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Identification new potential multidrug resistance proteins of *Saccharomyces cerevisiae*

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ABSTRACT

ABC (ATP-binding cassette) proteins can transport metabolic molecules and removes metabolic products and xenobiotics from the cell. The important problem is to study activity and search inhibitors of ABC proteins. There is a problem that ABC-proteins can transport hydrophobic drugs across the cell membrane due to their high substrate specificity. According to published data, *Saccharomyces cerevisiae* is an ideal model organism for analysis a lot of functional processes and gene activities of human cells. The aim of the present work is to reveal new potential yeast MDR proteins in *S. cerevisiae* with novel approach based on the cluster analysis.

According to the cluster analysis of yeast ABCB subfamily, STE6 protein is turned out to be the most related to human P-gp protein. The largest number of homologues with human MDR proteins was found in the yeast ABCC subfamily. Yeast BPT1 and YCF1 proteins are shown to be the most phylogenetically close to human MRP1. In the ABCG subfamily of yeast, PDR10, PDR12, PDR15 and PDR18 are turned out to be potential proteins of multidrug resistance. The future experimental study of these subfamilies should be conducted in order to confirm the role of STE6, YCF1, BPT1, PDR10, PDR12, PDR15 and PDR18 in MDR phenotype of yeast and to study their activity modulators.

1. Introduction

Members of the ATP binding cassette (ABC) protein superfamily, including human and yeast ABC transporters, transfer a variety of substances including ions, anticancer drugs, antibiotics, peptides, and phospholipids across biological membranes (Linton, 2007). ABC proteins fulfill a stunning variety of functions, ranging from ATP-driven transmembrane transport of great many different molecules, to the regulation of important cellular processes. In particular, ABC proteins can function as ion channels, channel regulators, receptors, proteases, as well as environmental sensors (Higgins, 1995; Dean and Allikmets, 1995; Kuchler and Thorner, 1992).

Most members of the ABC protein family share a similar molecular architecture and domain organization. But the typical ABC transporter contains two transmembrane domains (TMD1, TMD2) and two nucleotide-binding domains (NBD1, NBD2) (Bauer et al., 1999), (Dassa, 2003). The domain architecture of MDR proteins of both species includes full-size transporters (TMS6 − NBD)2 or (NBD − TMS6)2, half-size transporters (TMS6 − NBD) and members lacking obvious TMDs or NBDs (Gaur et al., 2005), (Tusnády et al., 2006). All ABC-transporters contain conserved regions (Hollenstein et al., 2007). NBD is approximately 200 residues in length and possesses six highly conserved motifs: the Walker A, Q-loop, ABC-signature, Walker B, D-loop and H-loop (Saurin et al., 1999). The main function of the NBDs is to bind and hydrolyze ATP or other NTPs, thereby fueling transport processes (Hollenstein et al., 2007). The most conserved features found in any given NBD are the Walker A and B motifs (Higgins, 1992). The Walker A motif has the sequence "GxxGxGKS/T," where x is any amino acid. Walker B reported the consensus sequence of this motif to be "xxxxD, where D denotes aspartic acid residues respectively, and x represents any of the 20 standard amino acids (Higgins, 1995), (Dean et al., 2001). Walker A and Walker B motifs are involved in the ATP binding (Kerr, 2002). ABC-signature is found only in ABC transporters (Kim and Chen, 2018). In general, particular positions of these motifs are invariantly conserved and occupied by different amino acids in each subfamily (Mishra et al., 2014). Another important domains in ABC-transporters are TMDs. These domains are the primary determinants of substrate specificity through specific substrate-binding sites (Fig. 1) (Hollenstein et al., 2007), (Lewis et al., 2012). Each TMD usually contains six predicted α-helical transmembrane-spanning segments (TMSs), although

Abbreviation: NBD, nucleotide-binding domain; TMD, transmembrane domain; TMS, transmembrane segment; P-gp, p-glycoprotein; MRP1, multidrug resistant protein; BCRP, breast cancer resistance protein.

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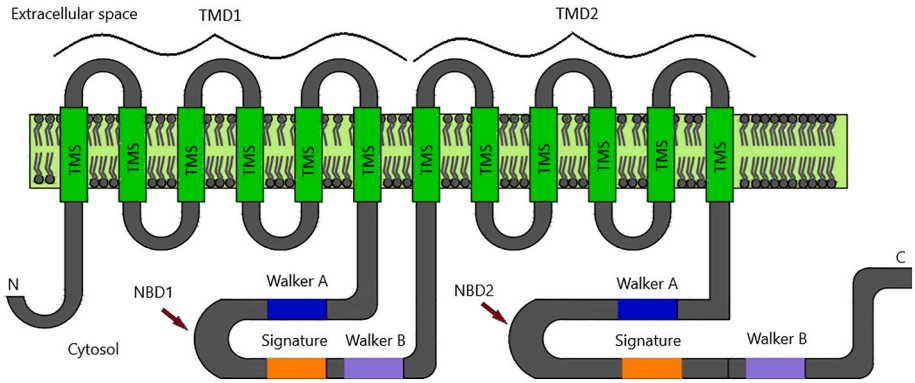

Fig. 1. Schematic model of ABC-protein of *Saccharomyces cerevisiae* According to literature *Saccharomyces cerevisiae* is an ideal model

in some cases four to eight predicted TMSs per TMD are also known (Biemans-Oldehinkel et al., 2006).

Despite their main functions in cell metabolism, ABC transporters can be harmful to humans. For instance, human diseases such as cystic fibrosis, adrenoleukodystrophy, Dubin-Johnson syndrome, Tangier disease are associated with mutations in human genes encoding ABC transporters. But one of the biggest problems with ABC proteins is their overexpression confers the resistance to hundreds of chemically unrelated drugs, including anticancer drugs and many others. Mainly these ABC-transporters localize in the plasma membrane and are called the multidrug resistant (MDR) proteins (Glavinas et al., 2005). For successful chemotherapy, it is necessary to search new inhibitors of MDR proteins in human cells. But the most important requirements are the low toxicity of inhibitors to the human cells. One of the main limitations of using human cells is different cell types need different expensive media to grow and survive, and most of the primary cells in culture have limited number of passages.

