Coursework 2

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github.com/jenjane118/ngs_tutorials/coursework_2.md

Question 1

Compile a set of virulence-related genes in the AFPN02.1 strain and other E. coli strains and compare them.

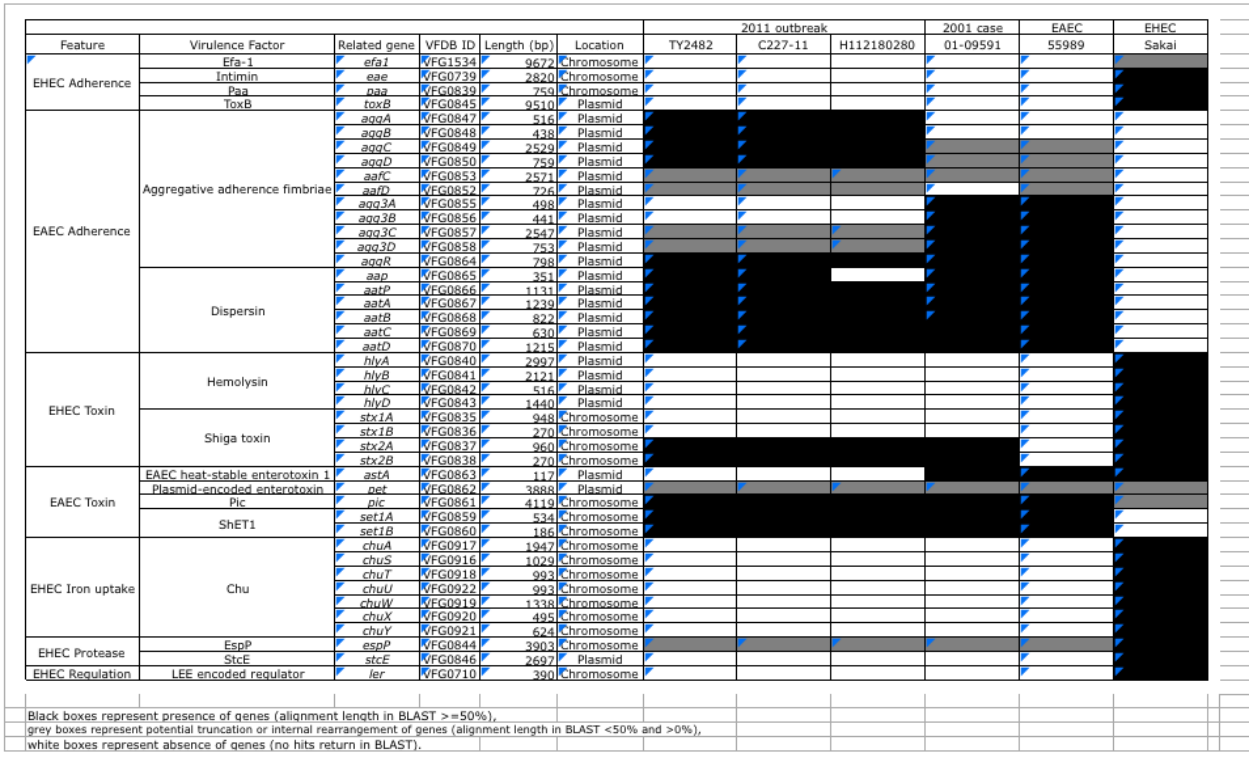
First I went to the VFPB website (http://www.mgc.ac.cn/VFs/main.htm) and looked up 'virulence factors' associated with E. coli strains. The most common of these are: adherence, toxin, invasion, Type III translocated protein, and immune evasion. I can use these terms to annotate genes associated with virulence.

VFDB website, Escherichia Coli

a) Assemble a set of virulence-associated genes from public source or publication

From VFDB I was able to download a database of DNA sequences of core set of experimentally-verified virulence-associated genes. This will be used to extract genes (and relevant sequences) for the associated adhesion genes for searching and annotating the AFPN02.1 genome. (VFDB_setA_nt.fas) (http://www.mgc.ac.cn/VFs/download.htm) (Jin, *et.al.*, 2007).

