using reduced thioredoxin as substrate. Consecutively, the subunit alpha of assimilatory sulfite reductase (AB282_01793), turns selenite into hydrogen selenide. Selenide is transformed into selenohomocysteine by the action of O-acetylserine-O-acetylhomoserine sulfhydrylase (AB282_03569).

The biosynthesis reaction of SeMet from selenohomocysteine is catalyzed by enzyme cobalamin-independent methionine synthase (AB282_01662), where selenohomocysteine undergoes a methylation process to create SeMet. There is a dependence on cobalamin in the activation of methyltransferases, as in that of MetH isolated from E. coli (Thomas and Surdin-Kerjan 1997). Still, both in S. cerevisiae and S. boulardii, homocysteine methyltransferase is independent of cobalamin. This is verified since none require vitamin B12 as growth factor.

Additionally, SeMet creates S-adenosyl-selenomethionine through enzyme S-adenosylmethionine synthetase (AB282_03468/AB282_00999), the catalyzer when the adenosyl group of ATP is transferred to the selenium atom of methionine. There, selenohomocysteine is created again by enzyme S-adenosyl-L-homocysteine hydrolase encoded by AB282_01610, catabolizing S-adenosyl-L-homocysteine formed after the donation of the activated methyl group of S-adenosyl-L-methionine to a receptor. The substitution of methionine by SeMet in proteins does not significantly alter the kinetic properties of the enzymes (Kitajima and Chiba 2013).

On the other hand, the biosynthesis of SeCys from selenohomocysteine starts with the conversion of selenohomocysteine into selenocystathionine through the reaction catalyzed by the enzyme cystathionine β-synthase encoded by AB282_01996. The reaction is reversible by the action of the enzyme peroxisomal cystathionine β-lyase (AB282_02293) converting selenocystathionine into selenohomocysteine. A later step is the transformation of selenocystathionine into SeCys by the enzyme
cystathionine γ-lyase (AB282_00053). In addition, SeCys is transformed into γ-glutamyl-selenocysteine, the first step in the biosynthesis of selenogluthathione. Finally, selenogluthathione is formed by the action of glutathione synthetase (AB282_04624), which catalyzes the synthesis of ATP-dependent selenogluthathione from γ-glutamyl-selenocysteine and glycine (Fig. 2).

Conclusions

Due to the identification of orthologous genes between *S. cerevisiae* and *S. boulardii*, we established the biochemical pathway this probiotic yeast follows for the biotransformation of inorganic selenium into selenomethionine and selenocysteine. *In silico* studies allow for a theoretical approach of the biochemical mechanisms of a yeast like *S. boulardii*, which are greatly important to the technological use this yeast offers. The addition of *S. boulardii* to the processing of fermented foods has advantages, besides the tested probiotic capability, given the relevance of the study of organic selenium as a highly bioaccessible and bioavailable metabolite in the human body, as compared against inorganic selenium usually consumed. The bioaccumulation of selenium by this yeast could create seleno nanoparticles, whose use opens a field of opportunities and challenges in medicine as alternative therapies against diseases like cancer.

Declartions

Founding

Authors declare that this work did not have any funding

Conflicts of interest/Competing interests

Authors declare any conflict of interest

Availability of data and material

All materials and data are available for your consultation and use

Code availability (software application or custom code)

All the software used is free to use

Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lourdes González Salitre, Luis Guillermo González Olivares and Alma Delia Román Gutiérrez. The first draft of the manuscript was written by Luis Guillermo González Olivares and Gabriela Mariana Rodríguez Serrano. The guide for the use of the software and the interpretation of data was made by Judith Jaimez Ordaz and Mirandeli bautista Ávila. All authors read and approved the final manuscript.

**Ethics approval**

Not applicable

**Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**References**

1. Bartosiak M, Giersz J, Jankowski K (2019) Analytical monitoring of selenium nanoparticles synthesis using photochemical vapor generation coupled with MIP-OES and UV–Vis spectrophotometry green. Microchemical Journal. 145, 1169-1175.

2. Bhagwat M., Aravind L. (2007) PSI-BLAST Tutorial. In: Bergman N.H. (eds) Comparative Genomics. Methods in Molecular Biology™, vol 395. Humana Press.

3. Bierla K, Bianga J, Ouerdane L, Szpunar J, Yiannikouris A, Lobinski R (2013) A comparative study of the Se/S substitution in methionine and cysteine in Se-enriched yeast using an inductively coupled plasma mass spectrometry (ICP MS)-assisted proteomics approach. Journal of proteomics. 87, 26-39.

4. Czerucka D, Piche T, Rampal P (2007) yeast as probiotics–Saccharomyces boulardii. Alimentary pharmacology & therapeutics. 26(6), 767-778.

5. Fietto JL, Araújo RS, Valadão FN, Fietto LG, Brandão RL, Neves MJ, Castro IM, et. al (2004) Saccharomyces boulardii. Canadian journal of microbiology, 50(8), 615-621.

6. Fisk DG, Ball CA, Dolinski K, Engel SR, Hong EL, Issel-Tarver L, Michael Cherry J, et al (2006) Saccharomyces cerevisiae S288C genome annotation: a working hypothesis. Yeast. 23(12), 857-865.

