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Full Length Research Paper

Investigating the role of *Candida glabrata SLN*1 gene in stress adaptation: *In silico* and molecular analysis

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The orthologous gene to histidine kinase *SLN*1 of *Saccharomyces cerevisiae* was found in the *Candida glabrata* genome database; we performed both *in silico* and molecular analyses of this gene. A phylogenetic analysis confirmed the close relatedness between these orthologous genes. We found that *C. glabrata* Sln1p presents two functional domains: a histidine kinase domain, and a response regulator domain, both similar to *S. cerevisiae*. Eight possible nitrogen source-activated transcription-factor binding sites and one for Mns4p/Mns2p were found. Expression analyses by semi-quantitative RT-PCR revealed that this gene was down-regulated when the yeast was cultured under osmotic and oxidative stress conditions, different nitrogen sources and acidic pH; but in alkaline pH, the expression of the *SLN*1 gene was recovered after 6 h. Hence, our results suggested that the *SLN*1 gene is involved in adaptation to different environmental stress conditions, similarly to the orthologous genes present in *S. cerevisiae* and *Candida albicans*

Key words: *Candida glabrata*, histidine-kinase Sln1p, osmotic and oxidative stress.

INTRODUCTION

The genus *Candida* comprises several species. *Candida albicans* is the most important among them because it is the most common pathogenic yeast in mucosal and systemic fungal infections. However, in recent years, an increase in reports on non-*albicans Candida* (NAC) species, such as *Candida glabrata*, *Candida krusei*, *Candida lusitanie* and *Candida dubliniensis* place these as the fourth most common cause of nosocomial infections (Fridkin and Jarvis, 1996; Pfaller et al., 1999, 2009; Pinjon et al., 2005; Sullivan et al., 1995). This has led to the consideration of these *Candida* species as opportunistic pathogens. The relevance of NAC species lies in two very important reasons: first, the virulence and pathogenicity of some NAC species in the immunocompromised host, resulting in significant mortality, and second, the rate of resistance to currently used antifungal drugs (Krcmery and Barnes, 2002).

In recent years, *C. glabrata* has increased its prevalence in systemic infections (Clark and Hajjeh, 2002). It is the

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second most prevalent fungal pathogen in humans after *C. albicans* and shows the highest mortality rate among NAC species (Krcmery and Barnes, 2002).

C. glabrata is a haploid yeast that is more phylogenetically related to *Saccharomyces cerevisiae* than to *Candida* species (Fitzpatrick et al., 2006). Some of the most virulent factors studied in *C. albicans* do not appear to be conserved in *C. glabrata* (Castaño et al., 2006; Cormack, 2004). These include hyphal formation, mating and secreted proteolytic activity (Calderone and Fonzi, 2001; Bairwa and Kaur, 2011). Nevertheless, they share other important characteristics and cause very similar infections because of adaptation to adverse environmental conditions during the infectious process. This is an essential survival mechanism of pathogenic fungi. Such adaptation is mediated by signal transduction pathways known as the two-component system, which usually consists of two proteins: one is a membrane-bound sensor protein histidine kinase (Sln1p), and the second, is a cytoplasmic response regulator protein (Ssk1p) (Cashin et al., 2006). This system activates the intracellular pathways mediated by Mitogen-Activated Protein Kinases (MAPK) (Appleby et al., 1996; Hohmann et al., 2007; Loomis et al, 1997), which are important components of the cellular adaptation system in changing environmental conditions. The two-component signal transduction system of *C. albicans* includes three histidine kinases (CaSln1p, CaNik1p and CaChk1p), two response regulator proteins (CaSsk1p and CaSkn7p) and a cytoplasmic intermediate protein denominated phosphoryl-histidine (CaYpd1p) (Calera and Calderone, 1999; Santos and Shiozaki, 2001). The three histidine kinases are orthologous to proteins involved in the MAPK and HOG1 pathways of *S. cerevisiae* related with osmoadaptation processes (Hohmann, 2002); but in *C. albicans*, these proteins are related to phenomena such as oxidative stress, morphogenesis, dimorphism, biofilm formation, adherence and cell wall biosynthesis (Calera and Calderone, 1999; Alfonso-Monge et al., 2003; Bernhardt et al., 2001; Calera et al., 2000; Chauhan et al., 2003, 2007; Kruppa et al., 2003, 2004; Li et al., 2002; Selitrennikoff et al., 2001; Singh et al., 2004; Torosantucci et al., 2002; Yamada-Okabe et al., 1999). Studies on the histidine kinases have shown the relevance of these proteins in cellular differentiation, antifungal resistance and the expression of the principal virulence factors related to yeast pathogenesis in *Coccidioid*es *immitis*, *Paracoccidiodes brasiliensis*, *Penicillium marneffei*, *Histoplasma capsulatum*, *Sporothrix schenckii* and *Blastomyces dermatitidis* (Nemecek et al., 2006). This information suggests that the pathogenesis process of many fungi can be regulated by the signal transduction system of two components (Bahn et al., 2006; Viaud et al., 2006; Yoshimi et al., 2005).

The complete genome of *C. glabrata* was sequenced by Dujon et al. (2004). This has allowed its analysis and comparison with related yeasts such as *Schizosaccharomyces pombe* and *S. cerevisiae*.

