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Confirmation that mrub_1751 is homologous to *E. coli* xylF, mrub_1752 is homologous to *E. coli* xylH, and mrub_1753 is homologous to *E. coli* xylG

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Confirmation that mrub_1751 is homologous to *E. coli* xyIF, mrub_1752 is homologous to *E. coli* xyIH, and mrub_1753 is homologous to *E. coli* xyIG.

Introduction

It had been hypothesized that the ABC transporters that function in *E. coli* have homologs in a variety of other microbes. By studying the transporters in *E. coli*, we should be able to recognize the corresponding transporters in other genomes. These are important to locate because of the many substrates that they carry, ranging from the uptake of special sugars or antibiotic protection, secreting toxins, or even, in the case of some mutations, causing some genetic disorders (Moussatova *et. al.* 2008). For this particular study we are focusing on the D-xylose transporters, found in *E. coli*, and the proposed homologs in *Meiothermus ruber*, a Gram-negative bacterium discovered in hot springs ranging across northern Europe and Asia (Tindall *et. al.* 2010). This bacterium is being used as our study organism because it has not been well studied, though a majority of its genes (71.8%) have been given putative functions, which gives some idea of what each gene is most likely to be (Tindall *et. al.* 2010).

The genes in question that we are studying are the putative D-xylose transport genes of *M. ruber*, Mrub_1751, Mrub_1752, and Mrub_1753. The D-xylose system is important for the cell because it is used in the pentose phosphate pathway to produce D-xylulose 5-phosphate (Song and Park 1997). The corresponding genes, in order are xylF, xylH, and xylG in *E. coli*, and are part of an operon, as also discussed in Song and Park (1997). The transporter made from these three genes is embedded in the cell

1

membrane, with XyIF filling the role of the solute binding protein, XyIH the transmembrane domain, and XyIG being the nucleotide/ATP binding protein.

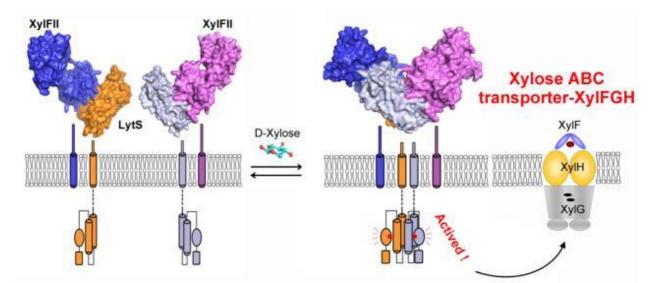


Figure 1. Activation and embedding of Xylose ABC transporter in cell membrane. Activation is initiated when a complex of the membrane-associated sensor protein XylFII and a transmembrane histidine kinase LytS senses d-xylose in the environment, activating the ABC transporter. Taken from Jia (2017).

Via Blast, it was noted that each gene had paralogs within the *M. ruber* genome, with Mrub_1751 only having 2 significant hits, Mrub_1752 having 6 significant hits, and Mrub_1753 having many, many hits (Altschul *et. al.* 1990). This study seeks to prove that Mrub_1751 is a homolog to *E. coli* xylF (b_3566), Mrub_1752 is homologous to *E. coli* xylH (b_3568), and Mrub_1753 is homologous to *E. coli* xylG (b_3567).

Materials and Methods

This study was completed by utilizing the *M. ruber* genome stored on KEGG (Kanehisa *et. al.* 2016). The gene in both nucleotide and amino acid chain form were obtained, which were then BLASTed (Altschul *et. al.* 1990) against the *E. coli* genome to discover likely homologs. The same process was repeated to discover homologs from other species, and fifteen homologous genes were selected. The results were