Using the information on the VFDB website (http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Escherichia), Brzuszkiewicz, *et. al.* (2011), and the spreadsheet of virulence genes from EHEC and EAEC strains from Cheung, *et.al*, (2011), I compiled a list of 18 pertinent virulence-related genes. There were many more genes available in the database, but these genes were selected because they are related to adhesion and toxin production. I used the gene names to select virulence-related genes from the VFDB.



						2011 outbreak			2001 case	EAEC	EHEC
Feature	Virulence Factor	Related gene	VFDB ID	Length (bp)	Location	TY2482	C227-11	H112180280	01-09591	55989	Sakai
EHEC Adherence	Efa-1	efa1	VFG1534	9672	Chromosome						
	Intimin	eae	VFG0739	2820	Chromosome						
	Paa	paa	VFG0839	759	Chromosome						
	ToxB	toxB	VFG0845	9510	Plasmid						
EAEC Adherence	Aggregative adherence fimbriae	aggA	VFG0847	516	Plasmid						
		aggB	VFG0848	438	Plasmid						
		aggC	VFG0849	2529	Plasmid						
		aggD	VFG0850	759	Plasmid						
		aafC	VFG0853	2571	Plasmid						
		aafD	VFG0852	726	Plasmid						
		agg3A	VFG0855	498	Plasmid						
		agg3B	VFG0856	441	Plasmid						
		agg3C	VFG0857	2547	Plasmid						
		agg3D	VFG0858	753	Plasmid						
		aggR	VFG0864	798	Plasmid						
	Dispersin	aap	VFG0865	351	Plasmid						
		aatP	VFG0866	1131	Plasmid						
		aatA	VFG0867	1239	Plasmid						
		aatB	VFG0868	822	Plasmid						
		aatC	VFG0869	630	Plasmid						
		aatD	VFG0870	1215	Plasmid						
EHEC Toxin	Hemolysin	hlyA	VFG0840	2997	Plasmid						
		hlyB	VFG0841	2121	Plasmid						
		hlyC	VFG0842	516	Plasmid						
		hlyD	VFG0843	1440	Plasmid						
	Shiga toxin	stx1A	VFG0835	948	Chromosome						
		stx1B	VFG0836	270	Chromosome						
		stx2A	VFG0837	960	Chromosome						
		stx2B	VFG0838	270	Chromosome						
EAEC Toxin	EAEC heat-stable enterotoxin 1	astA	VFG0863	117	Plasmid						
	Plasmid-encoded enterotoxin	pet	VFG0862	3888	Plasmid						
	Pic	pic	VFG0861	4119	Chromosome						
	ShET1	set1A	VFG0859	534	Chromosome						
		set1B	VFG0860	186	Chromosome						
EHEC Iron uptake	Chu	chuA	VFG0917	1947	Chromosome						
		chuS	VFG0916	1029	Chromosome						
		chuT	VFG0918	993	Chromosome						
		chuU	VFG0922	993	Chromosome						
		chuW	VFG0919	1338	Chromosome						
		chuX	VFG0920	495	Chromosome						
		chuY	VFG0921	624	Chromosome						
EHEC Protease	EspP	espP	VFG0844	3903	Chromosome						
	StcE	stcE	VFG0846	2697	Plasmid						
EHEC Regulation	LEE encoded regulator	ler	VFG0710	390	Chromosome						

Black boxes represent presence of genes (alignment length in BLAST >=50%),
grey boxes represent potential truncation or internal rearrangement of genes (alignment length in BLAST <50% and >0%),
white boxes represent absence of genes (no hits return in BLAST).

Virulence genes (Brzuszkiewicz, et al, 2011)

```
## help for this code came from (https://infoplatter.wordpress.com/2013/10/15/
## extracting-specific-fasta-records-from-a-multi-fasta-file/)
## grabs all ecoli files and sequences
awk 'BEGIN {RS=">"}/Escherichia/{print">"$0}' VFDB_setA_nt.fas \
> ecoli_vfdb_genes.fas
## grabs all files with these genes
cat ecoli_vfdb_genes.fas | awk \
'BEGIN {RS=">"}/aggR|agg3B|aat|astA|hlyA|sepA
|aggA|setA|espP|setC|aat|fimH|fimA|cpxA|cpxR|csgA|elfA|stx/
{print">"$0}' > virulence_vfdb_genes.fa
```