According to literature, *Saccharomyces cerevisiae* is an ideal model organism for the functional dissection of disease-related genes such as those of the ABC superfamily, because they grow easily, rapidly, cheaply, and have well understood genome (Karathia et al., 2011), (Wolters et al., 2015). Also, for example, up to 30% of genes implicated in human disease may have orthologs in the yeast proteome.

Genome of *S. cerevisiae* is shown to contain 30 ABC proteins which consists of six subfamilies: MDR (ABCB), MRP/CFTR (ABCC), ALDP (ABCD), RLI (ABCE), YEF3 (ABCF) and PDR5 (ABCG) (Bauer et al., 1999), (Piecuch and Obłak, 2014). Among these subfamilies, there is a network of genes involved in the multiple drug resistance phenotype, called the pleiotropic drug resistance (PDR) proteins in yeast (Piecuch and Obłak, 2014). Currently, yeast PDR network is shown to consist of PDR5 (ABCG), SNQ2 (ABCG), and YOR1 (ABCC) genes (Yibmantasiri et al., 2014). Likely, other ABC subfamily proteins of yeast cells can also perform the function of MDR under certain conditions (Bauer et al., 1999). The function of many ABC-transporters of *S. cerevisiae* has not been established yet. In the literature is shown that attempts to identify multidrug resistance functions of yeast ABC-proteins are being made by using quantitative reverse transcription PCR (RT-qPCR) analysis, GFP accumulation assay and flow cytometry (Galkina et al., 2018).

Besides the fact that multidrug resistance proteins of ABC superfamily are actively studied in clinical area, some of them are widespread in nature and have agricultural or biotechnological implications including agricultural fungicides, azoles, mycotoxins, herbicides and many others. For example, PDR18 of *S. cerevisiae* features the resistance to chemical stress agents, including herbicides, agricultural fungicides, and some metals (Teixeira et al., 2012). Also based on a genome-wide screening, PDR18 expression was also found to confer the resistance to the anticancer drugs cisplatin and carboplatin and the antifungal drug nocodazol (Teixeira et al., 2012). Pdr18 was found to play a role in plasma membrane sterol incorporation, and this physiological trait is proposed to contribute to its action as a multidrug resistance determinant (Cabrito et al., 2011). In the same time, PDR18 gene overexpression increases yeast ethanol tolerance and fermentation performance at more highly inhibitory concentrations of ethanol (Cabrito et al., 2011). PDR18 overexpressing in industrial yeast strains appears to be a promising approach to increase the bio-ethanol production (Godinho et al., 2018).

Despite the biological importance of the MDR phenotype, the identification of MDR functions of ABC proteins is carried out either by using time-consuming and expensive methods, or by establishing homology between proteins of different organisms using BLAST algorithms. Conventionally, only one specific part of the polypeptide chain is used for BLAST analysis (for example, the full protein sequence). By this way, the high level of homology of ABC-transporters of *S. cerevisiae* with *Candida albicans, Schizosaccharomyces pombe, Drosophila melanogaster, Mycobacterium tuberculosis, Staphylococcus aureus* has shown in previous researches (Shukla et al., 2003), (Christensen et al., 1997), (Braibant et al., 2000), (Burnie et al., 2000). The disadvantage of this method is that the established homology does not allow to assume the functional similarity of the studied proteins, since it relies on one parameter.

In our article, we proposed novel approach for identification potential MDR proteins using cluster analysis. For our analysis we use a number of parameters, namely cell localization and several homology parameters in the functionally important regions of ABC proteins, which are localized in the substrate-binding, ATP-binding and conserved sites of the protein molecules. In addition, to reveal functional similarity among ABC-proteins we use not only yeast ABC proteins that having proven PDR phenotype, but also human ABC proteins with a well-established MDR function, which increases the probability of establishing the MDR activity and functional analogs of yeast ABC proteins.

As a result of applying this approach, we have revealed new functional analogues of human MDR proteins in *S. cerevisiae* cells, that can be used to model and study the activity of MDR human proteins using cheaper yeast cells. Also, the data obtained might be used to develop new directions of modulating the activity of these proteins in yeast, that is aimed at ethanol bio-production increase.

Base on the literature review this analysis has not been done before and represents a new method identification of intraspecific and interspecies functional analogs of proteins. In addition, this method is easy to use and no time-consuming, and allows to minimize the range of proteins for experimental validation of their predicted functions in future.

2. Materials and methods

The protein sequence of ABC-transporters of *S. cerevisiae* and MDR proteins of human identified from UniProt. Information about the

amino acid sequence position of NBDs and TMDs of human MDR proteins (p-glycoprotein, MRP1, BCRP) and yeast ABC proteins are presented in the Table A1 (Appendices).

The full sequences, NBDs, TMDs, Walker A and Walker B motifs, Q-loop were used for local alignment withBLASTp.InformationabouttheaminoacidsequencepositionofAB Csignature, Walker A and Walker B motifs, Q-loop is confirmed by using the Conserved Domain Database of NCBI. Homology of ABC-transporters of *S. cerevisiae* and human MDR proteins is estimated by the percentage of query cover and e-value less than e^{-20}.

Cluster analysis were performed with STATISTICA 12 software. Parameters used for clustering are percent of homology of the full amino acid sequence of proteins, NBDs, Walker A, Walker B, Q-loop, ABC-signature and localization in the cell. Euclidean distance metric and single linkage amalgamation method was used.

3. Results

3.1. Identification of potential MDR proteins of yeast ABCB subfamily

Four ABC proteins of *S. cerevisiae* MDL1, MDL2, ATM1 and STE6 belong to ABCB subfamily. MDL1exports peptides formed upon proteolysis of mitochondrial proteins, whereas the function of MDL2 remains unknown (Paumi et al., 2009). ATM1 performs biogenesis of iron-sulfur (Fe/S) clusters, STE6takes part in mating of α factor secretion protein (Srinivasan et al., 2014), (Kölling and Hollenberg, 1994). Recently, functioning of members of ABCB subfamily as MDR proteins has not been covered in the literature. Yeast ABCB proteins were compared with human P-gp, which also belongs to the same subfamily (Sarkadi et al., 2006). According to UniProt database, the P-gp is full-size transporter with 1280 amino acid residue. STE6 is also full-sized transporter, while MDL1, MDL2 and ATM1 are half-size transporters (Table A1 (Appendices)) (Higgins, 1992). Based on this data, amino acid sequence of NBD1 with its conservative sites (ABC signature, Walker A, Walker B motifs and Q-loop) and TMDs domains of yeast ABCB proteins were local aligned with appropriate sequences of human P-gp (Young et al., 2001). Results of BLASTp analysis yeast ABCB proteins with P-gp of human are presented in Table 1. and yeast proteins of ABCB subfamily identity with each other and with P-gp of human showed in Table A2 (Appendix). The table shows the percentage of yeast ABCB proteins homology to P-gp of human and cellular localization. TMDs domains of yeast and human showed low identity value and are not used in further cluster analysis (data not shown).