2

aligned by T-Coffee (Notredame *et. al.* 2000) and a Weblogo (Crooks *et. al.* 2004) was created to find highly conserved residues. This was followed up with TMHMM (Krogh *et. al.* 2001, Krogh and Rapacki 2016, Sonnhammer *et. al.* 1998) to determine the number of transmembrane helices, SignalP (Petersen *et. al.* 2012) to determine the probability of having a signal peptide, LipoP (Juncker *et. al.* 2003) to determine the most likely ending point of the protein if it had a signal peptide, PsortB (Yu *et. al.* 2010) to determine the most likely ending location of the protein, and Phobius for the same (Kall *et. al.* 2004, Kall *et. al.* 2007). The gene was then BLASTed again to determine the CDD (Marchler-Bauer *et. al.* 2015), TIGRFAM (Haft *et. al.* 2001), and PFAM (Finn *et. al.* 2014, Fin *et. al.* 2016) groups. The amino acid sequence was entered into the Protein Data base (PDB) (Berman *et. al.* 2000) to determine the closest crystallized known protein. Finally, IMG/M (Markowitz *et. al.* 2012) was consulted to determine the likelihood that the protein was part of an operon.

Results

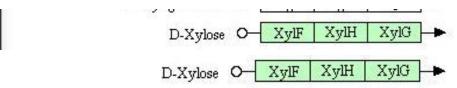


Figure 2. KEGG pathways monosaccharide ABC transporters for *M. ruber* (Top) and *E. coli* (Bottom). Note that both include the full D-xylose pathway.

The KEGG results showed that both organisms included the D-xylose

transporters, the first piece of evidence that the genes may be homologs. This was

followed by the BLAST of each gene, pictured below.

Download - GenPept Graphics	Vex	kt 🔺 Previous 🛓 Descriptions	Bownload + GenPept G	raphics		Vext 🔺 Previous 🛔 Description
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	Query 61 GETTAGET INSOLTAGY TRADI Solt 59 GOT/MORE/STOTA-VGO Solt 59 GOT/MORE/STOTA-VGO Solt 19 LEARTINEAU/CLAYSISTOTA-VGO Solt 19 LEARTINEAU/CLAYSISTOTA-VGO Query 29 VG 240 Solt 238 IG 239 Range 2: 20 to 234 Sinches Grandho Solt 238 IG 239 Range 2: 20 to 234 Sinches Grandho Gorey 207 WG/SISKELIGALAUAGE Solt 238 IG 239 Range 2: 20 to 234 Sinches Grandho Gorey 277 WG/SISKELIGAUAGE Solt 230 IG 240 Sinches Grandho Solt 230 IG 237 GIANNEDRIAGET/PHILIPSOL Query 237 VG/SISKELIGAUAGE Solt 230 IG 237 GIANNEDRIAGET/PHILIPSOL Query 237 VG/SISKELIGAUAGE Solt 20 IG/SISKELIGAUAGE Solt 20 IG/SISKELIGAUAGE Query 237 VG/SISKELIGAUAGE Query 237 VG/SISKELIGAUAGE Query 237 VG/SISKELIGAUAGET/PHILIPSOL	Identities Peak matrix adjust 92/242(38%) 146/ CLRIANGEYUSCHEDGSCHTWINCOGE SMILVREINGLOHIGGSCHTWINCOGE SMILVREINGLOHIGGSCHTWINCOGE HITTGLIALADULAWWINFLEIGENENGEN USGOOGRAVIAAUNGUNUTUREST USGOOGRAVIAAUNUNUNUTUREST USGOOGRAVIAAUNGUNUTUREST USGOOGRAVIAAUNUNUNUNUNUNUNUNUNUNUNUNUNUNUNUNUNUNU	242(00%) 5/242(2%) 9/45/1 60 000 000 000 000 000 000 000	Related Information Identical Protains : Identical proteins to VP_013014015.1		
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Figure 3. BLASTs of the *E. coli* amino acids chains against *M. ruber* genome. Top left being xylF and Mrub_1751, top right being xylH and Mrub_1752, and bottom being xylG and Mrub_1753.

The e-values for each of these BLASTs was, in order, 9e-61, 1e-58, and split

gene at 1e-48 and 2e-24 for each section. Tables for each of the matchups below show

the similarities between the genes.

