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Identification and evaluating the role of metal ion transporters in *Dienococcus radiodurans* using *insilico* tools

Bioinformatics

KEYWORDS : *Dienococcus radiodurans*, radiation resistance, metal ion transporters, iron transporters

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ABSTRACT

Most radiation resistant bacterium Dienococcus radiodurans accomplishes its ability to resist the toxic effects of metals by transporting and transforming them to less toxic metal ions utilizing metal ion transporters. Identification and understanding the role of metal ion transporters in D. radiodurans helps us to know their mechanism of maintaining ion level concentrations in adverse conditions and their role in bioremediation process. In the present study, a complete catalogue of 33 metal ion transporters was identified. Of these 33 metal transporters, the interactions and interacting partners for manganese, iron and mercury transporters were analyzed using STRING, as these metal ions are the remnants in the spent decontaminating solutions released from many industries and nuclear power plants which are hazardous. The interaction studies show that the three metal ion transporters are interacting with each other which might play a crucial role in imparting radiation resistance to this organism.

Introduction:

Metal ion transporters play a major role in maintaining the correct concentrations of the various metal ions in the different cellular compartments. (Nelson N,1999). Recent studies on metal ion transporters painted a picture of coordinated action of uptake and secretion systems for achieving the proper homeostasis for individual tissues. In some of the cellular organelles and the plasma membrane, low and high affinity transporters act in concert to maintain the right balance of metal ion concentrations (Hediger, 1997; Eide, 1998; Radisky and Kaplan, 1999). Moreover, highly specific transport systems function along with a wide range of metal ion transporters to achieve the right concentration balance. The complete suite of genes involved in transport of metal ions in *Neurospora crassa* is referred to as 'Metal Transportome' (KiranMayi and Maruthi Mohan. 2006).

Deinococcus radiodurans was discovered in 1956 by Arthur W. Anderson at the Oregon Agricultural Experiment Station in Corvallis, Oregon. *D.radiodurans* belongs to the *Deinococcaceae*, a family of bacteria characterized by an exceptional ability to withstand the lethal effects of DNA-damaging agents, including ionizing radiation, ultraviolet (UV) light, and desiccation (Rainey *et al*., 1997; Battista and Rainey, 2001). It is often found in habitats rich in organic materials, such as soil, feces, meat, or sewage, but has also been isolated from dried foods, room dust, medical instruments and textiles. The *D. radiodurans* genome was sequenced in 1999 (White et al., 1999). The genome consists of two chromosomes, a mega plasmid and a smaller plasmid encoding for a total of approximately 3200 proteins.

The most radiation resistant organism yet discovered is the bacterium *D.radiodurans,* and its genome (strain R1) has recently been sequenced and annotated; it consists of 4–10 identical copies of a chromosome (2.65 Mbp), two megaplasmids (412 and 177 kbp), and a plasmid (46 kbp). *D. radiodurans* is a non-pathogenic, solvent tolerant, soil bacterium that can grow continuously in the presence of 60 Gy/h (a dose rate that exceeds those in most radioactive waste sites) with no effect on either its growth rate or its ability to express foreign genes. Recent research showed that Mn(II) ions protect proteins from being damaged by super radicals, which was proposed to be the main mechanism of radiation resistance in *D. radiodurans* (Daly et al., 2007 , 2009).

D.radiodurans is the world's toughest and the most radiation resistant organism known. It can tolerate radiation levels at 1000 times the levels that would kill a human and has been genetically engineered for use in bioremediation to consume and digest solvents and heavy metals, even in highly radioactive site. *Insilico* techniques offers possibilities for bioremediation from environment protection point of view as the Genomic and Bioinformatics data provides a wealth of information that can be greatly enhanced by structural characterization of some of these proteins in them. Based on the above background this study was undertaken to identify the complete complement of metal ion transporters that are involved in maintaining the metal homeostasis *D.radiodurans* and modeling of mercury, manganese and iron transporters for further interaction studies.

Material and Methods:

The availability of the online genome has made possible to identify the metal ion transporters in *D.radiodurans.*

Identification of Metal Ion transporters and their Sub cellular localization

TransportDB (http://membranetransport.org/) a relational database describes the predicted cytoplasmic membrane transport protein complement for organisms whose complete genome sequence are available. For each organism, its complete membrane transport complement was identified, classified into protein families according to the TC classification system, and functional predictions are provided. In the organism menu by selecting the desired organism (*D.radiodurans*) a list of protein transporters have been obtained, the protein transporters that have transmembrane helices are identified using a tool TMHMM (TransMembrane prediction using Hidden Markov Models) an option present in TransportDB. The transporters with transmembrane helices are now subjected for the identification of protein subcellular localization using PSORTdb (http://db.psort.org/).