The yeast's Sln1p sensor kinase protein is the initiating member of a two-component system and is best known as an osmosensor that is involved in the regulation of the hyperosmolarity glycerol mitogen-activated protein kinase cascade (MAPK). Down-regulation of Sln1p kinase activity occurs under hypertonic conditions and leads to phosphorylation of the Hog1p mitogen-activated protein kinase and to increased osmotic stress-response gene expression. Conditions leading to kinase up-regulation include osmotic imbalance caused by glycerol retention in a glycerol channel mutant of *S. cerevisiae* (Shankarnarayan et al., 2008; Tao et al., 1999). In *S. cerevisiae*, loss of Sln1p kinase activity (null mutant) leads to inappropriate and lethal activation of the HOG pathway (Ostrander and Gorman, 1999; Ota and Varshavsky, 1992). If *SLN1* is deleted, then the Ssk1p response regulator is constitutively active, because it is unphosphorylated in both stressed and unstressed cells and leads to inviability in *S. cerevisiae*, but not in *C. albicans*. Thus, in the *C. albicans SLN*1 mutant, Ssk1p is presumably unphosphorylated, resulting in the down-regulation of histidine kinase protein (Chk1p) transcription. In *C. albicans*, this protein is not critical for viability, as in *S. cerevisiae*, Nevertheless, the *SLN*1 gene from *C. albicans* complements a conditional mutant in *S. cerevisiae* (Nagahashi et al., 1998), suggesting that this protein works in a similar way in both microorganisms; but in *S. cerevisiae*, its function is critical (Kruppa and Calderone, 2006). The HOG signaling in *C. glabrata* mediates the response to multiple stress conditions, not only to osmostress adaptation (Gregori et al., 2007).

The proteins of this system have been proposed as possible therapeutic targets, especially the histidine kinase protein, because this protein has not been found in mammalian cells and due to its role in different processes that are influenced by environmental factors, such as temperature, pH, nitrogen and carbon sources (Ernst, 2000). Thus, the main aim of the present study was to analyze if the expression of the gene *SLN*1 gene in *C. glabrata* is affected by different culture conditions. We analyzed the complete genome of *C. glabrata* and found a nucleotide sequence of a putative*SLN*1 gene, which is closely related to *S. cerevisiae*. The presence of this gene in the genome of *C. glabrata* was confirmed by PCR. Using bioinformatics, we found 8 different transcription factors binding sites related to different environmental conditions in a putative promoter region 1000 bp upstream the start codon. Finally, we evaluated *SLN*1 gene expression utilizing a semi-quantitative RT-PCR method under different environmental and stress conditions (pH, nitrogen sources, osmotic and oxidative stresses). This work is, to our knowledge, the first to

report the regulation of the *SLN*1 gene of *C. glabrata* under different culture conditions.

MATERIALS AND METHODS

Strains and growth conditions

C. glabrata strain CBS138 (kindly donated by Professor Bernard Dujon from the Institute Pasteur, France) was routinely cultivated in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 37°C under agitation (200 rpm). Strains were sub-cultivated in solid YPD media with 2% agar.

Bioinformatics analyses

The putative *C. glabrata SLN*1 gene was identified by its similarity with the *S. cerevisiae SLN*1 gene. Sequence data for *C. glabrata* were obtained from the Genolevures Consortium website (http://www.genolevures.org/download.html). Isoelectric point, hydrophobicity region prediction, and helical membranous regions were determined with Antheprot 2000 software program version 5.0 employing the protein sequences Sln1p. Motif prediction was performed with Prosite (http://www.expasy.org). Subcellular localization was determined with PSORTII at http://www.psort.org/ and with Softberry (http://www.softberry.com). Prediction of the transcription factors binding sites was performed with the MatInspector program (http://www.gsf.de/biodv/matinspector.html) analyzing putative regulatory *SLN*1 gene sequences 1000 pb upstream of the starting codon (ATG). A phylogenetic tree of the *C. glabrata* Sln1p and histidine kinase proteins of different *Candida* species and *S. cerevisiae* was constructed with MEGA program version 3.1 (Molecular Evolutionary Genetics Analysis) using the Maximum Parsimony model. Percentage similitude and identity was calculated utilizing MatGat. Analysis was based on multiple alignment (data not shown) employing neighbor-joining grouping method (Saitou and Nei, 1987). Statistical evaluation included 1,000 bootstrap resamplings.

Detection of the *SLN*1 gene in the *C. glabrata* genome by PCR

Using the sequence that encodes for the *SLN*1 gene obtained from the genome of *C. glabrata*, specific primers were designed utilizing the DNAman program (Lynnon BioSoft 1994-1997, Pointe-Claire, Quebec, Canada). The primers used were *SLN*1Fw 5´TGCTCTCGACCAACACTACGC3´ and *SLN*1Rv 5´CGGCTTTAGTTGCTTCCTCG3´ (expected product size 572 bp). DNA was extracted employing the CTAB method (Allers and Lichten, 2000). The *SLN*1 gene was amplified by PCR, utilizing Taq polymerase (Invitrogen, Carlsbad, CA, USA). The reaction mixture was performed with 1X buffer, 3 mM $MgCl_2$, 0.2 mM dNTP, 20 pmol of each primer *SLN*1Fw/*SLN*1Rv, and 50 ng genomic DNA. Amplification conditions were as follows: 94°C, 5 min; 35 cycles of denaturation at 94°C, 1 min; annealing at 62°C, 1 min; polymerization at 72°C, 1 min, and final polymerization at 72°C, 10 min. PCR products were separated on 2% Agarose gels and purified.

Cg*SLN*1 gene expression analysis by semi-quantitative RT-PCR

Semi-quantitative reverse transcription (RT)-PCR analyses of *C. glabrata* putative*SLN*1 gene were used to study its response to different stress conditions. The osmotic stress was analyzed in YPD medium plus NaCl (1.0, 1.5 and 2.0 M). The oxidative stress was analyzed in YPD medium plus H_2O_2 (5, 10, 15 and 20 mM). The different pH (3 and 9) was adjusted with HCl and NaOH to YPD medium. The three different nitrogen sources were analyzed using 0.17% YNB medium adding 2% proline, 0.5% $(NH_4)_2SO_4$, and without nitrogen source. The *C. glabrata* CBS138 was preincubated for 12 h in liquid YPD medium. This was done considering time zero to the expression of putative Cg*SLN*1 gene. The cells were recovered and resuspended in sterile water. The inocula were adjusted at 9×10^8 colony forming unit (CFU)/mL concentrations. Then YPD and YNB media with different stress conditions were inoculated and incubated at 37°C for 5, 30, 60, 90, 180 and 360 min. YPD medium was used as control.