Table 1.	b 3566	and Mrub	1751

Table 1. D_3566 and Wrub_1751	D 0500			
	B_3566	Mrub_1751		
BLAST	9e-61			
CDD	COG4213 ABC-type	COG4213 ABC-type		
	xylose transport system,	xylose transport system,		
	periplasmic component	periplasmic component		
Localization	Periplasmic	Periplasmic		
TIGRFAM	TIGR02634 D-xylose ABC	TIGR02634 D-xylose ABC		
	transporter, substrate	transporter, substrate		
	binding protein	binding protein		
PFAM	PF13407 Periplasmic	PF13407 Periplasmic		
	binding domain	binding domain		
PDB	3M9W Open ligand free	3M9W Open ligand free		
	crystal structure of Xylose	crystal structure of Xylose		
	binding protein from	binding protein from		
	Escherichia coli	Escherichia coli		
Table 2. b_3568 and Mrub_1752				
	B_3568	Mrub_1752		
BLAST	1e	e-58		
CDD	COG4214 ABC-type	COG4214 ABC-type		
	xylose transport system,	xylose transport system,		
	permease component	permease component		
Localization	Cell Membrane	Cell Membrane		
TIGRFAM	No matches	No matches		
PFAM	PF02653 Branched-chain	PF02653 Branched-chain		
	amino acid transport	amino acid transport		
	system/permease	system/permease		
	component	component		
PDB	No matches	No matches		
Table 3. b_3567 and Mrub_1753				
	B_3567	Mrub_1753		
BLAST	1e-	-48		
	2e-	-24		
CDD	COG1129 ABC-type sugar	COG 1129 ABC-type sugar		
	transport system, ATPase	transport system, ATPase		
	component	component		
Localization	Cytoplasmic Membrane	Cytoplasmic Membrane		
TIGRFAM	TIGR02633 D-xylose ABC	TIGR02633 D-xylose ABC		
	transporter, ATP-binding	transporter, ATP-binding		
	protein	protein		
PFAM	PF00005 ABC transporter	PF00005 ABC transporter		
PDB	1G9X An ATP binding	1G9X An ATP binding		
	cassette of an ABC	cassette of an ABC		
	transporter	transporter		
		transporter		

Based on the information included in these tables, it appears that we have genes that are homologous here. A point of interest related to Table 3 is that there were two hits for Mrub_1753 within the same gene when BLASTed against the *E. coli* genome. This is likely because *E. coli* xylG is a fused gene, containing two ATP-binding domains (Keseler *et. al.* 2013), which indicates that *M. ruber* may require two of Mrub_1753 to be translated to yield one complete protein complex, as opposed to the one likely required by *E. coli*. One additional point of interest within these tables is that b_3567 and Mrub_1753 are listed as predominantly in the cytoplasmic membrane. This is different from other ABC transporters, which are in the cytoplasm, which means that part of the protein may be partially embedded in the cell membrane. The PSORTb results that gave this answer are shown below.

PSORTb Resu	lts (<u>C</u>	lick here for an ex	PSORTb Results	(Click here for an ex
	PLVEMR Unk Unk Unk Unk Unk Unk Unk Unk Unk Unk	NISLRFGGNQALDKVSVNLY nown nown nown nown nown nown nown oplasmicMembrane nown nown	SeqID: b_3567 MPYLLEMK Analysis Report: CMSVM- CytoSVM- ECSVM- ModHMM- Motif- OMPMotif- OMSVM- PPSVM- Profile- SCL-BLAST- SCL-BLAST-	NI TKTFGSVKAI DNVCLRLN/ Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown CytoplasmicMembrane Unknown 2.11
Periplasmic		0.00	OuterMembrane	0.00
OuterMembrane		0.00	Extracellular	0.00
Extracellular		0.00	Final Prediction:	0.00
Final Prediction:		0.00	CytoplasmicMembran	e 7.88
CytoplasmicMemb		7,88	Cycopiasiii chenoran	e 7.00

Figure 4. PSORTb results for b_3567 and Mrub_1753. Contrary to expectations, the proteins appear to be majority embedded in the membrane, rather than in the cytoplasm. From Yu *et. al.* (2010).

Additionally, both sets of genes show evidence of being operons based on the position of the genes in relation to each other in the genome, as well as the fact that *E. coli* is confirmed to utilize the D-xylose operon. Images of the genomes around these genes are shown below.