7. Khatri I, Akhtar A, Kaur K, Tomar R, Prasad GS, Ramya TNC, Subramanian S (2013) Gleaning evolutionary insights from the genome sequence of a probiotic yeast Saccharomyces boulardii. Gut
pathogens. 5(1), 1-8.
8. Khatri I, Tomar R, Ganesan K, Prasad GS, Subramanian S (2017) Complete genome sequence and comparative genomics of the probiotic yeast Saccharomyces boulardii. Scientific reports. 7(1), 1-12.
9. Kieliszek M, Błażejak S Placzek M (2016) Spectrophotometric evaluation of selenium binding by Saccharomyces cerevisiae ATCC MYA-2200 and Candida utilis ATCC 9950 yeast. Journal of Trace Elements in Medicine and Biology. 35, 90-96.
10. Kieliszek M, Błażejak S, Gientka I, Bzducha-Wróbel A (2015) Accumulation and metabolism of selenium by yeast cells. Applied microbiology and biotechnology. 99(13), 5373-5382.
11. Kieliszek M, Błażejak S, Kurek E (2017) Binding and conversion of selenium in Candida utilis ATCC 9950 yeasts in bioreactor culture. Molecules. 22(3), 352.
12. Kieliszek M, Błażejak S (2013) Selenium: significance, and outlook for supplementation. Nutrition. 29(5), 713-718.
13. Kieliszek M, Dourou M (2020) Effect of Selenium on the Growth and Lipid Accumulation of Yarrowia lipolytica Yeast. Biological Trace Element Research. 1-12.
14. King ZA, Lu J, Dräger A, Miller P, Federowicz S, Lerman JA, Lewis NE, et al (2016) BiGG Models: A platform for integrating, standardizing and sharing genome-scale models. Nucleic acids research. 44(D1), D515-D522.
15. Kitajima T, Chiba Y (2013) Selenomethionine metabolism and its toxicity in yeast. Biomolecular concepts. 4(6), 611-616.
16. Lazard M, Dauplais M, Plateau P (2018) Contribution of the Yeast Saccharomyces cerevisiae Model to Understand the Mechanisms of Selenium Toxicity. In Selenium. Springer, Cham. pp. 71-87.
17. Martiniano SE, Philippini RR, Franco-Marcelino PR, da Silva SS (2020) Effect of selenium uptake on growth metabolism in yeasts for the production of enriched single-cell protein using agro-industrial by-products. Biomass Conversion and Biorefinery. 1-9.
18. McCullough MJ, Clemons KV, McCusker JH, Stevens DA (1998) Species Identification and Virulence Attributes of Saccharomyces boulardii (nom. inval.). Journal of clinical microbiology. 36(9), 2613-2617.
19. Moon JE, Heo W, Lee SH, Lee SH, Lee HG, Lee JH, Kim YJ (2020) Trehalose Protects the Probiotic Yeast Saccharomyces boulardii against Oxidative Stress-Induced Cell Death. Journal of Microbiology and Biotechnology. 30(1), 54-61.
20. NCBI. Available from https://www.ncbi.nlm.nih.gov/genome. Accessed Jan. 24, 2021.
21. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic acids research. 27(1), 29-34.
22. Ouerdane L, Mester Z (2008) Production and characterization of fully selenomethionine-labeled Saccharomyces cerevisiae. Journal of agricultural and food chemistry. 56(24), 11792-11799.
23. Patel N, Kaler A, Jain S, Chand Banerjee U (2013) Biosynthesis of selenium nanoparticle by whole cells of Saccharomyces Boulardi and its evaluation as anticancer agent. Current Nanoscience. 9(4),
24. Pérez-Corona MT, Sánchez-Martínez M, Valderrama MJ, Rodríguez ME, Cámara C, Madrid Y (2011) Selenium biotransformation by Saccharomyces cerevisiae and Saccharomyces bayanus during white wine manufacture: Laboratory-scale experiments. Food chemistry. 124(3), 1050-1055.

25. Prange A, Sari M, von Ameln S, Hajdu C, Hambitzer R, Ellinger S, Hormes J (2019) Characterization of selenium speciation in selenium-enriched button mushrooms (Agaricus bisporus) and selenized yeasts (dietary supplement) using X-ray absorption near-edge structure (XANES) spectroscopy. Journal of Trace Elements in Medicine and Biology. 51, 164-168.

26. Rajashree K, Muthukumar T (2013) Preparation of selenium tolerant yeast Saccharomyces cerevisiae. Journal of Microbiology and Biotechnology Research. 3(3), 46-53.

27. Schrauzer GN (2000) Selenomethionine: a review of its nutritional significance, metabolism and toxicity. The Journal of nutrition. 130(7), 1653-1656.

28. Stephen FA, Thomas LM, Alejandro AS, Jinghui Z, Zheng Z, Webb M, David JL (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402

29. Suhajda A, Hegoczki J, Janzso B, Pais I, Vereczkey G (2000) Preparation of selenium yeasts I. Preparation of selenium-enriched Saccharomyces cerevisiae. Journal of Trace Elements in Medicine and Biology. 14(1), 43-47.

30. Thomas D, Surdin-Kerjan Y (1997) Metabolism of sulfur amino acids in Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 61(4), 503-532.

31. Wang T, Lou X, Zhang G, Dang Y (2019) Improvement of selenium enrichment in Rhodotorula glutinis X-20 through combining process optimization and selenium transport. Bioengineered. 10(1), 335-344.

Figures
Figure 1

Circular representation to identify orthologous genes in Saccharomyces boulardii (nom. inval.) ASM141397v1 (green) and Saccharomyces cerevisiae s288c (blue)
Figure 2

Proposed metabolism pathway of selenium by Saccharomyces boulardii (nom. inval.) ASM141397v1

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