Further, the data from Tables A1 and A2 (appendices) are used as parameters for cluster analysis of yeast ABCB proteins and human P-gp (ABCB1). Walker A and Q-loop were excluded from cluster analysis because their homology values were the same among all ABCB proteins of yeast (Walker A - 75% and Q-loop - 50%) (Table 1).

The results of analysis are presented on Fig. 2.

According to the results of cluster analysis the ABC-proteins were divided into three classes: P-gp and STE6, MDL1 and MDL2, ATM1.

Table 1
Analysis of aminoacid sequence homology (%) by BLAST and cellular localization of yeast ABCB proteins in comparison with human P-glycoprotein.

Criteria	ABCB subfamily			
	MDL1	MDL2	ATM1	STE6
Full sequence	15	15	13	22
NBD	46	50	45	40
Walker A	75	75	75	75
Walker B	50	67	33	67
Q-loop	50	50	50	50
ABC-signature	100	100	60	100
Localization	Inner mitochondrial membrane	Inner mitochondrial membrane	Inner mitochondrial membrane	Plasma membrane

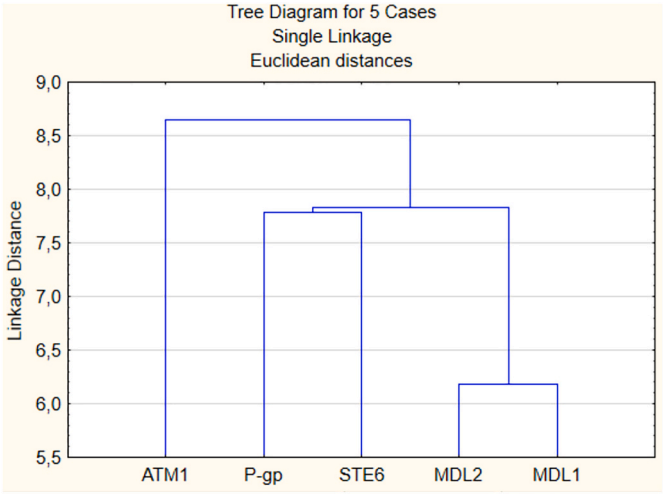

Fig. 2. Cluster analysis of yeast ABCB transporters and human P-gp

The first cluster is formed by STE6 and P-gp due to a higher level of homology for the full sequence and Walker B (22% and 67%, respectively) among all studied yeast ABCB proteins. In addition, only STE6 has NBD2 domain which shown 37% ($p = e^{-54}$) of identity with appropriate domain of P-gp. Besides, both STE6 and P-gp are localized in the plasma membrane, and mainly they transport hydrophobic substrates (McGrath and Varshavsky, 1989). Earlier, existence of a similar cluster was shown by local alignments with ClustalW software (Wilks, 2011).

The second cluster is consisted from MDL1 and MDL2 proteins of yeast. These proteins have the highest homology in full sequence (38% identical amino acid residues), Walker B (100%), and are localized in the inner mitochondrial membrane. These proteins have structural and functional similarity, and they exhibit activity as homodimers (Park et al., 2014). The above mentioned proteins are in a separate cluster due to less homology in full sequence, Walker B, and different localization regarding compared to cluster P-gp and STE6. But MDL1 and MDL2 have high homology of NBD1 (46% for MDL1 and 50% for MDL2, ($p = e^{-67}$ and $p = e^{-56}$, respectively)) and TMD1 (22% for MDL1 and 20% for MDL2 ($p = e^{-27}$ and $p = e^{-19}$, respectively)) to the human P-gp (Table 1). Percent of the NBD1 and TMD1 identity of MDL1 and MDL2 proteins with P-gp are higher than among STE6 and P-gp. It is likely that these proteins also confer drug resistance, which has not been previously shown.

The third cluster consist of only yeast ATM1 protein. ATM1 has the lowest percentage of identity for all studied parameters (full sequence, ABC signature, Walker B motifs) and different localization compared with other ABCB proteins (Table 1). The function of this member of the yeast ABCB subfamily is not well understood. Due to mitochondrial localization, ATM1 probably participates in the transport of essential metabolic compounds across mitochondria membranes, or it removes toxic metabolites out of mitochondria (Chloupková et al., 2003).

According to the data obtained the cluster analysis showed that among four ABC-transporters of ABCB subfamily of *S. cerevisiae* only STE6 can have MDR activity. The main results in this conclusion that the STE6 protein of yeast have a high identity with P-gp in NBD1 and the total protein sequence. Also an important aspect that STE6 is full-sized transporter and have general localization with P-gp in the plasma membrane.

3.2. Identification potential MDR proteins of ABCC subfamily of S. cerevisiae

Yeast ABCC subfamily (formerly designated the MRP/CFTR

Table 2
Analysis of aminoacid sequence homology (%) by BLAST and cellular localization of yeast ABCC proteins in comparison with human MRP1.