These have really elaborate headings, so I needed to make them much more simple:

```
## removes part of heading before gene name, replaces with '>'
cat virulence_vfdb_genes.fa | sed 's/^>.*(.*)\s(/>/g' > temp.fa
## removes rest of heading after gene name
cat temp.fa | sed 's/)\s.*$//g' > virulence_vfdb_editedgenes.fa
## remove extra blank lines between records
cat virulence_vfdb_editedgenes.fa | sed -e 's/^ *//; s/ *$//; /^$/d' \
> vfdb_virulence_genes.fa
```

b) Build a separate set of virulence-associated genes in the annotation file created for AFPN02.1

The next step is to work with the annotation.gff file to retrieve annotations based on

'virulence', 'adherence', 'toxin', 'invasion' and 'Type III' (short for Type III aggregative adherence fimbriae) and convert into .bed format.

```
cat annotation.gff | grep -E 'virulence|adherence|toxin|invasion|Type III' |
awk 'BEGIN {FS="\t"}  split($9, captured, /[(=);]/) >=10  {print "sequence1"
"\t" $4 "\t" $5 "\t" captured[10] "\t" captured[4] "\t" $7}'
> present_in_AFPN02_virulence_genes.bed
```

Then we have to retrieve the DNA sequence for these genes and save in fasta format:

```
/s/software/bedtools/v2.27.1/bin/bedtools getfasta -name -s -fi \
${st_path}/results_GC/annotation/genome.fna -bed \
present_in_AFPN02_virulence_genes.bed -fo present_in_AFPN02_virulence_genes.fasta
```

A bit more formatting to get rid of extra elements in the heading:

```
cat present_in_AFPN02_virulence_genes.fasta | sed 's/(.*//g' \
> present_in_AFRN02_virulence_genes.fasta
# to remove extra info in square brackets
cat present_in_AFPN02_virulence_genes.fasta | sed '/[.*]/d' > \
present_in_AFPN02_virulence_genes.fasta
#
```

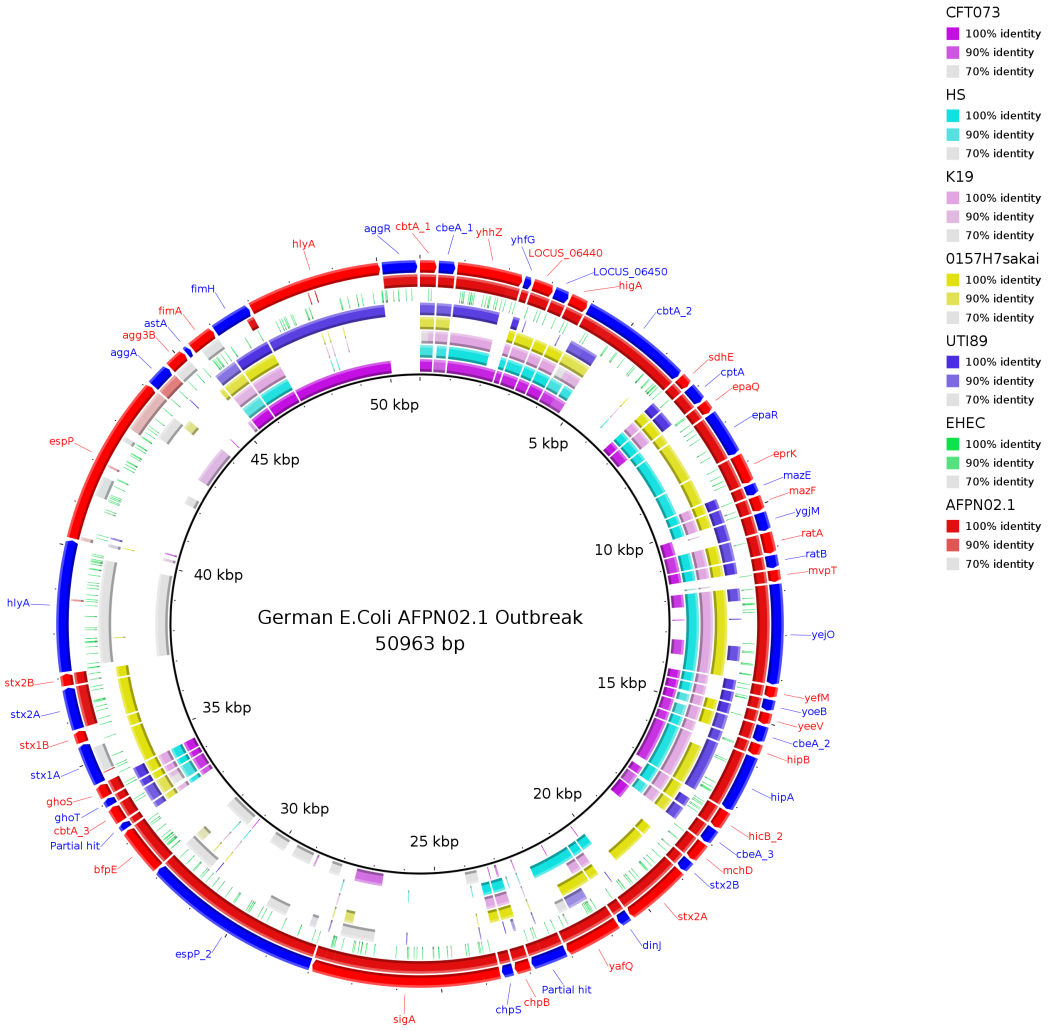
Then we have to combine the two files:

```
cat present_in_AFPN02_virulence_genes.fasta vfdb_virulence_genes.fa \
> final_comparison_virulence.fasta
cp present_in_AFPN02_virulence_genes.fasta vfdb_virulence_genes.fa \
final_comparison_virulence.fasta ${st_path}/results_GC/wholeGenomeExamples
```