Total RNA was extracted from cell pellets by the Farrell (2009) method. The quality of RNA was confirmed in agarose gels with formaldehyde 0.6 M. The quantity and purity of RNA was estimate using absorption of light 260/280 nm. Just the RNA´s with a 260/280 ratio close to 2 was used. To minimize the risk of DNA contamination, 10 U of DNAse I amplification grade (Invitrogen) was utilized. The elimination of all DNA was confirmed applying on the samples the PCR reaction for the *SLN*1 gene described above. Oligo (dT) 500 µg/mL primer was employed to amplify total mRNA. The cDNA was synthesized by using SuperScript III Reverse Transcriptase (Invitrogen) following manufacturer's instructions. PCR was performed with Taq DNA polymerase (Invitrogen) and the primers *SLN*1Fw/*SLN*1Rv and 18S rRNA Fw 5´CAATTGGAGGGCAAGTCTGG3´ and 18SrRNA Rv 5´TAAGAACGGCCATGCACCAC3´. *SLN*1 gene amplification was performed as mentioned previously. The 18S rRNA gene amplification conditions were as follows: 94°C, 5 min; 35 cycles of denaturation at 94°C, 1 min; annealing at 65°C, 1 min; polymerization at 72°C, 1 min, and final polymerization at 72°C, 10 min. The PCR products were visualized by electrophoresis in 2% agarose gels. Expression of the *SLN*1 gene was normalized against levels of 18S rRNA cDNA using the program SigmaGel version 1.0 (Jandel Scientific, San Rafael, CA, USA). Extensive experimentation was done to define the best conditions to perform the analysis. Once these conditions were set, we ran biological duplicates (from culture until RT-PCR). RNA extractions and RT-PCR were done twice.

RESULTS

Bioinformatic analyses

A complete search was conducted on whole genome sequences using the histidine kinase domain. In *C. glabrata*, only one sequence was found which encodes a very similar protein to histidine kinase Sln1p Genebank ID 2888762. This putative gene (*SLN*1) was found on chromosome H, its length is 3,510 bp, and no introns were present. The translated sequence showed that this gene encodes for a membranal protein of 1,169 amino acid residues with a calculated molecular weight of 130.7 kDa and a pI of 5.945. It was observed, as occurs in *S. cerevisiae*, that the *C. glabrata* Sln1p protein presents two functional domains: the histidine kinase domain in the region between nucleotides 536 and 879, and a receiving domain from nucleotide 1021 to 1142 (Figure 2). Also, the orthologous sequences of *C. albicans*, *S. cerevisiae* and *Kluyveromyces lactis* were analyzed. A total of eight