Prediction of Interaction and interacting partners of the metal ion transporters

The Mercury, Iron and Manganese are the major contituents in most of the hazardous wastes released by most of the industries and hence the transporters that are capable to transport these metal ions are subjected to protein-protein interaction using STRING database (http://string-db.org/) which provides the information about their interaction and interacting partners which helps us in identification of their functional partners involved during the mechanisms of metal ion transport.

Prediction of 3D Structure and Domain analysis

After STRING analysis, the selected transporters that are involved in transport of Mercury, Iron and Manganese are subjected for homology modeling and their 3D models are built by selecting the probable template using Swiss-Model (http://swissmodel.expasy.org/). The 3D structures that were built are subjected for verification using RAMPAGE (http://mordred.bioc.cam.ac.uk) and active domains are analyzed using ProDom (http://prodom.prabi.fr).

Results and Discussion

A set of metal ion transporters with their sub-cellular localization are identified from TransportDB and PSORTdb respectively and a total of **33** metal ion transporters are tabulated table 1. A total of 33 metal ion transporters are identified which includes 3 cobalt transporters, 3 iron transporters, 1 molybdenum transporter, 2 manganese transporter, 1 calcium transporter, 1 chromate transporter, 1 chloride transporter, 1 mercury transporter, 8 sodium transporter, 4 potassium transporter and remaining 8 share commonality for sodium and potassium.

From this data the metal ion transporters that include Mercury (DR-2452), Manganese(DR-2284, DR-1709) and Iron (DR-2590, DRD-0121, DR-1219) are subjected to STRING analysis and their interactions and interacting partners are identified. The interactions indicate their functional protein partners are involved in the mechanism of transport of metal ion are interacting with each other as shown in table 2.

After STRING analysis these proteins are subjected to homology model and their 3D structures are obtained using the templates searched from Swiss Model tool and later these structures are validated using RAMPAGE are illustrated in table 3. Then to know their functionality the domains are identified using PRODOM and from the domain analysis it is clear that these proteins transports the metal ions as their functional domains are ABC transport related cell, Manganese Transporting domain and iron binding domain as shown in table 4.

Conclusion

The present *insilico* study reveals the complete suite of Metal Transporters in *D.radiodurans*, of which the metal ion transporters that transport Mercury, Ferrous and Manganese ion are well studied as they are the major constituents of the most hazardous wastes released from most of the industries. The STRING analysis defines that the functional partners involved in transport of metal ions. The domains predicted conclude that they are mainly involved in the transport of Manganese, Ferrous and Manganese and maintain the concentration of metal ions through their cytoplasmic membrane by taking up them and oxidizing it in their body. As this metal ion can transport such hazardous metal ions they are used in bioremediation process and help protect the environment.

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Table 1. Set of Metal ion transporters identified in *D.radiodurans*

S. No	Metal	Subcellular Localization	ORF ID	Protein Family
1.	Cobalt	Cytoplasmic Membrane	DR2469, DRA0269	The ATP Binding Cassette (ABC) Super Family
2.	Ferrous/Iron	Cytoplasmic Membrane	DR2590, DRB0121, DR1219	The ATP Binding Cassette (ABC) Super Family and the Ferrous Iron uptake (FeoB) Family
3.	Manganese	Cytoplasmic Membrane	DR2284, DR1411	The ATP Binding Cassette (ABC) Super Family and The Meal Ion (Mn^{2+} iron) transporter (Nramp) Family
4.	Molybdenum	Cytoplasmic Membrane	DR2145	The ATP Binding Cassette (ABC) Super Family
5.	Sodium	Cytoplasmic Membrane	DR0927, DRB0133, DRB0119, DR0652, DR2395, DR1149, DR0748a, DR0447	The ATP Binding Cassette (ABC) Super Family, The Alanine or Glycine: Cation Symporter (AGCS) Family, The Monovalent Cation:Proton Antiporter-1 (CPA1) Family, The Monovalent Cation:Proton Antiporter-2 (CPA2) Family, The Divalent Anion:Na+ Symporter (DASS) Family and The Solute:Sodium Symporter (SSS) Family
6.	Magnesium	Cytoplasmic Membrane	DR2399	The CorA Metal Ion Transporter (MIT) Family
7.	Potassium	Cytoplasmic Membrane	DR2336, DR2367, DR1667, DR1668	The Voltage-gated Ion Channel (VIC) Superfamily, The Monovalent Cation:Proton Antiporter-2 (CPA2) Family and The K+ Transporter (Trk) Family
8.	Mercury	Unknown	DR2452	The MerTP Mercuric Ion (Hg2+) Permease (MerTP) Family
9.	Sodium/Potassium	Cytoplasmic Membrane	DR1149, DR0880, DR0881, DR0882, DR0883, DR0884, DR0885, DR0886	The Monovalent Cation (K+ or Na+):Proton Antiporter-3 (CPA3) Family
10.	Chromate	Cytoplasmic Membrane	DR2413	The Chromate Ion Transporter (CHR) Family
11.	Calcium	Cytoplasmic Membrane	DR0373	The Ca2+:Cation Antiporter (CaCA) Family
12.	Chloride	Cytoplasmic Membrane	DR1752	The Chloride Carrier/Channel (ClC) Family