Criteria	ABCC subfamily				
	VMR1	YBT1	YCF1	BPT1	YOR1
Full sequence	29	28	39	34	27
NBD1	44	49	41	52	46
Walker A 1	62	62	62	75	62
Walker B 1	50	50	50	83	83
Q-loop 1	50	50	75	50	0
ABC-signature 1	100	100	100	100	100
NBD2	46	43	61	54	48
Walker A 2	100	100	100	100	100
Walker B 2	67	67	100	100	87
Q-loop 2	100	100	100	100	100
ABC-signature 1	60	80	100	100	40
Localization	Vacuole	Vacuole	Vacuole	Vacuole	Plasma membrane

[a] the number shows the percent of homology of yeast ABCC proteins with respect to MRP1 (ABCC1) of human;
[b]NBD1, nucleotide binding protein in the first half of ABCC proteins sequence; NBD2, nucleotide binding protein in the second half of ABCC proteins sequence;
[c]Walker A 1, Walker B 1, Q-loop 1, ABC-signature 1 are located in the NBD 1;
[d]Walker A 2, Walker B 2, Q-loop 2, ABC-signature 2 are located in the NBD 2.

subfamily), includes six members (YCF1, BPT1, YBT1, NTF1, VMR1, and YOR1) (Borst et al., 2000). It is known that only YOR1 is involved in multidrug resistance (Katzmann et al., 1995). Members of yeast ABCC subfamily are full size transporters with the domain architecture TMD1-NBD1-TMD2-NBD2. Human MRP1 (ABCC1) has the same domain architecture as the yeast proteins of ABCC subfamily and includes 1273 residues. MRP1 has the greatest clinical significance due to its expressing in 230 organs and involvement in cancer cell resistance to chemotherapy, unlike other proteins of ABCC subfamily of the human (Johnson and Chen, 2017). BLASTP analysis of yeast ABCC proteins with human MRP1 was carried out based on 13 parameters (Tables A2 and A3 (appendices)).

ABC-signature of NBD1, Walker A and Q-loop 2 of NBD2 were excluded from further cluster analysis because their values of homology were similar among all of human and yeast proteins (ABC-signature of NBD1–100%, Walker A and Q-loop - 100%) (Table 2).

Further, the data from Table A3 (appendices) are used as parameters for cluster analysis of yeast ABCC proteins and human MRP1 (ABCC1). As the result of cluster analysis the studied proteins were divided into three cluster groups (Fig. 3).

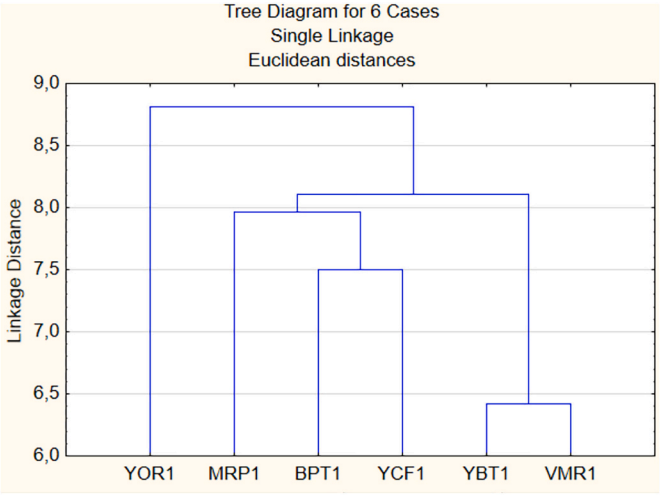

Fig. 3. Cluster analysis of ABC-transporters of yeast and human ABCC subfamily

The first cluster is consisted of yeast YCF1 and BPT1 proteins. YCF1 is 39% ($p = e^{-41}$) identical to MRP1 in full sequence, 50% in NBD 1 ($p = e^{-76}$) and 61% in NBD2 ($p = e^{-107}$). In addition, YCF1 as human MRP1 has an equivalent spacing of conserved residues, and is collinear with respect to the location, extent, and alternation of putative transmembrane and extramembrane domains (Li et al., 1996). Moreover, percent of identity in TMD1 and TMD2 of YCF1 and MRP1 are 43% ($p = e^{-80}$) and 41% ($p = e^{-75}$), respectively. Likely it is due to the possibility that yeast YCF1 and human MRP1 catalyze the same, or at least overlapping, reactions. According to literary data, they perform almost identical functions, namely, they are involved in the transport of bilirubin and heavy metal detoxification via glutathione conjugates. Based on this, YCF1 can be potential protein responsible for multidrug resistance in *S. cerevisiae* cells (Johnson and Chen, 2017).

BPT1 also shares with MRP134% identity in full sequence, 52% ($p = e^{-69}$) and 54% ($p = e^{-93}$) of NBD1 and NBD2, respectively. This protein involved in the transport of unconjugated bilirubin and heavy metal detoxification via glutathione conjugates. BPT1 like as YCF1 has high identity of TMD1 (33% ($p = e^{-51}$)) and TMD2 (38% ($p = e^{-61}$)) with MRP1. Moreover, Walker A region of BPT1 showed 75% of identity with MRP1 (contains a four conserved residues of the glycine, valine, lysine and serine).

Moreover, BPT1 and YCF1 form a subcluster due to higher level of homology among them in full sequence (40% identical amino acid residues), NBD1 and NBD2 (50% and 62% ($p = e^{-80}$ and $p = e^{-116}$, respectively)), Walker A 1 and Walker A2 (83% and 100%), Walker B 1 and Walker B2 (87% and 100%, respectively), Q-loop 1 and Q-loop 2 (75% and 100%, respectively) compared to MRP1. The BPT1 and YCF1 are localized in the vacuole unlike MRP1 (Petrovic et al., 2000).

The second cluster consists of yeastYBT1 and VMR1 proteins because they have a high percentage of identity across all criteria. In particular, these proteins have 49% identity throughout whole protein molecule. In the same time, these yeast proteins are in one more high level cluster with MRP1. YBT1, also known as BAT1, showed high level of structural and functional identity with human MRP1. The homology of NBD1 and NBD2 of YBT1 of yeast are 49% ($p = e^{-59}$) and 43% ($p = e^{-76}$) with human MRP1, respectively. VMR1 also has high percent of identity with human MRP1 (44% ($p = e^{-69}$) for NBD 1 and 46% ($p = e^{-79}$) for NBD2).

Besides sequence identity, both proteins show functional similarity to MRP1. VMR1 is known to exhibit a multidrug resistance phenotype (Wawrzycka et al., 2010a). YBT1 as well as human MRP1 and yeast BPT1 can transport glutathione conjugated substrates, bile pigments and acids (Gulshan and Moye-Rowley, 2011).