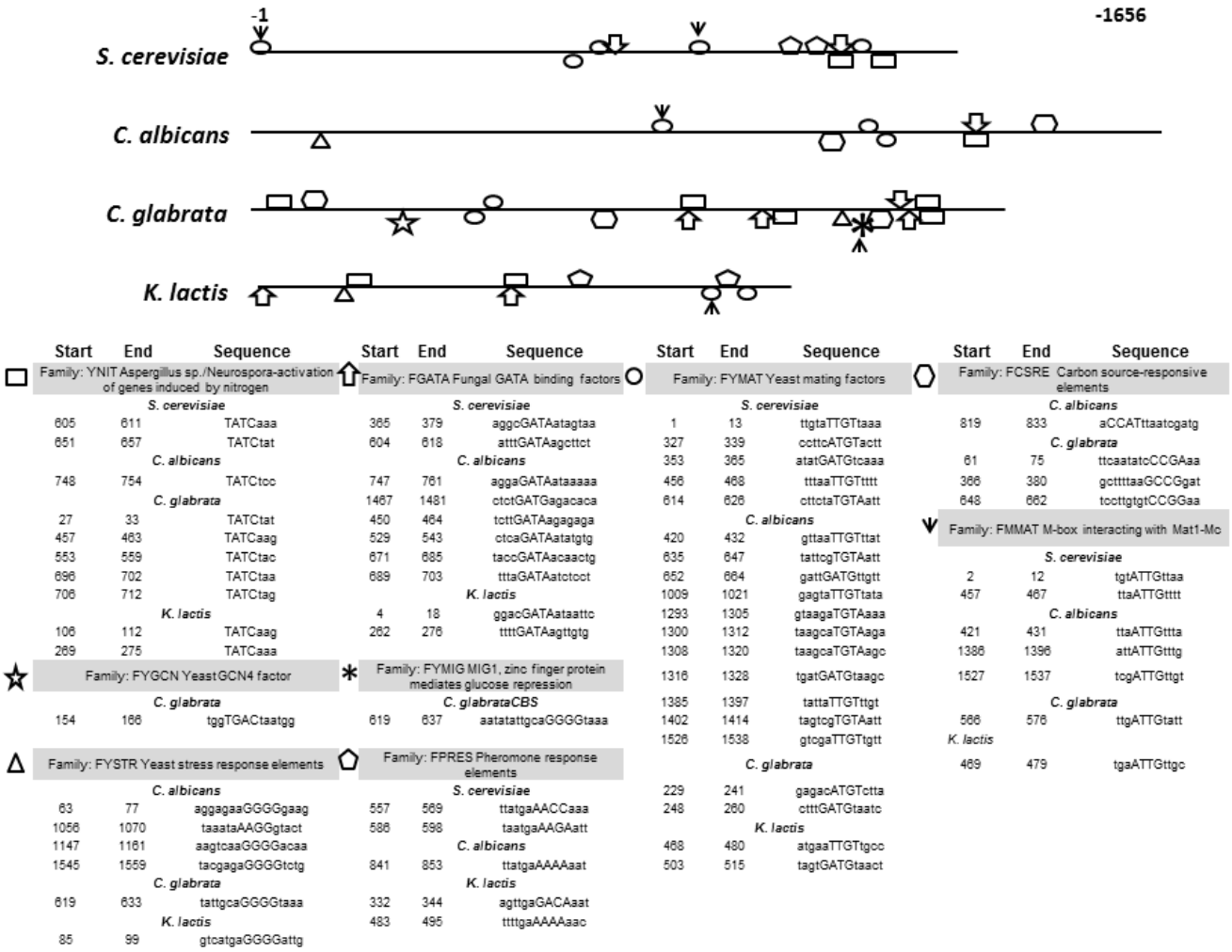

Figure 1. Possible binding site for transcription factors of the *SLN*1 gene from *C. glabrata* and related yeasts.

possible binding sites for transcription factors activated by nitrogen source and one by carbon source were recognized; in addition, one site was found to be related to the environmental stress response (EST). The possible binding sites that regulate adaptations to carbon and nitrogen sources are more numerous in *S. cerevisiae*, *K. lactis* and *C. glabrata*; moreover, the sequence for amino acid synthesis regulation is not present in *C. albicans*. Mating transcription-factor sites are present in the yeast analyzed. The EST binding site is shared only by *C. glabrata* and *C. albicans*. The possible binding-site sequences and its position are shown in Figure 1. Additionally, a Basic Local Alignment Search Tool (BLAST) analysis was performed utilizing the deduced amino acid sequences of the *SLN*1 gene from several yeasts. The functional domains HK (histidine kinase) and RR (response regulatory) are showed in Figure 2. A phylogenetic tree of the Sln1p was constructed (Figure 3), where the tree topology was similar to that obtained with other molecular markers. Thus, *C. glabrata* appears closer to *S. cerevisiae* than other *Candida* spp., confirming the results of Scannell et al. (2007) who used a different molecular marker.

*SLN*1 gene expression analysis by semi-quantitative RT-PCR

Employing the designed primers, the Cg*SLN*1 gene was detected by PCR from the chromosomal DNA of the *C. glabrata* CBS138 (data not shown). Four stress and nutritional conditions were chosen to analyze Cg*SLN*1expression: osmotic (Figure 5A) and oxidative stress (Figure 5B); pH (Figure 5C) and different nitrogen sources (Figure 5D). In general, we observed down-regulation of the putative Cg*SLN*1 gene when *C. glabrata* cells were resuspended in sterile water, indicating that CgSln1p acts as an immediate response protein.

