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Research paper

Inventory of ABC proteins and their putative role in salt and drug tolerance in *Debaryomyces hansenii*

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ABSTRACT

ATP-binding cassette (ABC) is one of the largest superfamily of proteins, which are ubiquitously present, performing variety of cellular functions. These proteins as drug transporters have been enticing substantial consideration because of their clinical importance. The present study focuses on genome wide identification of ABC proteins of an important halotolerant yeast *Debaryomyces hansenii* and explores their role in salt and drug tolerance. Our bioinformatics analysis identified a total of 30 putative ABC protein-coding genes whose expression at transcript level was confirmed by qRT-PCR. Our comparative phylogenetic analysis of nucleotide binding domains of *D. hansenii* and topology prediction categorized these proteins into six subfamilies; ABCB/MDR, ABCC/MRP, ABCD/ALDP, ABCF/YEF3, ABCE/RLI, and ABCG/PDR based on the nomenclature adopted by the Human Genome Organization (HUGO). Further, our transmembrane domain (TMD) predictions suggest that out of 30 ABC proteins, only 22 proteins possess either two or one TMD and hence are considered as membrane localized ABC proteins. Notably, our transcriptional dynamics of ABC proteins encoding genes following *D. hansenii* cells treatment with different salts and drugs concentrations illustrated variable transcriptional response of some of the genes, pointing to their role in salt and drug tolerance. This study first time provides a comprehensive inventory of the ABC proteins of a haploid *D. hansenii* which will be helpful for exploring their functional relevance.

1. Introduction

Lipid bilayer forms barriers between the cell and the external environment to maintain not only cell integrity but also provides the cell a protective environment to perform various physiological functions. For the maintenance of equilibrium conditions necessary for cell survival, the transport of organic and inorganic solute is required which is accomplished by a repertoire of transporters present on the cell membrane of an organism. ATP-binding cassette (ABC) proteins are one of the most abundant superfamilies of proteins which are present not only in unicellular organisms but occur widely in multi cellular eukaryotes, as well (Higgins, 1992). The majority of ABC proteins are ATP dependent membrane bound transporters helping in translocation of various substrate but some of the ABC proteins are also soluble in nature which performed specialized role in mRNA translation and ribosome biogenesis (Higgins, 1992; Kovalchuk and Driessen, 2010). Thus, their functional spectrum goes beyond transport, which justifiably matches their ubiquitous existence in multiples.

ABC transporter consists of functionally distinct transmembrane domain (TMD) and nucleotide binding domain (NBD) (Prasad and Goffeau, 2012). Each TMD has 6–7 transmembrane helices (TMHs) and are diverse in the context of sequences as well as in the nature of amino acids. The NBDs which powers drug transport, possess well-conserved sub-domains involved in ATP binding and hydrolysis. The highly conserved motifs of NBDs are Walker A, Walker B, signature sequence and Q loop. Out of these, the signature sequence exclusively present in ABC protein but the walker A and Walker B also exist in other ATP utilizing proteins (Strauss et al., 2014). A full size functional ABC transporter has

Abbreviations: ABC, ATP-binding cassette; MDR, multi drug resistance; TMDs, transmembrane domains; TMH, transmembrane helix; HUGO, Human Genome Organization; HMM, Hidden Markov Models; ROS, reactive oxygen species; FLC, fluconazole; TRB, terbinafine; AMPB, amphotericin B

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two NBDs and two TMDs. Most of ABC transporters usually show the forward topology (TMD-NBD) but in some cases, the ABC transporters are functional in reverse topology (NBD-TMD) where the NBD precedes TMD. The ABC transporters with each of a TMD and NBD are categorized as half transporters while an ABC protein without TMDs are grouped as soluble proteins (Xiong et al., 2015).

On the basis of sequence similarity between the nucleotide binding domain and their topology, the Human Genome Organization, (HUGO) has classified ABC proteins into 8 subfamilies (A-H) (Dean et al., 2001). ABCH is specifically present in arthropods and Zebra fish (Tian et al., 2017). Recently, the protein subfamilies are extended to ABCI subfamily to include the ABC proteins present in plants (Verrier et al., 2008). There is no report confirming existence of ABCA proteins in yeast, however, in higher animals, plants and various parasites, their presence is recorded (Kovalchuk and Driessen, 2010).

Debaryomyces hansenii is a halotolerant yeast which grows in hyper saline environment (Aggarwal et al., 2005; Minhas et al., 2012). The presence of salt in the growth medium appeared to stimulate its growth, but the mechanism underlying its halophillic nature in not very well understood (Prista et al., 2005). Additionally, *D. hansenii* cells can also accumulate lipids up to 70% of its total biomass thus it is also considered as an oleaginous yeast (Breuer and Harms, 2006). There are reports to point that salt stress could impact phospholipids composition of *D. hansenii* cells (Turk et al., 2007). The role of ABC transporter in the lipid transport and metabolism has been studied in different organisms (Prasad et al., 2016). In a recent study, exposure of salt stress to yeast *S. cerevisiae* cells resulted in the variable expression levels of large number of plasma membrane (PM) proteins that included ABC proteins as well (Szopinska et al., 2011). In *D. hansenii* genome approximate 10% of the genes encodes transmembrane proteins (De Hertogh et al., 2006). The phylogenetic tree based on 18S rDNA sequence revealed that it is closely related to pathogenic yeast *Candida albicans* and belong to CTG clade (Butler et al., 2009). The close resemblance of haploid yeast *D. hansenii* to *C. albicans* and its ability to withstand high salt concentrations makes it a preferable organism to explore the role of ABC transporters in drug and salt stress tolerance. Therefore, a detailed analysis of ABC superfamily proteins of *D. hansenii* is required to dissect their relevance in physiological stresses.