c) Use BRIG to visualise which of the virulence genes are present/absent in E. coli strains

To run brig, use the following command:

```
/s/software/brig/BRIG-0.95-dist/brig.sh
```



BRIG: Blast Ring Image Generator (brig.sourceforge.net)

Most of the virulence genes were present in AFPN02.1. However, hylA was only identified with a short sequence, aggA was completely missing, esp was mostly missing, though some parts were present at lower identity, fimH was completely missing, stxA was more weakly identified, and agg3B was more weakly identified. The other shiga-toxin genes, stx2A/B were present in the AFPN02.1 strain, as well as in the sakai strain, which is a EHEC (enterohemmoraghic) strain, but isn't present at all in the other strains, except for short regions of identity with the other EHEC strain. The EHEC strain seemed the most different from the other strains, with only short regions of identity spread out over the genome, however, it seemed there were hits in all of the genes.

Question 2

Select ONE of the virulence genes (or if you prefer one operon) present in AFPN02.1 and study this gene/operon in more detail including its biological action/mechanism phylogeny.

I have chosen to take a closer look at the gene *aggR*, 'aggregative regulator', a putative transcriptional activator that regulates many virulence factors including genes for adherence and toxin production (www.uniprot.org/uniprot/P43464). The *aggR* gene is part of a family of AraC transcriptional activators, regulatory proteins that bind DNA and control the expression of a whole host of other genes. The product of aggR is a DNA binding protein is aobut 30KDa in size, with a conserved helix-turn-helix motif (Navarro-Garcia, 2013).

AggR is called the 'master virulence regulator', as it regulates many other known virulence factors (Morin, 2013). Among the first of these identified are the genes encoding AAF, the aggregative adherence fimbriae. These proteins help the bacterium to adhere to the intestinal mucosa in a characteristic 'stacked-brick' pattern, which stimulates an inflammatory response in these cells, leading to diarrhea (Morin, et.al, 2013). The presence of AggR is essential for expression of AAF genes located on pAA plasmids. Another function is to regulate the expression and secretion of dispersin (by regulating aap and aat genes), another gene product involved in adherence to intestinal mucosa (Figure 1) (Sheikh, et al., 2001). In fact, microarray data has indicated that at least 44 genes are under the control of AggR, 23 of them on the bacterial chromosome, including many secretory proteins. 20 of these are found in what is called a 'pathogenicity island', a cluster of virulence genes that is obtained by a bacterium through horizontal gene transfer (Morin, 2013).