In contrast to BPT1 and YCF1, yeast YBT1 and VMR1 have lower level of whole protein sequence identity (28% ($p = e^{-25}$) and 29% ($p = e^{-27}$), respectively). It is likely because of more lowest level of TMD's homology of both proteins. In particular, TMD1 and TMD2 of YBT1 have 28% ($p = e^{-37}$) and 38% ($p = e^{-62}$) identity with MRP1, respectively. While TMD1 and TMD2 of VMR1 have 34% ($p = e^{-43}$) and 32% ($p = e^{-39}$) identity.

The YOR1 protein is the most phylogenetically distant from the previously described ones. But this ABC protein is known as a multidrug transporter. Namely,YOR1 mediates export of many different organic anions (Kolaczkowska et al., 2008). Its ABC protein highly homologous to mammalian transporters MRP1 both in full sequence homology (32% ($p = e^{-51}$)) and in NBD1, NBD2, TMS1, TMS2 (sequence homology are 46% ($p = e^{-64}$), 48% ($p = e^{-80}$), 24% ($p = e^{-20}$), 32% ($p = e^{-52}$)). But there is one advantage of this yeast ABC protein compared to other proteins of the same family namely it is localized in the plasma membrane, as well as MRP1 (Decottignies et al., 1998).

Based on the data the YCF1 and BPT1 of yeast are the most related to MRP1 of human, because its have ability to transport similar substrate. Also YCF1 and BPT1 of yeast have high homology of full protein sequence, NBD and binding sites (Walker A, Walker B, Q-loop, ABC-signature) with MRP1 of human.

3.3. Identification potential MDR proteins of ABCG subfamily of S. cerevisiae

ABCG of yeast is known to be the largest of the yeast subfamilies, containing nine members (PDR5, PDR10, PDR11, PDR12, PDR15, PDR18, SNQ2, ADP1 and AUS1) (Prasad and Goffeau, 2012). Based on literature data only SNQ2 and PDR5 have two MDR yeast ABC proteins (Decottignies et al., 1995). PDR5 and SNQ2 are the phenotypically best characterized PDR gene products. PDR5 is involved in the elimination of toxic compounds that could potentially accumulate within yeast cells during stationary phase growth. PDR5 confers resistance to various drugs, such as cycloheximide, trifluoperazine, and rodamine 6G (Mahé et al., 1996). SNQ2 is known to be as a homolog of PDR5 and encodes an ABC protein whose expression is associated with a MDR phenotype (Servos et al., 1993).

The architecture of PDR subfamily members is distinctive. Their domains are arranged in "reverse order" (NBD1-TMD1-NBD2-TMD2), and all members of the yeast PDR subfamily are full length proteins (with the exception of ADP1, discussed later) (Yibmantasiri et al., 2014).Yeast ABCG proteins were compared with human BCRP (ABC-G2).Human ABCG2 (BCRP) is half-size transporter with 655-residue and localizes to the mitochondria and plasma membrane.

BLASTP analysis of yeast ABCG proteins with human ABCG2 was carried out based on 6 parameters (Tables A3 and A4 (Appendices)). ABC-transporters of yeast ABCG subfamily showed a lower percentage of identity to ABCG2 of human in all studied parameters (Table 3). Although PDR5 is known as MDR protein of yeast, this protein gives a low percentage of identity with human ABCG2 protein.

Further, the data from Table A4 (Appendices) are used as parameters for cluster analysis of yeast ABCG proteins and human ABCG2 (BCRP).

Based on the cluster analysis yeast ABCG proteins were divided into five clusters (Fig. 4).

One of the clusters consists from PDR5, PDR15 and PDR10. PDR15 is the closest homolog to PDR5 of yeast, which shares 74% ($p = e^{-60}$) in full sequence identity and 82% ($p = e^{-166}$) in NBD1. In addition, identity in TMD1 of PDR5 and PDR15 is 57% ($p = e^{-47}$), which indicates a similar substrate specificity and, therefore, its capability to transport drugs. PDR10 has less homology compared to PDR5 and PDR15 than these proteins with each other. PDR10 has high level of identity with PDR5 (66% identity in full sequence and 78% ($p = e^{-154}$) in NBD1) and PDR15 (64% identity in full sequence and 76% ($p = e^{-154}$) in NBD1). This ABC transporter of yeast probably also have MDR activity. Besides, PDR10 as well as PDR5 and SNQ2 have Pdr1/Pdr3 target genes encoding the ATP-binding cassette proteins confer resistance against drugs compounds (Rockwell et al., 2009), (Egner et al., 1995). Interesting that PDR10 is associated with detergent-resistant domains in the plasma membrane and affects lipid distribution and maintenance of membrane micro-environment for the adequate functioning of membrane embedded proteins including the ABC-MDR transporter PDR12 (Piper et al., 1998).

The next cluster consists of SNQ2, PDR18 and PDR12. PDR18 is the closest homolog to SNQ2, and shares 63% in full protein sequence identity and 73% ($p = e^{-145}$) in NBD1. In addition, these proteins showed a high level in Walker A, Walker B, Q-loop and ABC-signature (100% in each sites). Based on these dates SNQ2 and PDR18 are close paralogs and, likely, PDR18 is able to transport drugs. PDR12 has less homology compared to level of identity of SNQ2 and PDR18. In particular, full protein sequence identity of PDR12 was 40% and 44% to PDR18 and SNQ2, respectively. While homology of NBD1 was shown to be only 52% ($p = e^{-103}$) and 57% ($p = e^{-113}$) with NBD1 of PDR18 and SNQ2, respectively. As result of these analysis, the PDR12 can also have MDR activity.

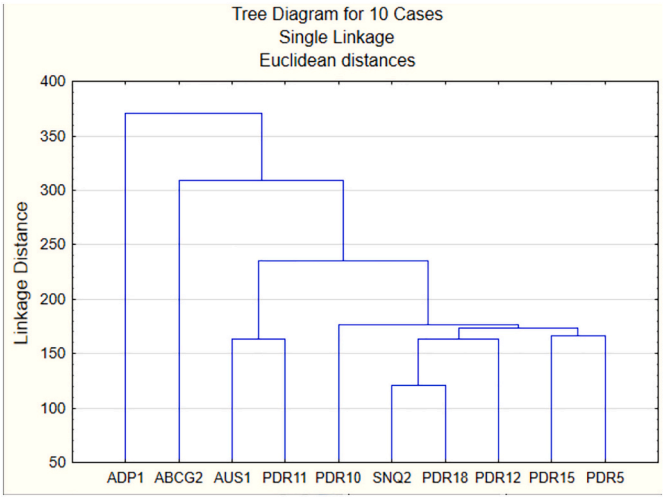

Fig. 4. Cluster analysis of ABC-transporters ABCG subfamily

The third cluster consists of yeast ABC proteins AUS1 and PDR11. Because there is high level of identity in the full protein (67%) and NBD1 (72%, $p = e^{-137}$) sequences among these proteins. AUS1 and PDR11 have identical function (i.e. both proteins transport sterol compounds from the cell) (Marek et al., 2011), (Rogers et al., 2001).