2737891 27408		2746891 274	3891 2752		
			∢ ⊂		
xylA : xylose isomerase, subunit of	xylose isomerase			xylG	xyiH
3,729,600 3,729,800 3,730,000 3,750,2 xvH	00 3,730,400 3,730,600 3,730,800 3	731,000 3,731,200 3,731,400 3,731,600 3,7	31,800 3,732,000 3,732,200 3,732,400	3,732,600 3,732,800 3,733,000 3,733,200	3,733,400 3,733,600 3,733,801

Figure 5. Genomes in locations of genes of interest in *M. ruber* (top) and *E. coli* (bottom) (taken from Keseler *et. al.* 2013). The genome for *E. coli* wraps around to the second line in the middle of the xylH gene, but it can still be seen that the genes are very close together, and even overlapping in *E. coli*, with an overlap of 22 nucleotides, as xylG ends at nucleotide position 3733742, and xylH starts at nucleotide position 3733720.

Additional research was done to examine whether the start codons for each of the genes examined were correct, yielding the following figure 6. This figure shows that for each gene it appears that the start codon has been correctly identified, as there was no evidence otherwise. The upstream region of each gene shows no likely replacements for the start codon, while the Weblogos show agreement in the starting amino acid. Of note is that one of the Weblogos for these genes has a large gap in the first row, due to one genome having a larger gene focused towards the beginning.

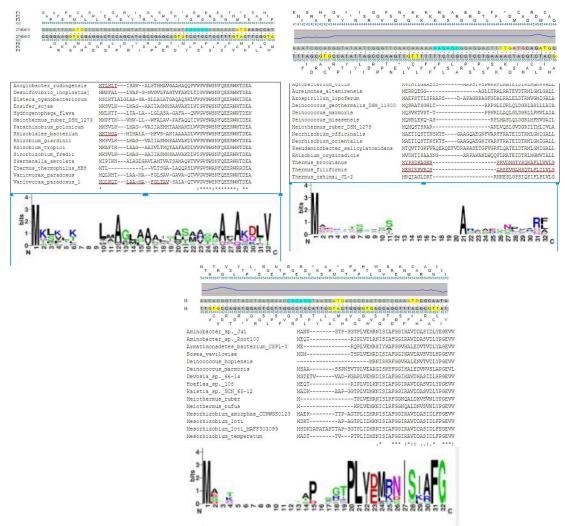


Figure 6. Start codon analysis of *M. ruber* genes. There is no evidence that would indicate incorrect start codon placement.

Finally, there was evidence that there were paralogs for each of the *M. ruber*

genes researched, as shown below in figure 7. Of note is the relatively low number of

paralogs for Mrub_1751 and Mrub_1752 compared to Mrub_1753.

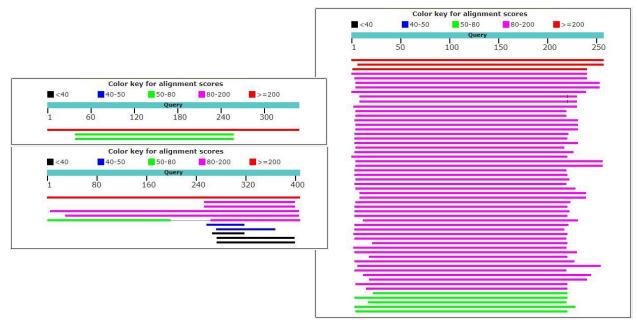


Figure 7. BLASTs of genes of interest against *M. ruber* genome. Displays paralogs of Mrub_1751 in the top left, Mrub_1752 in the bottom left, and Mrub_1753 on the right. Of note is the large number of paralogs for Mrub_1753, indicating a basic structure.

Conclusions

In conclusion, it would appear that our hypothesis is correct, as it appears that Mrub_1751 is homologous to b_3566, Mrub_1752 is homologous to b_3568, and Mrub_1753 is homologous to b_3567. Due to the low value of the e-values, which indicated the likelihood that two genes are similar due to random chance, with higher values indicating high likelihood of random chance, as well as the presence of paralogs within the genome that were similar to the *M. ruber* genes, but different enough from the *E. coli* genes, indicates these genes are orthologs. Additionally, both sets of genes are part of an operon, lending weight to the conclusion that they are orthologs.