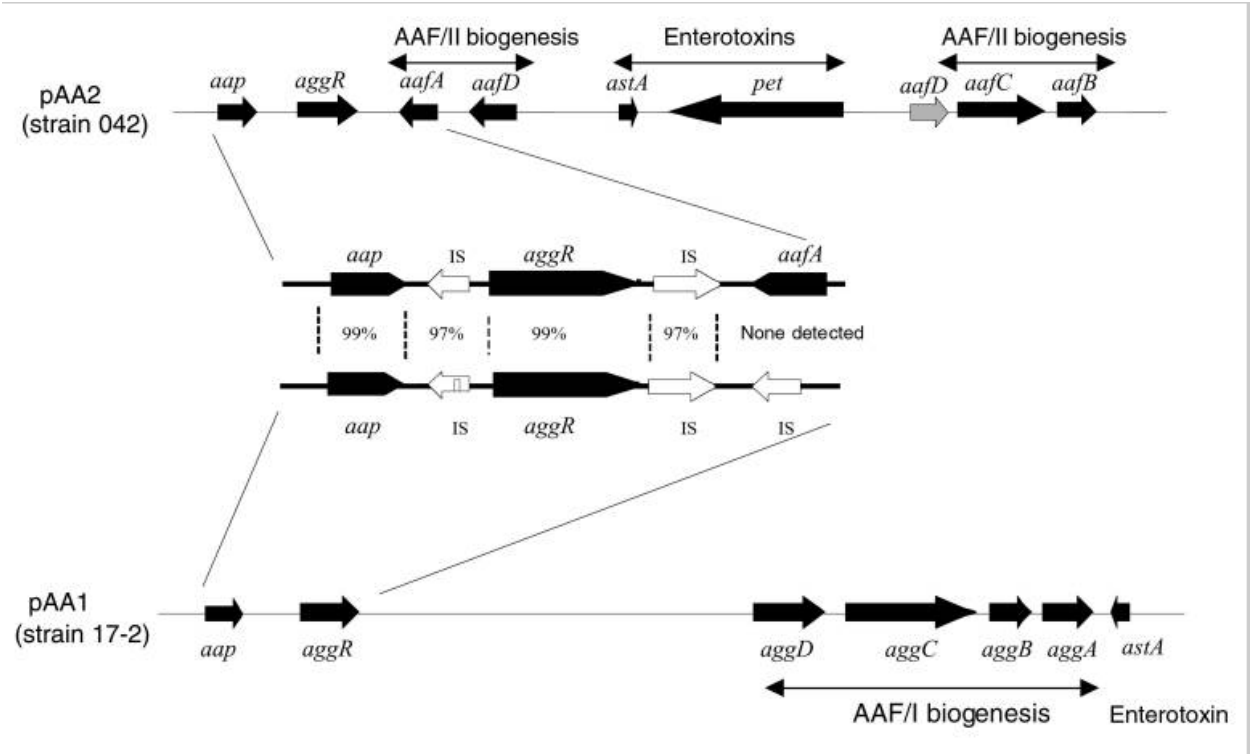


Figure1. Map of aap-aggR loci from two E. coli strains producting AAF/II and AAF/I (Sheikh, 2001)

To explore the phylogenetic origins of the aggR gene, I extracted the fasta sequence from the edited file of virulence genes I obtained from the Virulence Factor Database and ran a blast search.

```
cat vfdb_virulence_genes.fa | awk 'BEGIN {RS=">"}/aggR/{print">"$0}' > aggR.fa
```

I submitted this sequence to nBlast, using the nr/nt database, optimised for 'more dissimilar sequences'. All of the hits were to E. coli, many to various plasmids that have been sequenced (Figure 2). It seems to be a gene found predominantly in enterobacteria, specifically, E. coli. The presence of AggR in the genome, is sometimes considered indicative of a 'typical EAEC' strain (Navarro-Garcia, 2013).