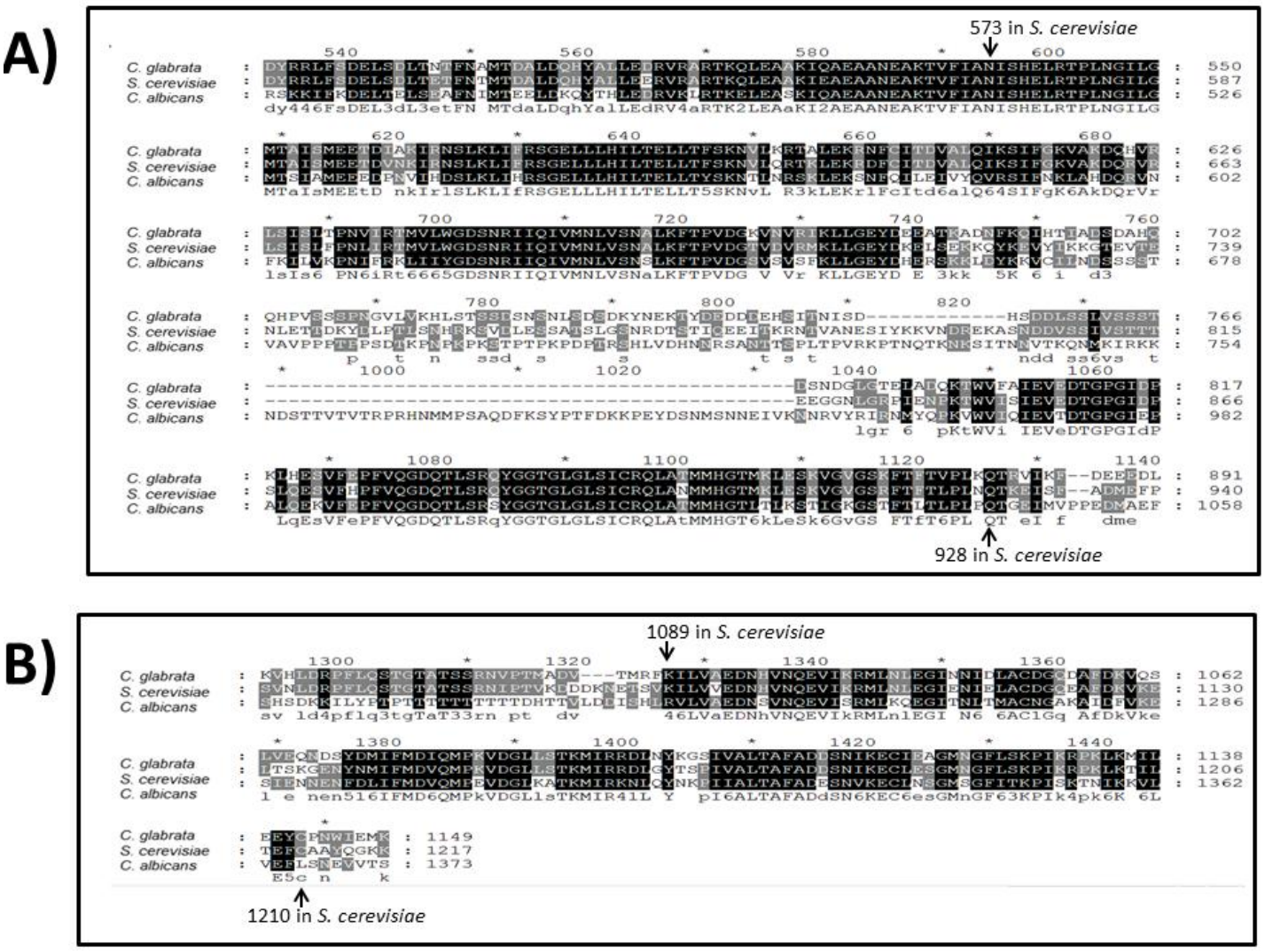

Figure 2. Alignment of histidine kinase osmosensor Sln1p of *S. cerevisiae*, and putative Sln1p of *C. glabrata* and *C. albicans*. A) Histidine kinase domain from amino acid 573 to amino acid 928 of *S. cerevisiae* Sln1 protein (black arrows). This domain receives the signal from the sensor partner in a two-component system. B) Response regulatory domain from amino acid 1089 to amino acid 1210 of *S. cerevisiae* Sln1 protein (black arrows). This domain have three activities: First, they interact with phosphorylated HKs and catalyze transfer of a phosphoryl group to one of their own Asp residues; second, they catalyze autodephosphorylation; and finally they regulate the activities of their associated effector domains in a phosphorylation-dependent manner.

Furthermore, down-regulation was maintained for 30 min under all conditions, YPD medium, osmotic stress (NaCl), oxidative stress (H_2O_2), and extreme pH (Figure 5A to D); however, at 60 min, *SLN*1 gene expression was recovered in the control YPD medium and at alkaline pH (pH 9) (Figure 5C), but not under any of the remaining conditions. The constitutive 18S rRNA gene was amplified under all conditions, ensuring the quality of the RNA (Figure 4). In particular, osmotic stress down-regulates expression of the Cg*SLN*1 gene. Especially under the NaCl 2 M condition (Figure 5A), expression is nearly undetected until 180 min; in addition to that, the level of expression is always lower than expression in the YPD medium in all NaCl concentrations tested (Figure 5A). The oxidative stress induced by H_2O_2 at different concentrations (5 to 20 mM) caused down-regulation of the Cg*SLN*1 gene; the level of expression of the gene was not recovered during the time of the experiment (360 min) at any of the concentrations used (Figure 5B). Figure 5C depicts the pH effect on expression of the Cg*SLN*1 gene; in this case, expression is down-regulated by the acid condition (Figure 5C) during the entire time of the experiment (360 min). However, expression is restored at a comparable level to the YPD control (neutral pH) under the alkaline condition (Figure 5C) from 90 to 360 min. Expression of the Cg*SLN*1 gene was down-

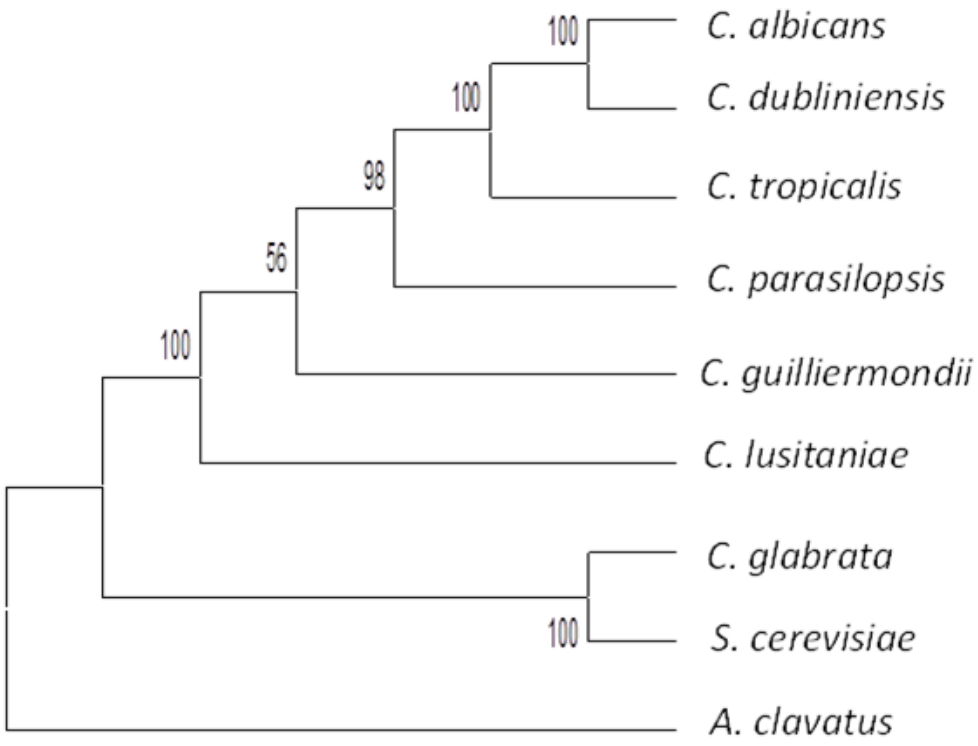

Figure 3. Phylogenetic tree based on amino acid sequences of *C. glabrata* Sln1p [Accession number XP_447081] and other histidine kinases described in *S. cerevisiae* [Accession number NP_012119 and XP_717880] and Non-*albicans Candida* [NAC], *Candida dubliniensis* [Accession number XM_002421651], *Candida lusitaniae* [ABO84859*], *Candida tropicalis* [CTRG00987*], *Candida parapsilosis* [CPAG02975*], *Candida guilliermondii* [GUG03258*] and *Asperguillus clavatus* [XP_001273137*]. The bar indicates number of nucleotide changes per 100 nucleotides. *These accession numbers are from the Broad Institute of MIT and Harvard. http://www.broadinstitute.org/regev/orthogroups/html/2/9/OG25229.html.

