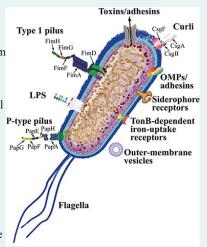
| Started on | Saturday, 21 October 2023, 8:31 AM |
|--------------|--|
| State | Finished |
| Completed on | Monday, 23 October 2023, 11:59 PM |
| Time taken | 2 days 15 hours |
| Marks | 9.50/12.00 |
| Grade | 7.92 out of 10.00 (79.17 %) |

Correct

Mark 1.00 out of 1.00

Escherichia coli is a significant cause of diarrheal illness and mortality worldwide each year, especially among children in developing countries. Pathogenic *E. coli* strains produce diverse virulence factors that allow them to overcome or subvert host defenses and to colonize, injure, and invade host cells or tissues. Today the existence of thousands of *E. coli* genomes in public databases has opened new perspectives for identification of novel virulence factors that could act as targets for treatment of infectious diseases. However, around 25% of the open reading frames (ORFs) in *E. coli* genomes remain as hypothetical genes without known function because they codify for uncharacterized proteins.

One of those ORF is the one that codifies for the uncharacterized protein YeeJ (UniProt P76347) in the *E. coli* K12 genome. Here, to better annotate function for this protein, we will combine similarity searches using protein databases with searches at pattern, domain and family databases.



Question 1. What can you say about the type of evidence that supports the existence of the protein P76347?

Select one:

- \odot a. The existence of this protein is probable because clear orthologs exist in closely related species. \checkmark
- O b. There are clear experimental evidences for the existence of this protein.
- o. The existence of this protein is unsure.
- Od. Don't know/no answer (without penalty).
- Expression data indicate the existence of a transcript for this protein.

https://www.uniprot.org/uniprotkb/P76347/entry

https://www.uniprot.org/help/protein_existence

The value 'Protein inferred by homology' indicates that the existence of a protein is probable because clear orthologs exist in closely related species.

The correct answer is: The existence of this protein is probable because clear orthologs exist in closely related species.

| Question 2 Correct Mark 1.00 out of 1.00 | Question 2. In what biological process does this protein seem to participate? Select one: a. Don't know/no answer (without penalty). b. Amino acid transporter c. Protein kinase signal transduction d. Cell adhesion c. DNA replication https://www.uniprot.org/uniprotkb/P76347/entry#function https://www.ebi.ac.uk/QuickGO/annotations?geneProductId=P76347 The correct answer is: Cell adhesion | | | | | | |
|--|---|--|--|--|--|--|--|
| Question 3 Correct Mark 2.00 out of 2.00 | Question 3. With which nucleotide entry at the NCBI RefSeq database has this UniProt protein cross-reference? (In case of multiple possibilities, use the oldest one) NC_000913 | | | | | | |
| Question 4 Correct Mark 1.00 out of 1.00 | Question 4. In other Escherichia coli strains different to K12, the function of this protein has been annotated as: Select one: a. VacA-like protein b. Inverse autotransporter adhesin YeeJ / Bacterial Ig-like domain family protein ✓ c. Agglomeration and penetration protein d. AIDA-1 autotransporter adhesion protein e. Don't know/no answer (without penalty) We can find it in similar proteins https://www.uniprot.org/uniprotkb/P76347/entry#similar_proteins | | | | | | |