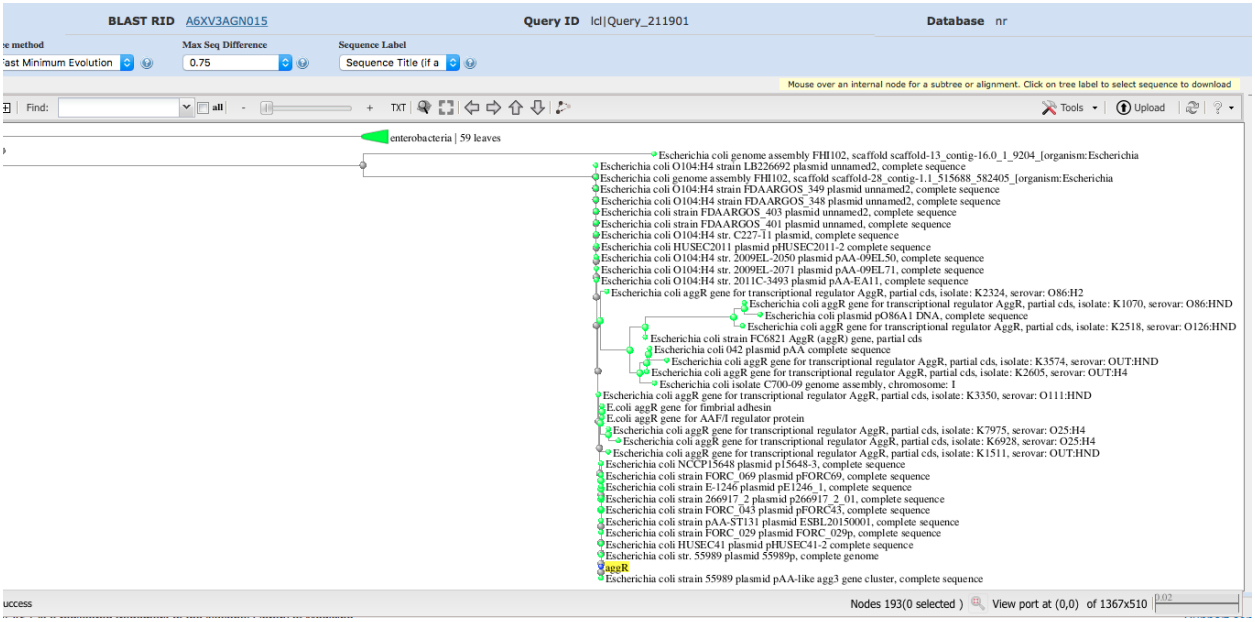


Figure 2. Phylogenetic tree showing evolutionary relationships of aggR gene among E. coli strains.

I thought it might be interesting to see if there were any more interesting results using the protein sequence. I obtained the FASTA protein sequence from uniprot (https://www.uniprot.org/uniprot/P43464)

```
>sp|P43464|AGGR_ECOLX Transcriptional activator AggR OS=Escherichia coli OX=562 GN=aggR
MKLKQNIEKEIIKINNIRIHQYTVLYTSNCTIDVYTKEGSNTYLRNELIFLERGINISVR
LQKKKSTVNPFIAIRLSSDTLRRLKDALMIIYGISKVDACSCPNWSKGIIVADADDSVLD
TFKSIDHNDDSRITSDLIYLISKIENNRKIIESIYISAVSFFSDKVRNTIEKDLSKRWTL
AIIADEFNVSEITIRKRLESEYITFNQILMQSRMSKAALLLLDNSYQISQISNMIGFSST
SYFIRLFVKHFGITPKQFLTYFKSQ
```

and used it in a psi-blast query (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=

blastp&PAGE_TYPE=BlastSearch&BLAST_SPEC=&LINK_LOC=blasttab&LAST_PAGE=blastn) on a standard nr protein database. The results were slightly more interesting, showing that the protein is from the AraC family of transcriptional regulators and related to other genes involved with adhesion, such as the CFA/I fimbrial subunit D. The phylogenetic tree shows the relationship between this family of DNA-binding transcriptional regulators among bacteria, with AraC protein family members from Salmonella and Citrobacter as distant homologues (Figure 3).