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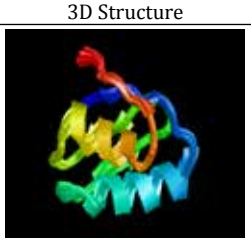
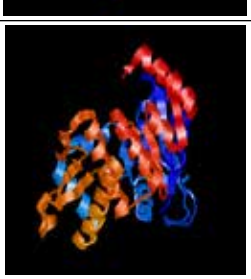
Table 2. Summary of Interacting Partners of Manganese, Iron and Mercury Transporters

S.NO.	ORF ID	PROTEIN NAME	INTERACTED PROTEINS	IP ORF ID	NO. OF A/A	SCORES
1	DR_2590	iron ABC transporter, ATP-binding protein (274 aa)	iron ABC transporter, permease protein, putative	DR_2589	338	0.988
			iron ABC transporter, periplasmic substrate-binding protein, putative	DR_2588	335	0.984
			iron ABC transporter, permease protein	DR_B0122	351	0.929
			hemin ABC transporter, permease protein, putative (354 aa)	DR_B0015	354	0.920
			iron ABC transporter, permease protein	DR_B0123	324	0.906
			metal binding protein, putative	DR_B0007	352	0.881
			hemin ABC transporter, periplasmic hemin-binding protein, putative	DR_B0014	302	0.859
			iron ABC transporter, periplasmic substrate-binding protein	DR_B0125	324	0.821
			metal binding protein, putative	DR_1373	253	0.802
			manganese ABC transporter, permease protein, putative	DR_2283	268	0.611
2	DR_B0121	iron ABC transporter, ATP-binding protein	iron ABC transporter, permease protein	DR_B0122	315	0.996
			iron ABC transporter, periplasmic substrate-binding protein	DR_B0125	324	0.993
			iron ABC transporter, permease protein	DR_B0123	324	0.989
			iron ABC transporter, permease protein, putative	DR_2589	338	0.933
			hemin ABC transporter, permease protein, putative	DR_B0015	354	0.931
			iron-chelator utilization protein, putative	DR_B0124	307	0.895
			iron ABC transporter, periplasmic substrate-binding protein, putative	DR_2588	335	0.887
			hemin ABC transporter, periplasmic hemin-binding protein, putative	DR_B0014	302	0.863
			metal binding protein, putative	DR_B0007	352	0.862
			metal binding protein, putative	DR_1373	253	0.806
3	DR_2284	manganese ABC transporter, ATP-binding protein, putative	manganese ABC transporter, permease protein, putative	DR_2283	268	0.992
			adhesin B	fimA	286	0.937
			A/G-specific adenine glycosylase	DR_2285	363	0.697
			hypothetical protein	DR_2286	502	0.554
			hemin ABC transporter, permease protein, putative	DR_B0015	354	0.504
			iron dependent repressor, putative	DR_2539	232	0.429
			iron ABC transporter, permease protein, putative	DR_2589	338	0.418
4	mntH	manganese transport protein MntH; H(+)-stimulated, highly selective, manganese uptake system (By similarity)	iron dependent repressor, putative	DR_2539	232	0.967
			hypothetical protein	DR_2106	468	0.811
			LamB/YcsF family protein	DR_A0284	300	0.790
			hypothetical protein	DR_A0285	227	0.786
			GufA protein	DR_1032	256	0.779
			glycogen branching enzyme; Catalyzes the formation of the alpha-1,6-glucosidic linkages in glyc[...]	glgB	705	0.652
			DNA-directed DNA polymerase; In addition to polymerase activity, this DNA polymerase 5' to 3' e [...]	polA	956	0.651
			aconitate hydratase	acn	906	0.648
			exodeoxyribonuclease III	DR_0354	283	0.610
			GrpE protein; Participates actively in the response to hyperosmotic and heat shock by preventin [...]	grpE	221	0.596