regulated when the nitrogen source was changed from complex (YPD) to simpler (proline and NH_4^+) and non-nitrogen source (YNB without nitrogen) (Figure 5D). Moreover, the growth of *C. glabrata* CBS138 under these nitrogen sources was one and half of that obtained in YPD medium, while there was no growth in YNB without nitrogen medium (data not shown). When the cells were not washed with water, the *SLN*1 gene expression was not lost in YPD medium, but in the osmotic, oxidative and under different nitrogen sources. pH was not tested (data not shown).

DISCUSSION

The main conclusion drawn from this study is that the Cg*SLN*1 gene is related to the response to osmotic and oxidative stress, as well as to different pH values and nitrogen sources. The close phylogenetic relationship exhibited by *C. glabrata* with *S. cerevisiae* leads to the assumption that the Sln1p could possess a similar function in both yeasts. In this work, we used bioinformatic tools and found that there is only one orthologous gene to the *SLN*1 gene of *S. cerevisiae* that encodes only one putative membrane histidine kinase protein in *C. glabrata*. The CgSln1p presents very similar characteristics with those demonstrated by *S. cerevisiae* and *C. albicans* (Román et al., 2005). Our phylogenetic analysis of this protein reflects a similar topology to those obtained with other molecular markers, confirming a tight phylogenetic relation between *C. glabrata* and *S. cerevisiae* (Fitzpatrick et al., 2006), that is, protein CgSln1p is phylogenetically related to the Sln1p of *S. cerevisiae* than to that of other pathogenic *Candida* species.

The *SLN*1 gene encodes a sensor hybrid histidine kinase with two membrane-spanning regions and an extracellular sensing domain (Ota and Varshavsky, 1992). Prediction of possible elements that regulate expression of this gene served to establish the environmental conditions that could affect its expression. It possesses binding sites activated by the nitrogen source, which is interesting because nutritional stress leads to filamentous growth in *S. cerevisiae* (Hohmann et

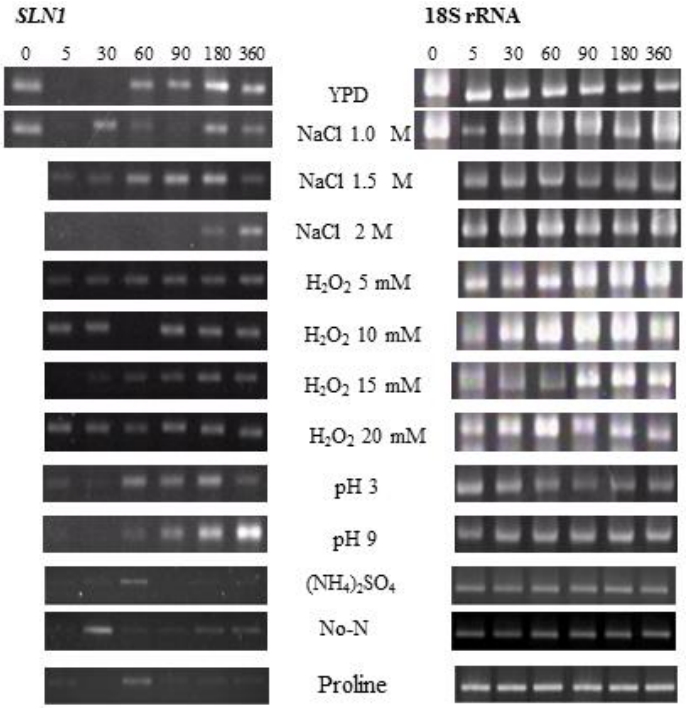

Figure 4. Differential expression of the Cg*SLN*1 gene in *C. glabrata* CBS138 during osmotic stress by NaCl, oxidative stress by H_2O_2, acid and alkaline pH and different nitrogen sources by RT-PCR technique.

al., 2007), and *C. albicans*, this nutritional condition favors dimorphism and generation of pseudohyphae, which has been considered a virulence factor (Gow, 1997). In *C. glabrata*, when grown in solid medium with deficient nitrogen source, important morphological changes take place, including the formation of structures resembling pseudohyphae (Csank and Haynes, 2000). Additionally, a binding site to factors Msn2p/Mns4p was found; these are normally detected in the cytoplasm and are transported to the nucleus during stress conditions. Studies with mutants lacking *MNS2/MNS4* genes in *S. cerevisiae* and *C. glabrata* confirmed that these factors activate the responses shared by stress conditions (Causton et al., 2001; Cuellar-Cruz et al., 2008). Interestingly, a binding site for Msn2p/Mns4p was found for Cg*SLN*1, a characteristic shared with *C. albicans*, but not with *S. cerevisiae*; we think that this is the reason why when we washed the cells with cold water, the Cg*SLN*1 expression was down-regulated, indicating a general stress response.