All proteins of above mentioned yeast ABCG subfamily clusters have a very good level of identity among themselves in all studied parameters and form a cluster of a higher level. But one of yeast protein ADP1 has the lowest value in compare with other yeasts proteins. In particular, its full protein sequence, NBD1, Walker A, Walker B, Q-loop and ABC-signature have less than 26% identity with appropriate sequences of other yeast ABCG proteins. Furthermore, ADP1 is not a full-sized transporter and locates in the endoplasmic reticulum compared with other ABCG subfamily proteins (Metzgar et al., 2004).

Human ABCG2 did not form any cluster with another ABCG subfamily proteins of yeast ABCG subfamily proteins in compare with ABCB and ABCC protein subfamilies of yeast. It is due to low value of identity in all studied parameters (Table 3). In particular, human ABCG2 has less than 19% identity in full protein sequence and 40% in NBD1 with all yeast ABCG subfamily proteins. While in ABCC

Table 3
Analysis of aminoacid sequence homology (%) by BLAST and cellular localization of yeast ABCG proteins in comparison with human BCRP.

Criteria	ABCG subfamily								
	PDR5	PDR10	PDR11	PDR12	PDR15	PDR18	AUS1	ADP1	SNQ2
Full sequence	11	11	11	11	10	11	11	19	12
NBD	31	32	35	31	31	35	37	43	36
Walker A	50	50	50	37.5	50	50	25	50	50
Walker B	0	33	67	67	50	67	67	17	67
Q-loop	50	50	50	75	50	75	50	0	75
ABC-signature	0	0	0	0	0	0	0	0	0
Localization	Plasma membrane	Plasma membrane	Plasma membrane	Plasma membrane	Plasma membrane	Plasma membrane	Plasma membrane	Endoplasmic reticulum	Plasma membrane

subfamily, for instance, the human protein MRP1 showed 39% in full protein sequence and more than 50% in NBD1 with yeast ABCC proteins.

We conclude that ABC proteins of *S. cerevisiae* PDR15 and PDR18 have an ability to transport drugs from cell due to the high level of homology with yeast PDR5 and SNQ2. Because, PDR5 and SNQ2 have Pdr1/Pdr3 target genes encoding the ATP-binding cassette proteins confer resistance against drugs compounds.

4. Discussion

The most convenient approach for the identification of potential MDR proteins has been found because there is the growth of data on the functions of ABC-transporters in human cells, namely, their participation in drug transfer. The disadvantages of using animal and human cell lines are the necessity of standardization of the environment, difficulties of medium preparation and a limited number of passages. An attempt of conduct a cluster analysis of three subfamilies of ABC-transporters of *S. cerevisiae* and known human MDR proteins (P-gp, MRP1 and BCRP) was made to identify potential MDR proteins in yeast cells. The third-party software was used to carry out cluster analysis where the results of alignment of the complete protein sequence, nucleotide-binding domain and its functional important sites (Walker A, Walker B, ABC-signature and Q-loop), as well as localization of ABC-transporters in the cell were used as parameters.

According to the data cluster analysis, potential MDR proteins are identified in *S. cerevisiae* cells, which can serve as cheap protein models to study of various drugs and develop the strategies to increase ethanol resistance. In particular, in the ABCB subfamily, the yeast STE6 protein is turned out to be the most related to human P-gp protein. This is probably due to the fact that STE6 has the highest identity in terms of the total protein sequence and NBD1 binding sites. Moreover, it is full-sized transporter like P-gp. Additional feature supported STE6 drug export function is same localization in the plasma membrane and ability to transport of hydrophobic substrate (McGrath and Varshavsky, 1989).

In addition, it could also be suggested that MDL1 and MDL2 proteins are also phylogenetically close to the human P-gp due to their high identity in Walker B (100%) and NBD1 (46% ($p = e^{-67}$) for MDL1 and 50% ($p = e^{-56}$) for MDL2, respectively). In the same time due to the fact that MDL1 and MDL2 are localized in the mitochondria and also are not full-length transporters unlike STE6, probably, their ability to transfer drugs is low or absent.

In the ABCC subfamily, the level of identity of ABC-transporters of *S. cerevisiae* with human MRP1 was analyzed. It should be noted that all transporters of ABCC yeast subfamily have various degree of homology compared to that among another ABC subfamilies. BPT1 and YCF1 are shown to be the most phylogenetically close to human MRP1 one because the homology of full protein sequence and all nucleotide-binding domains was not lower than 30% and 40%, correspondingly. Moreover, the protein sequence of important binding sites (Walker A, Walker B, Q-loop, ABC-signature) showed almost 100% identity when compared with MRP1. Previously it was shown that that yeast YCF1 and human MRP1 probably have the similar binding site for MK571 but no binding site for verapamil, that confirms our results of cluster analysis (Ren et al., 2000).

YCF1 and BPT1 have a high level of identity across the transmembrane domains (at least 40%) and overall functional activity. It is likely that proteins also have binding sites similar to MRP1. In the literature, there is no evidence that BPT1 may be a potential multidrug resistance protein.

Another potential MDR proteins are YBT1 and VMR1. These proteins are shown to have high level of identity to MRP1 in all studied parameters and similar functional activity, in particular, transport glutathione out of cells (Chang, 2003). Earlier, it was shown that VMR1 exhibits increased sensitivity to at least 11 amphiphilic drugs of unrelated structure. Moreover, despite the close phylogenetic similarity of VMR1 and YBT1, no difference was found in the VMR1 strain for taurocholic acid and glycochenodeoxycholic acid sensitivity, which are characteristic substrates of YBT1. And also VMR1 also contributes to cadmium and mercury resistance (Wawrzycka et al., 2010b). It is to confirm our results of cluster analysis.