5	DR_1219	ferrous iron transport protein B	ferrous iron transport protein A	DR_1220	76	0.998
			hypothetical protein	DR_1218	114	0.865
			iron dependent repressor, putative	DR_2539	232	0.829
			manganese ABC transporter, permease protein, putative	DR_2283	268	0.705
			hypothetical protein	DR_1221	245	245
			glycine cleavage system protein H; The glycine cleavage system catalyzes the degradation of gly [...]	gcvH	281	0.576
			hypothetical protein	DR_0982	203	0.548
			manganese transport protein MntH; H(+)-stimulated, highly selective, manganese uptake system (B [...]	mntH	436	0.530
			aconitate hydratase	acn	906	0.526
6	DR_2452	hypothetical protein	cation-transporting ATPase	DR_2453	847	0.987
			hypothetical protein	DR_2449	102	0.971
			hypothetical protein	DR_2451	187	0.873
			hypothetical protein	DR_2450	185	0.865
			cation-transporting P-type ATPase	DR_A0073	728	0.855
			hypothetical protein	DR_A0156	108	0.785
			cation-transporting ATPase	DR_1440	538	0.594
			HSP20 family protein	DR_1114	182	0.546
			transcriptional activator TipA	tipA	185	0.532
			hypothetical protein	DR_2288	170	0.500

Table 3. Prediction of 3D structure and Validation of Manganese, Iron and Mercury Transporters

S.No	Metal	ORF ID	3D Structure	RAMPAGE Validation
1.	Manganese	DR-2284		97.7% in Favored region
2.	Manganese	DR-1709		93.5% in Favored region
3.	Ferrous	DR-2590		86.9% in Favored region
4.	Ferrous	DRD-0121		98% in Favored region

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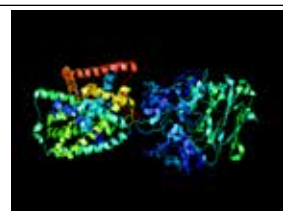
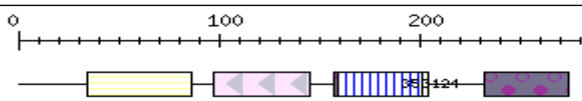
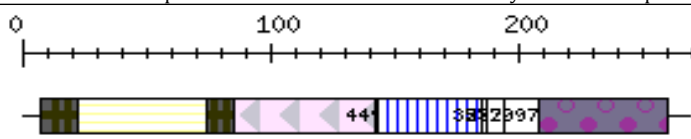
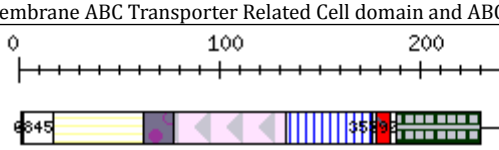
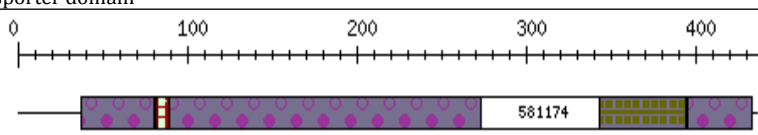
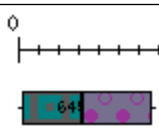
5.	Ferrous	DR-1219		98.8% in Favored region
6.	Mercury	DR-2452		90.1% in Favored region

Table 4. Active Domains identified in Manganese, Ferrous and Mercury ion transporters

ORF ID	Domain
DR2590	 ABC Membrane ABC Transporter Related Cell domain and ABC Hydrolase Transporter domain
DRB0121	 ABC Membrane ABC Transporter Related Cell domain and ABC Hydrolase Transporter domain
DR2284	 ABC Membrane ABC Transporter Related Cell domain, ABC ABC Transporter Cell domain and ABC Hydrolase Transporter domain
DR1709	 ABC Hydrolase Transporter domain and Manganese domain
DR1219	 ABC Hydrolase Transporter domain, Probable ENGB Cycle domain, Ferrous GTP-Binding Membrane Ferrous Transporter domain and Ferrous Transporter domain
DR2552	 Copper Translocation domain and ABC Hydrolase Transporter domain

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