During NaCl-induced hyperosmotic stress conditions, it was found that Cg*SLN*1decreases its expression (Figure 5A). This is directly related to findings in *S. cerevisiae* (Causton et al, 2001), in which this gene was found to be of utter relevance, in that, it is vital for the survival of the yeast because osmoregulation is an active cellular process exerted to maintain the water balance in the cell, in addition to involving crucial homeostatic processes (Hohmann et al., 2007). We also observed that diminution in gene expression occurs almost immediately. *S. cerevisiae* reacts almost immediately after the osmotic stress signal, both at the transcriptional and protein level. In fact, in less than one minute, the glycerol Fps1p-exporting channels closed rapidly to prevent the loss of intracellular glycerol under hyperosmotic stress conferring osmotolerance (Tamás et al., 2003); at the same time, the HOG1 pathway was activated (Hohmann, 2002).

Similar results were obtained with the oxidative stress down-regulation of gene *SLN*1 of *C. glabrata*; this decrease persisted under different H_2O_2 concentrations (Figure 5B). The decrease in gene *SLN*1 under oxidative stress conditions in *S. cerevisiae* was described previously by Causton et al. (2001), and because the yeast *C. glabrata* is phylogenetically very close to *S. cerevisiae*, it is reasonable to think that it will react similarly. Again, it was observed that the response occurs immediately and the gene recovers its expression slowly during the time of analysis. Responding rapidly to oxidative stress conditions is fundamental to pathogenic microorganisms, in that, it is directly related to evasion of the host immune response. Polymorphonuclear leukocytes are the first line of defense against candidosis; these cells generate reactive oxygen species

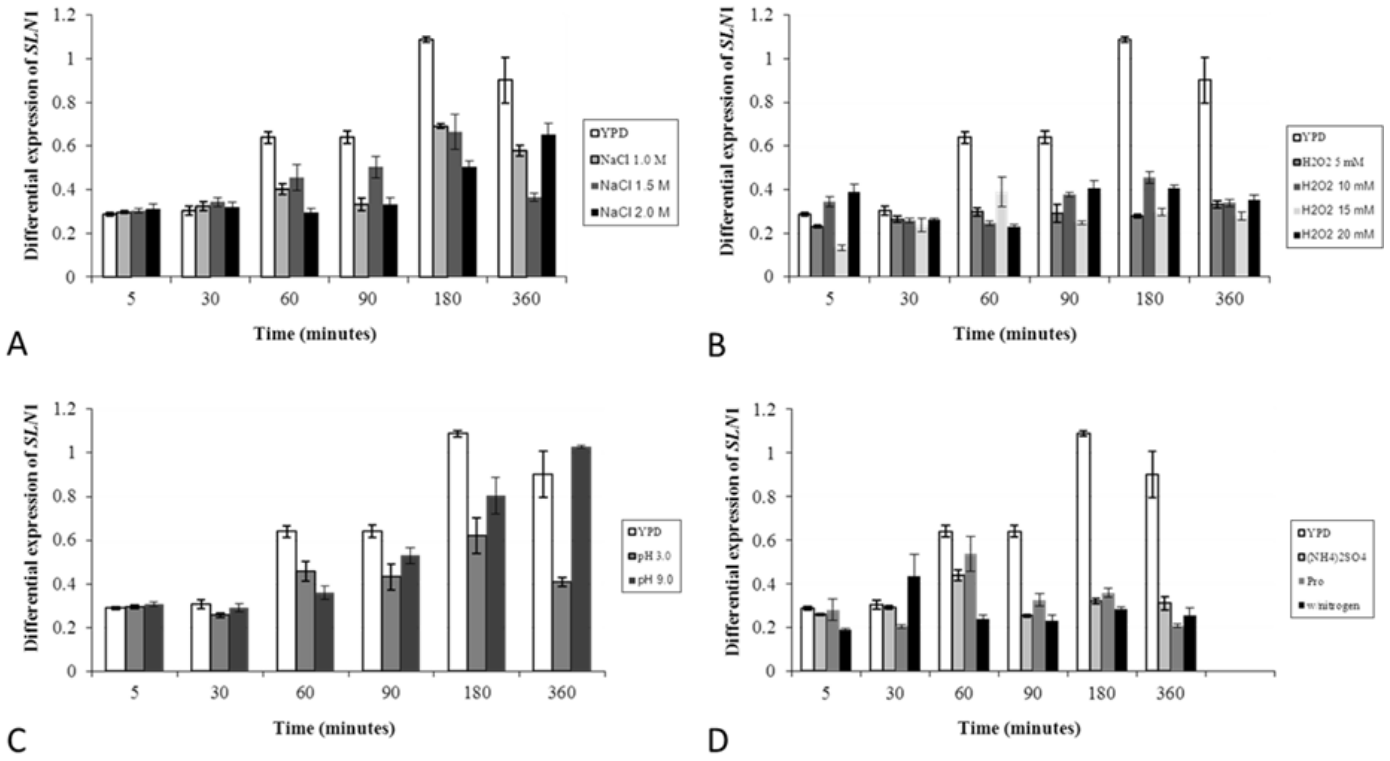

Figure 5. Differential expression of the Cg*SLN*1 gene in *C. glabrata* CBS138: A) during osmotic stress with NaCl (1.0; 1.5 and 2.0 M). B) during oxidative stress with H_2O_2 (5; 10; 15 and 20 mM). C) under different pH (3 and 9). D) under different nitrogen source (proline; $[NH_4]_2SO_4$ and YNB without nitrogen source). YPD medium was used as control. The intensity of the Cg*SLN*1 cDNA bands was normalized to the intensity of 18S rRNA cDNA obtained in the same stress condition. Error bars indicate the range of two independent experiments (biological duplicates) and four replicates.

