Qeios

Peer Review

Review of: "Multinuclear Non-Heme Iron-Dependent Oxidative Enzymes: Landscape of Their Substrates, Partner Proteins"

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This study explores the diversity of MNIO BCGs, the variety of substrates, and partners associated with the MNIO superfamily through in silico analysis.

The research uncovers that MNIO clusters include different gene partners that can be regrouped as DUF2063 domain-containing partner proteins or others. Different subgroups emerged. Diverse common points are shed light on:

- One MNIO BCG subgroup carrying PiPP with a signal peptide, including Buf1, Buf 2, and the 2 Buf EGKCG. This family belongs to the PME-Type 1 type, which means it includes a DUF2063 domain partner « fused » to an RRE domain partner.

- One MNIO subgroup carrying PiPP without a signal peptide, including TIGR04222 and the partner proteins carrying the CxxxxC motif. This family can be clustered in the PME Type 2 Sub-Family, which means it includes a DUF2063 domain protein partner without an RRE domain partner protein.

Major remarks :

The purpose of this study is to analyze and determine the outcomes of gene in silico analysis of NMIO OGCs. The article is well explained and easily trackable even if an amazing quantity of information is being read. But that’s what we can expect from in silico analysis.

I. However, the tracking could be easily facilitated by adding a table summarizing all the partners of the operons and their respective identification names. As an example:

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Protagonist	« names »	PFAM	+/- signal peptide	Sub family	Predicted lenght	Partner association	… As Genome numbers, localization compartment
MNIO	Fe oxydase	DUF692					
	MbnB TglH CHrH	PF05114					
RiPP	MbnA						
	BUF 1	DUF2282	+	PME Type 1			
	BUF 2	DUF2063	+	PME Type 1			
	BUF EGKCG/oxazoline		+	PME Type 1	31 à 506		Reapeted motif Glu-Gly-Lys-Cys-Gly Or EGKCG motif Folding in beta helix
	BUF EGKCG/EF-Hand 5		+	PME Type 1			Ca binding site
	Buf1|Cytochrom_C|FGE-sulfatase	PF00034					
	Buf1|Cytochrom_C|FGE-sulfatase	PF03781					
	MerC|Buf1	PF03203					

	MerC|Buf2						
	TIGR04222		-	PME Type 2	70 à 643		Large periplasmic dom
	CxxxxC		-	PME Type 2			
	Chryebassin						
	Minor RiPPs						
Other Partners	Anthrone oxy	PF08592					
	TIGR04160 MbnC Meth bact						
	DUF2063	PF09836					
	-RRE dom -C Rich beta-helix						
	PME Type 1				30 à 835	DUF2063+ RRE dom	
	PME Type 2				51 à 367	DUF2063- RRE dom	
	DUF2282						
	DoxX	PF07681					
	Sigma-70 region 4	PF08281					
	Sigma-70 region 2	PF04542					
	NrsF	PF06532					
...							

II. Are all metal-oxidizing bacteria equipped with this MNIO cluster?

III. The text does not explain why RiPPs need a signal peptide motif to function. We do not have an idea of the functioning mode of RiPP. You write « The bufferin's action mode involves trapping copper metal into the periplasm when it is in excess in the medium ». But there are not enough details to go around. As:

“These RiPPs should be involved in the excess Metal surviving”. Does it happen inside the cell, outside, or both?

IV. Tell us more about RiPP substrates featuring export signal sequences or other secretion systems. A table summarizing the predicted final probable location of the RiPPs may be a plus (inner membrane (for the lipoproteins ones?), periplasm (as in ascomycetes), or even extracellular environment (via Transporter ABC or others?)

You mention some exportation system across the inner membrane; do you have an idea of who they are?

After your genomic analysis, do TAT signal sequences appear or other transport signals on the BGCs ?

You announce ABC transporters in the text. Does it mean that some RiPPs could be secreted into the bacteria’s environment?

Moreover, cysteine residues seem important for the MNIO function. Do we have more information about it? And what can be the role of the other cysteines? Can we expect some SH oxidation of PiPPs via MNIO into the cytoplasm and C-C bond formation after inner translocation of the other cysteines? Information about it can be evaluated on an application dedicated to predicted bond formation (with expected length and others).

Displaying a prediction model of the MNIO-PiPP complex that includes the predicted binding pocket and cysteine residues could be intriguing.

Even though the functions of the two domains seem totally different, one is dedicated to binding NMIO and the other is directed towards the inner membrane. Given that the DUF2063 domain’s function has opposite interdependency with the RiPP carrying the signal sequence, what are your expectations?

Little Remarks :

I. Fig 1B : Does cluster 11 refer to MbnB (as mentioned in the figure) or a Pearline (as mentioned in the legend)?

II. I don’t see an abbreviation list summary used in the text; could you add it, please?

III. What is a b-helix fold ? A β-helix fold? If it is, please change the b to β.

IV. At the beginning of your discussion, you mention only eubacteria group studies. But in the supplement data, it seems that you extend your study to other bacteria such as archaea. It's not clear. Please be precise.

V. It's not clear if Gram-positive bacteria's group carry MNIO BGCs. Do they ?

VI. In the Mat and met parts, you mention a « RODEO approach » and a "home-made RODEO approach" was used. What do they mean exactly? Add more explanation and details.

VII. In the supplement material Table S1 (and also p29), you mention DUF692. It will be easier to read and faster to understand if you remind in the Table S1 title that DUF692 corresponds to the NMIO gene

Same remark for DUF2282 (BUF 1) and DUF2063 (PTM domains) that would serve as protein partners for MNIOs.

VIII. Finally, I am looking for the Excel file link for Tables S3 and S4. How can I access it, please?

("Table S3. Accession numbers of all proteins identified in this work (Excel file)

Table S4. Analysis of complete genomes: genome identification, accession numbers of

the proteins of interest, and numbers of paralogs in the genome (Excel file)").

IX- In the discussion, the perspectives of the study are missing; what is next?

Declarations

Potential competing interests: No potential competing interests to declare.