The YOR1 protein of ABCC subfamily is in the farthest cluster to human MRP1, despite this ABC transporter has MDR activity in yeast cells. Compared with other proteins of the same subfamily, the identity of all studied parameters was lower. In the same time, YOR1 is the only protein among the ABCC family that is located in the plasma membrane like MRP1. Moreover, the general functional activity of YOR1 and MRP1 is associated with glutathione transport (Leier et al., 1994). According to the published data, the transport of substances in the form of conjugates with glutathione is a key step in MRP-mediated detoxification (Burnie et al., 2000). In addition, YOR1 has Pdr1/Pdr3 target genes, which are responsible for drug resistance (Katzmann et al., 1995). As a result, the similarity of functional activity of YOR1 and MRP1 proteins is key in determining MDR function of this protein, but this was not enough to combine them in one cluster. In addition, there is evidence that this protein is mutant for the above genes, as a result of which, our studied protein of the wild strain *S. cerevisiae* was in a distant cluster from MRP1 (Liesa et al., 2012).

In the ABCG subfamily of yeast, PDR12, PDR15, PDR18 and PDR10 are turned out to be potential proteins of multidrug resistance. This is due to the fact that they showed a high identity in all studied parameters (at least 65%) with the known MDR proteins of yeast (PDR5 and SNQ2). In addition, yeast PDR5 and SNQ2 have Pdr1/Pdr3 target genes (Chloupková et al., 2003).

PDR12 is another protein in the same cluster with SNQ2, which also can be attributed to potential drug transporter. This protein showed high homology with SNQ2 in the full protein sequence, as well as in nucleotide-binding sites (52% ($p = e^{-103}$) and 57% ($p = e^{-113}$), respectively). Also, PDR12 protein provides resistant to sorbate, benzoate and acetate, what is an important determinant of the MDR phenotype (Balzi and Goffeau, 1995). Probably, this ability can protect *S. cerevisiae* cells against the toxicity of high organic acid levels. That can be applied to biotechnological processes with yeast. In addition, like PDR12, SNQ2 is also localized in plasma membrane (Rockwell et al., 2009).

PDR10 is also the potential transporter of MDR proteins, which as well as PDR5 and SNQ2 have Pdr1 / Pdr3 target genes (Rogers et al., 2001). Also cluster analysis showed, that PDR10 has a high level of identity with PDR5 (66% identity in full sequence and 78% ($p = e^{-154}$) in NBD1) and PDR15 (64% identity in full sequence and 76% ($p = e^{-154}$) in NBD1).

The PDR18 is in cluster with PDR10 and, probably, this protein also has resistance to chemical stress agents, including herbicides, agricultural fungicides, and some metals. Likely, this is ability determines resistant of *S. cerevisiae* expressing this protein is to higher concentrations against the more highly inhibitory concentrations of ethanol.

Thus, seven new potential MDR proteins in yeast were identified after cluster analysis of three large ABC protein subfamilies of *S. cerevisiae* and human proteins was performed. STE6, YCF1, BPT1, PDR10, PDR12, PDR15 and PDR18 are proposed to have MDR activity. The data can be used for the further study of the functionality of potential yeast MDR proteins and to modulate their activity, which may be applied in medicine and agriculture. Moreover, experiments with the usage of special probes and inhibitors to test the activity of MDR proteins of the corresponding families should be conducted in order to confirm the role of STE6, YCF1, BPT1, PDR10, PDR12, PDR15 and PDR18 in MDR phenotype of yeast.

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Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Declaration of Competing Interest

Authors declare that they have no conflict of interests.

Appendix A

Table A1
Positions TMDs and NBDs of yeast and human ABC proteins in the protein sequence.

№	ABC-trans-porter	Length	TMD1	NBD1	TMD2	NBD2
Human						
1	ABCB1	1280	51–357	392–628	711–1000	1035–1273
2	ABCC1	1531	325–608	644–868	975–1256	1293–1527
3	ABCG2	655		37–286		389–651
S. cerevisiae						
1	MDL1	695	103–398	432–673		
2	MDL2	773	119–413	493–733		
3	ATM1	690	119–409	443–697		
4	STE6	1290	27–319	357–603	717–1007	1052–1287
5	VMR1	1592	338–632	664–908	981–1282	1323–1572
6	YBT1	1661	354–662	694–935	1026–1345	1381–1646
7	YCF1	1515	287–590	626–853	951–1235	1272–1507
8	BPT1	1559	292–578	639–871	980–1265	1302–1553
9	YOR1	1477	207–493	581–808	897–1175	1213–1464
18	PDR 5	1511	161–410	869–1112		
19	PDR 15	1529	171–420	884–1127		
20	PDR 10	1564	174–430	923–1166		
21	PDR 18	1333	30–281	729–971		
22	PDR12	1511	144–397	836–1084		
23	PDR11	1411	31–273	751–979		
24	AUS 1	1394	33–273	751–978		
25	ADP1	1049	384–631	793–1044		
26	SNQ 2	1501	161–410	853–1095		

Table A2
Analysis of aminoacid sequence homology (%) by BLAST of between yeast ABCB subfamily proteins and in comparison with human P-gp.

ABC proteins	MDL1	MDL2	ATM1	STE6	Pgp
ABCB subfamily					
The full sequence					
MDL1	100	38	24	12	15
MDL2		100	22	13	15
ATM1			100	11	13
STE6				100	22
P-gp					100
NBD1					
MDL1	100	32	38	37	46
MDL2		100	27	23	50
ATM1			100	37	45
STE6				100	25
P-gp					100
Walker B					
MDL1	100	67	33	50	50
MDL2		100	33	67	67
ATM1			100	50	33
STE6				100	67
P-gp					100
ABC-signature					
MDL1	100	100	60	100	100
MDL2		100	100	100	100
ATM1			100	60	60
STE6				100	100
P-gp					100

Table A3
Analysis of aminoacid sequence homology (%) by BLAST of between yeast ABCC subfamily proteins and in comparison with human MRP1.