(ROS), which include hydroxyl radicals, superoxide anion and hydrogen peroxide (H_2O_2), which can kill the fungal cells (Yale and Bohnert, 2001; González-Párraga et al., 2003). *C. glabrata* is capable of tolerating high H_2O_2 concentrations (Thorpe et al., 2004) as compared to *C. albicans* and *S. cerevisiae*. This resistance depends on transcription factors Yap1p, Skn7p, and Mns4p; in fact, for the latter one, a possible binding site to gene *SLN*1 of *C. glabrata* was found by the *in silico* analysis. It has been observed that the main transcription factor related to this response is Skn7p, which is part of the two-component system and is activated by the histidine kinase protein Sln1p and, as mentioned, this protein is a negative regulator in this system (Cuellar-Cruz et al., 2008).

Culturing *C. glabrata* under different nitrogen sources also induced a decrease of gene *SLN*1 expression (Figure 5D); this correlates with data obtained in the bioinformatic analysis, which revealed that this gene has eight possible binding sites for the nitrogen source-activated transcription factors. Also, the two-component system is implicated in the development of pseudohyphae; however, *C. glabrata* is not dimorphic, but when the yeast is cultured in solid medium with deficient nitrogen source, it is capable of developing pseudohyphae-like structures (Csank and Haynes, 2000); therefore, it is important to understand how this protein interacts with the nitrogen metabolism of the yeast.

The signaling system that responds to the extracellular pH has been identified in *S. cerevisiae*, *A. nidulans* and *C. albicans* (Roetzer et al., 2011). These pathways are specific for each fungus and have not been found in animal cells; therefore, the components of the response to the pH have been suggested as therapeutic targets, similar to those of the two-component system. In *S. cerevisiae*, this response is composed of the transcription factor Rim101p/PacC, which must be anchored in its active form to allow for growth of the yeast under different pH (Roetzer et al., 2011; Davis, 2003). The change in pH is an inducer of cellular differentiation and development. *C. albicans* requires growth in an alkaline pH to be able to develop hyphae and dimorphism is essential for the pathogenesis of this microorganism. *C. glabrata* does not present dimorphism, but, similar to *C. albicans*, it strives to grow in environments with very varied pH; hence, it is critical for it to possess one or more adaptation mechanism to these variations. Performing a search in the genome, we found that *C. glabrata* possesses all the

RIM genes that have been described for *S. cerevisiae* as necessary for adaptation to different pH. Expression analyses revealed that, as under the previously described conditions, the gene *SLN*1 decreases its expression at an acidic pH; however, at alkaline pH, there is a recovery of the expression, the expression level was nearly reached under optimal conditions (Figure 5C). It has not been described yet how the signal transduction pathway of the two-component system interacts with the pH-regulation signaling pathway; however, we suggested that there might be a probable relation or connection between both pathways, because it has been observed that both the two-component system and the pH signaling pathways are related with some processes, such as pseudohyphae formation and virulence factor expression, which favor the infectious process (Calera et al., 2000). Hence, it would be interesting to continue with studies that tries to elucidate the role of histidine kinases in other signaling pathways. In this work, we observed that the *SLN*1gene of *C. glabrata* decreases its expression under diverse environmental stress conditions, evidencing its possible participation in the environmental adaptation process of this yeast, as occurs in *S. cerevisiae*. *C. glabrata* has been considered as normal microbiota of healthy individuals, occasionally causing infections in humans (Kullas et al., 2007). However, the frequency of mucous and systemic infections has increased significantly (Haley, 1961). It is difficult to control infections caused by this yeast due to its innate resistance to the conventionally employed antifungals, mainly fluconazole (Kaur et al., 2005). In addition, the histidine kinase proteins have not been found in the human genome; therefore, their study is interesting, mainly in the search of new antifungal compounds for treatment of these infections (Barrett and Hoch, 1998; Bourret and Silversmith, 2010; Chauhan and Calderone, 2008; Koretke et al, 2000; Matsushita and Janda, 2002). Additionally, we suppose that this protein can exert certain additional functions in *C. glabrata*, because it has been observed that these proteins are involved in fundamental processes of virulence and pathogenicity in both *C. albicans* and in *C. lusitaniae* (Bernhardt et al., 2001; Kruppa et al., 2003, 2004; Li et al., 2002; Singh et al., 2004; Nagahashi et al., 1998; Kaur et al., 2005; Boisnard et al., 2008; Chapelan-Leclerc et al., 2007), and it has been reported that histidine kinase proteins are directly implicated in fungal dimorphism. It has been demonstrated in *C. albicans* and *C. lusitaniae* that the three histidine kinase proteins that they possess, play a fundamental role in the transition from yeast to hyphae; deletion of genes *NIK*1 and *SLN*1 attenuates virulence, and deletion of gene *CHK*1 abolishes virulence (Calera and Calderone, 1999; Yamada-Okabe et al., 1999; Chapelan-Leclerc et al., 2007). Also, in both *C. albicans* and *C. lusitaniae*, Sln1p importantly affects biosynthesis of the functional and structural components of the cell wall (Calera and Calderone, 1999; Chapelan-Leclerc et al., 2007; Kruppa et al., 2003).

This research suggests that the adaptation response to stress conditions in *C. glabrata* is similar to that described in its closer related yeast, *S. cerevisiae*. However, to demonstrate similar function, an experiment attempting to delete Cg*SLN*1 in a strain containing a plasmid expressing Sc*SLN*1 gene should be done. This would address whether deletion of *SLN*1 is lethal in *C. glabrata* and whether the *S. cerevisiae* gene complements it. Therefore, pathogenicity analyses are needed to elucidate the involvement of this gene during expression of virulence factors; obtaining null mutants and constitutive expression would help to clarify the role of this gene in the pathogenesis of *C. glabrata*.

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