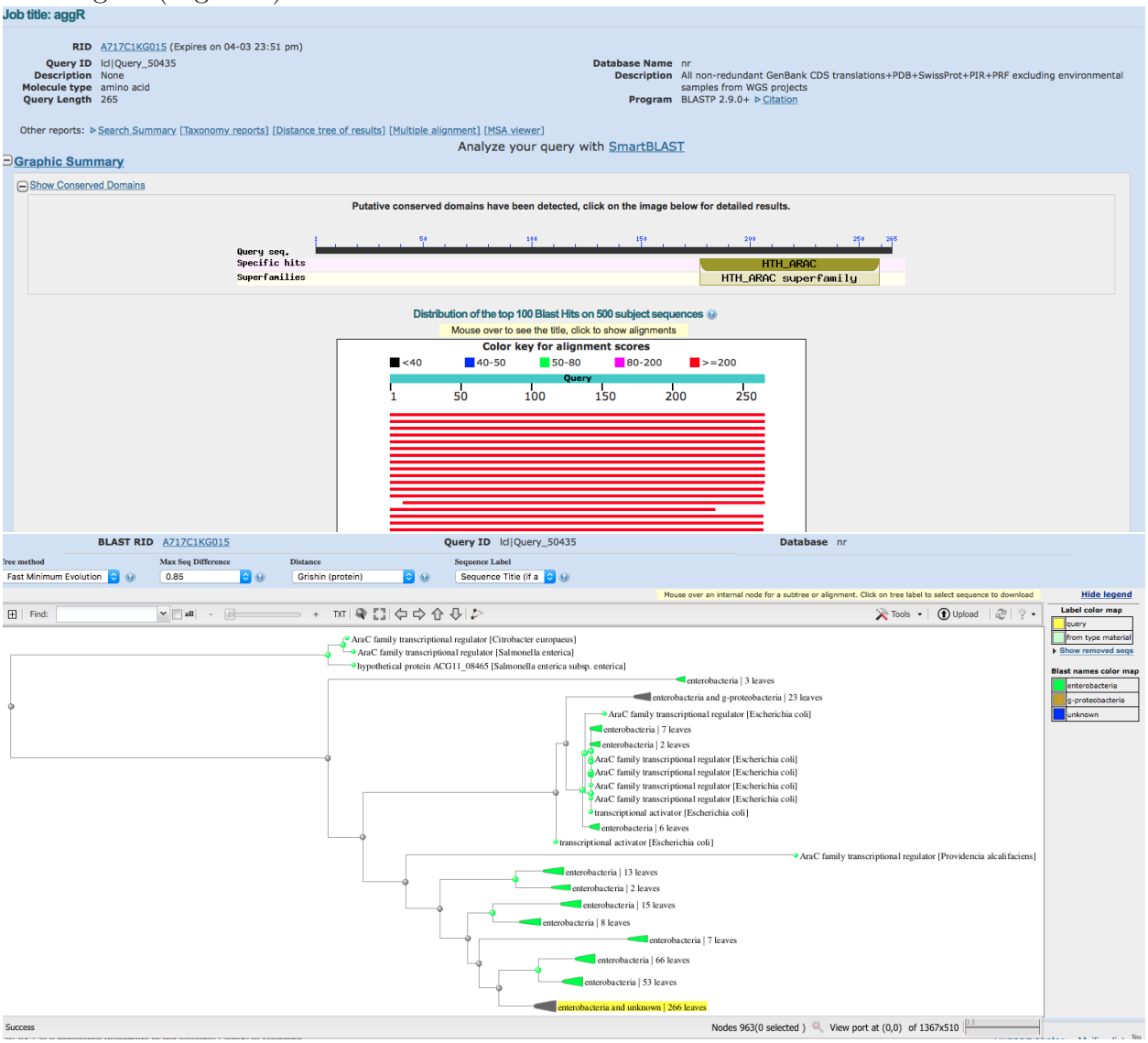


Figure 3. Psi-blastp results and phylogenetic tree for AggR protein.

References

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Sheikh J, Czeczulin JR, Harrington S, et al. (2002). A novel dispersin protein in enteroaggregative Escherichia coli. J Clin Invest., 110(9), 1329–1337. doi:10.1172/JCI16172

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