The present study focuses on genome wide identification and investigation of ABC proteins of halotolerant yeast *D. hansenii*. Our bioinformatics analysis identified a total of 30 putative ABC proteins in *D. hansenii* which based on comparative analysis and topology predications are categorized into six subfamilies ABCB/MDR, ABCC/MRP, ABCD/ALDP, ABCF/YEF3, ABCE/RLI, and ABCG/PDR according to the nomenclature adopted by HUGO. Further, our transmembrane domain (TMD) predictions suggest that out of 30 ABC proteins only 22 proteins contain TMDs and are considered as membrane localized proteins. The expression of these ABC protein-coding genes at transcript level was confirmed by qRT-PCR wherein the level of some of these transcripts showed variable expression in response to salt and drug stresses.

2. Materials and methods

2.1. Materials

The growth media YEPD (yeast extract/peptone/dextrose) was purchased from Difco (Sparks, MD, USA). The NaCl, KCl and LiCl were purchased from Qualigens (Mumbai, India). The antifungal drugs FLC, TRB and AMPB were purchased from the Sigma Chemical Co. (St. Louis, MO). The oligonucleotides used in this study were purchased from Sigma Genosys, India.

2.2. Strains and culture conditions

D. hansenii strain CBS767 was grown and maintained in YEPD media. The *D. hansenii* strain CBS767 Strain stock was prepared in 15% glycerol at store at −80 °C. Before experiment culture was freshly revived in YEPD media.

2.3. Identification of ABC proteins

The whole genome sequences of *D. hansenii* strain CBS767 was retrieved from NCBI genome database. This *D. hansenii* genome was sequenced by was sequenced by Genolevures (Dujon et al., 2004). For identification of ABC proteins ABC-tran model (accession PF00005) of the Pfam database (http://pfam.sanger.ac.uk/) was used to build HMM profile using the HMM search program from the HMMER package (http://hmmer.org/) using the default settings (Finn et al., 2016). Positive hits above the default threshold were further filtered based on a cutoff, defined from the plot of scores and e-values (Supplementary Fig. 1). Hits with domain score > 42.7 and E-value < 2.60E−11 were considered true positives containing the NBD domain and procured as potential ABC sequences for further analysis.

2.4. Phylogenetic analysis

The amino acid sequences of ABC proteins were aligned using the ClustalW software of MEGA6.06 package with default parameters (Tamura et al., 2013). These alignments further subjected to phylogenetic analysis using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The confidence of the tree topology done by boot straps analysis taking 1000 replicates.

2.5. Identification of TMDs and NBDs

To predict transmembrane region we used Topcons (http://topcons.net/) web based tool, which combines different prediction tools and gives a consensus prediction (Tsirigos et al., 2015). The ScanProsite tool (http://prosite.expasy.org/scanprosite/) was used to identify the number and positions of NBDs in each predicted protein sequence. Domain architecture of the ABC proteins was drawn using my domain creator tool (http://prosite.expasy.org/mydomains/).

The complete amino acid sequences of ABC proteins of *S. cerevisiae, C. albicans, and Kleyvuromyces lactis* were taken from previously published reports (Gaur et al., 2005; Kovalchuk and Driessen, 2010).

2.6. Chromosomal location

The chromosomal locations of *D. hansenii*'s ABC genes were drawn from bottom to top by taking co-ordinate from NCBI gene database. All the genes were marked by taken scale of 0.5 MB.

2.7. Subcellular localization

To predict subcellular localization of ABC proteins we used the WoLF PSORT (https://wolfpsort.hgc.jp/) and LocTree3(https://rostlab.org/services/loctree3/) (Goldberg et al., 2014; Horton et al., 2007). Based on homology these localization prediction result were further compared with experimentally verify localization data of *S. cerevisiae* ABC proteins (Snider et al., 2013).

2.8. RNA isolation and cDNA synthesis

The primary culture of *D. hansenii* CBS767 strain was grown in YEPD media until saturation reached. For secondary culture $O.D_{600}$ of 0.2 inoculated in 50 ml of YEPD media and cells were grown for 7 h to reach log phase. In case of treatment after 6 h of growth, cells were treated for 60 min at non-lethal concentrations of different salts (NaCl 1 M, LiCl 100 mM and KCl 50 mM) and drugs, {Fluconazole (FLC) 0.25 μg/ml, Terbinafine (TRB) 4 μg/ml and amphotericin B (AMPB)