Additionally, for Mrub_1751, the shared COG hit COG4213: ABC-type xylose transport system, periplasmic component, PFAM hit PF13407: Periplasmic binding domain, and TIGRFAM hit TIGR02634: D-xylose ABC transporter, substrate binding protein, shared with b_3566 all indicate that the genes are likely paralogs.

For Mrub_1752, the shared COG hit COG4214: ABC-type xylose transport system, permease component, PFAM hit PF02653: Branched-chain amino acid transport system/permease component, and lack of a TIGRFAM hit with b_3568 all indicate that the genes are likely paralogs.

For Mrub_1753, the shared COG hit COG1129: ABC-type sugar transport system, ATPase component, PFAM hit PF00005: ABC transporter, and TIGRFAM hit TIGR02633: D-xylose ABC transporter, ATP-binding protein with b_3567 all indicate that the genes are likely paralogs.

Finally, for a future study, I will propose the change of the proline at position 106 to an alanine in Mrub_1751 to determine whether that will change the functionality of the protein. This will likely cause some sort of conformational change as alanine is a very small amino acid, with an r-group composed of a single carbon, whereas proline is a complicated amino acid that connects the r-group to the n-side of the amino acid (Betts and Russel 2003). The figure detailing how we would do that is below.

Click and drag to set m	utagenesis region		M	rub_1751 1038	3 bp	
>Mrub_1751 1038 bp		100			1	
	STAATGGTGTTGTTGTGGTTTGCACTGGCCGT			Substitution	Insertion	Deletion
	GATCATCGTGGGGGGTAAGCTGGGCCAACTTCC					
	ITGACGAGCGGGCCATCCGCGAGCAGTTGGGC					
	ATCAGCGCCGACGCCCAAAGCTCGTCCGAGAA		F	ind:		
	CGCCCTGATTGCGCGCGGGGCCAAGGCCCTGA		5	ina:		
	AAGACGCCATCCTGCCCGCCATTGACAAGGCC		S	tart and end po	sitions include	d in substitution
AGGCTGCGGGCATCCC <mark>G</mark>	STGGTGGCCTACGACCGCCTCATCGAAAACGA		c	tart (5') 318	End (3')	220
TACGCCTACTACATCAC	CTTCGACAACGTGGAGGTAGGCCGGATGCAGG		3	(art (5) 510		320
CCGCGAGGTCTATAAGG			D	esired Sequend	e	
GGGCCAACACCGACCCC	AACGCCGACTTCCTGCATAAAGGGCAGCTCGA			GA		
GTGCTCGATGCCGCCAT	CAAGCGCGGCGACATCAAGGTGGTGGGCAAAC		20	27.000 C		2000
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TCCTCACCGCCAACGGC	AACAAAGTGGACGCGGTGGTGGCCTCCAACGA					
GGCACCGCCGGCGGTGT	GGTGGCTGCGCTGGCCGCGGTGGGCCTGGCCG					
CAAGGTGCCGGTCTCGG	SCCAGGACGGCGACCAGGCCGCCCTCAACCGG					
TGGCGCGTGGGCTGCAA	ACCGTTAGCGTCTGGAAGGATGCCCGCGAGCT					
GGCCGCCGCGCTGCCGA	GGCCGCGGTGCTGCTGGCCCGTGGTACCCCCA	•				
Result						
LTRPR	L R A S P R W P					
IDKAKA	AGIPEVAY					
H * Q G Q G	CGHPRGGL					
ATTGACAAGGCCAAGGC	IGCGGGCATCCCcgaGGTGGCCTAC					
TAACTGTTCCGGTTCCG	ACGCCCGTAGGGGCTCCACCGGATG					
Required Primers						
Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm Ta*		
5SDM_2/10/2018_F	CGGGCATCCCcgaGGTGGCCTAC	23	74	68°C		
255DM_2/10/2018_R	CAGCCTTGGCCTTGTCAATG	20	55	66°C		

* Ta (recommended annealing temperature)

Figure 8. Changing of the highlighted GGT to a CGA would yield the change from a proline to an alanine, something that will change the conformation of the protein.

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