ABC proteins ABCC subfamily	VMR1	YCF1	YBT1	BPT1	YOR1	MRP1
The full sequence						
VMR1	100	27	49	28	25	29
YCF1		100	27	40	26	39
YBT1			100	27	23	28
BPT1				100	26	34
YOR1					100	27
MRP1						100
NBD1						
VMR1	100	38	64	38	35	44
YCF1	38	100	34	50	37	41
YBT1	64	34	100	34.5	36	49
BPT1	38	50	34.5	100	35	52
YOR1	35	37	36	35	100	46
MRP1	44	41	49	52	46	100
NBD2						
VMR1	100	43	68	50	48	46
YCF1		100	44	62	48	61
YBT1			100	49	45	43
BPT1				100	51	54
YOR1					100	48
MRP1						100
Walker A 1						
VMR1	100	62,5	87.5	62.5	62.5	62.5
YCF1		100	62.5	62.5	100	62.5
YBT1			100	62.5	75	62.5
BPT1				100	62.5	75
YOR1					100	62.5
MRP1						100
Walker A 2						
VMR1	100	100	100	100	100	100
YCF1		100	100	100	100	100
YBT1			100	100	100	100
BPT1				100	100	100
YOR1					100	100
MRP1						100
Walker B 1						
VMR1	100	67	67	100	67	50
YCF1		100	67	83	83	50
YBT1			100	67	67	50
BPT1				100	100	83
YOR1					100	83
MRP1						100
Walker B 2						
VMR1	100	67	83	100	87	67
YCF1		100	67	100	87	100
YBT1			100	67	67	67
BPT1				100	87	100
YOR1					100	87
MRP1						100
Q-loop 1						
VMR1	100	50	100	100	25	50
YCF1		100	75	75	0	75
YBT1			100	100	25	50
BPT1				100	25	50
YOR1					100	0
MRP1						100
Q-loop 2						
VMR1	100	100	100	100	100	100
YCF1		100	100	100	100	100
YBT1			100	100	100	100
BPT1				100	100	100
YOR1					100	100
MRP1						100
ABC-signature 1						
VMR1	100	100	100	100	100	100
YCF1		100	100	100	100	100
YBT1			100	100	100	100
BPT1				100	100	100
YOR1					100	100

(*continued on next page*)

Table A3 (*continued*)

ABC proteins ABCC subfamily	VMR1	YCF1	YBT1	BPT1	YOR1	MRP1
MRP1						100
ABC-signature 2						
VMR1	100	80	100	60	40	60
YCF1		100	80	100	40	100
YBT1			100	80	40	80
BPT1				100	40	100
YOR1					100	40
MRP1						100

*the number shows the percent of homology of yeast ABCC proteins with respect to MRP1 (ABCC1) of human;
*NBD1, nucleotide binding protein in the first half of ABCC proteins sequence; NBD2, nucleotide binding protein in the second half of ABCC proteins sequence;
*Walker A 1, Walker B 1, Q-loop 1, ABC-signature 1 are located in the NBD 1;
*Walker A 2, Walker B 2, Q-loop 2, ABC-signature 2 are located in the NBD 2.

Table A4
Analysis of aminoacid sequence homology (%) by BLAST between yeast ABCB subfamily proteins and in comparison with human BCRP.

ABC proteins ABCG subfamily	PDR5	PDR10	PDR11	PDR12	PDR15	PDR18	AUS1	ADP1	SNQ2	ABCG2
The full sequence										
PDR5	100	66	27	35	74	35	27	12	37	11
PDR10		100	27	34	65	35	26	13	37	11
PDR11			100	29	26	33	67	13	31	11
PDR12				100	35	40	28	17	44	11
PDR15					100	34	26	13	36	10
PDR18						100	34	15	63	11
AUS1							100	15	30	11
ADP1								100	13	19
SNQ2									100	12
ABCG2										100
NBD1										
PDR5	100	75	29	43	82	46	30	26	42	31
PDR10		100	32	44	76	45	33	26	44	32
PDR11			100	34	30	35	72	21	35	35
PDR12				100	35	46	31	26	44	31
PDR15					100	46	31	26	44	31
PDR18						100	39	23	73	35
AUS1							100	24	36	37
ADP1								100	25	43
SNQ2									100	36
ABCG2										100
Walker A 1										
PDR5	100	87.5	50	50	100	87.5	12.5	87.5	87.5	50
PDR10		100	37.5	50	100	87.5	12.5	87.5	87.5	50
PDR11			100	87.5	50	50	37.5	50	62.5	50
PDR12				100	62.5	62.5	25	87.5	62.5	37.5
PDR15					100	87.5	12.5	87.5	87.5	50
PDR18						100	12.5	87.5	100	50
AUS1							100	12.5	12.5	25
ADP1								100	75	50
SNQ2									100	50
ABCG2										100
Walker B 1										
PDR5	100	33	83	83	100	83	83	0	83	50
PDR10		100	33	33	33	33	33	0	33	33
PDR11			100	100	83	100	100	17	100	67
PDR12				100	83	100	100	17	100	67
PDR15					100	83	83	17	83	50
PDR18						100	100	17	100	67
AUS1							100	17	100	67
ADP1								100	17	17
SNQ2									100	67
ABCG2										100
Q-loop 1										
PDR5	100	100	50	50	100	75	50	0	75	50
PDR10		100	50	50	100	75	50	0	75	50
PDR11			100	50	50	75	100	0	75	50
PDR12				100	50	75	50	0	75	75
PDR15					100	75	50	0	75	50
PDR18						100	75	0	100	75

(*continued on next page*)

Table A4 (*continued*)

ABC proteins ABCG subfamily	PDR5	PDR10	PDR11	PDR12	PDR15	PDR18	AUS1	ADP1	SNQ2	ABCG2
AUS1							100	0	75	50
ADP1								100	0	0
SNQ2									100	75
ABCG2										100
ABC-signature 1										
PDR5	100	100	40	40	100	100	0	100	0	0
PDR10		100	40	100	100	100	60	0	100	0
PDR11			100	40	40	40	75	0	40	0
PDR12				100	100	100	60	0	100	0
PDR15					100	100	40	0	100	0
PDR18						100	60	0	100	0
AUS1							100	0	60	0
ADP1								100	0	0
SNQ2									100	0
ABCG2										100

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