Partially correct

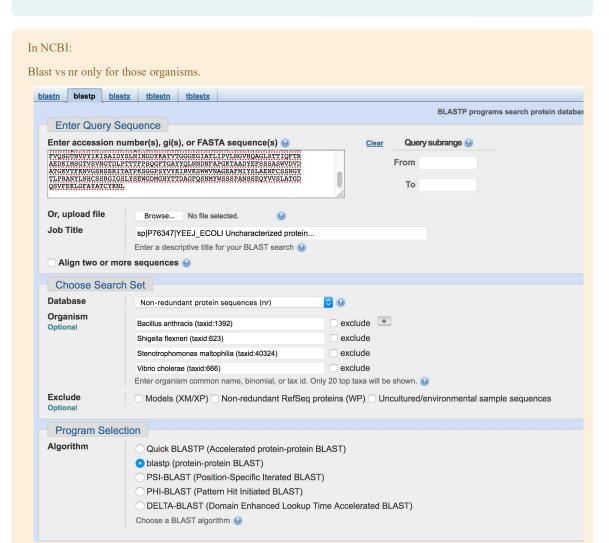
Mark 0.75 out of 2.00

Question 5. Based exclusively on our protein sequence (UniProt P76347), which of these bacterial pathogens seems to be the closest to *E. coli* from an evolutionary point of view? Shigella flexneri ◆

Bacterial motility \$

What function has been assigned to the homologous protein in the selected closest species?

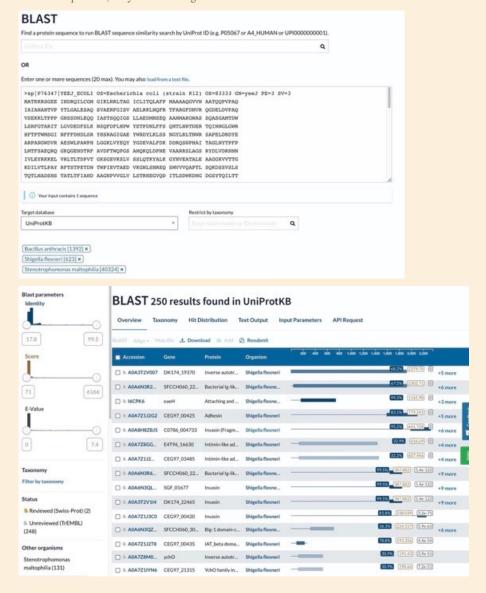
×



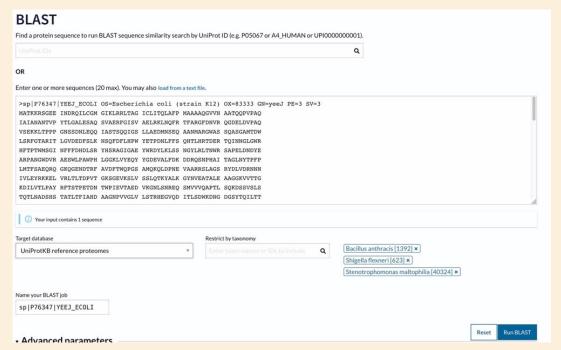
| select all 100 sequences selected | | GenPept Graphics | GenPept Graphics Distance tree of res | | results | ults Multiple alignment | | | Mew MSA Viewer |
|-----------------------------------|--|-------------------|---------------------------------------|----------------|---------|-------------------------|---------|-------------|----------------|
| | Description | Scientific Name | Max Score | Total Score | Query | E value | Per. | Acc. Len | Accession |
| Z | TPA: inverse autotransporter adhesin YeeJ [Shigella flexneri] | Shigella flexneri | 4789 | 4789 | 100% | 0.0 | 100.00% | 2358 | HBD6189621.1 |
| 2 | TPA: inverse autotransporter adhesin YeeJ [Shigella flexneri] | Shigella flexneri | 4785 | 4785 | 100% | 0.0 | 99.87% | 2358 | HBD4498613.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2559 | 4989 | 100% | 0.0 | 73.32% | 2660 | EFP8747227.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2515 | 4936 | 100% | 0.0 | 72.07% | 2660 | EFW8300347.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2433 | 4538 | 100% | 0.0 | 65.74% | 2668 | EFY9118459.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2410 | 2410 | 81% | 0.0 | 68.26% | 1928 | EFZ4075408.1 |
| 1 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2407 | 2407 | 81% | 0.0 | 68.16% | 1937 | EAA0483991.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2407 | 3608 | 81% | 0.0 | 68.14% | 1956 | RIE63217.1 |
| 2 | lg-like domain-containing protein [Shigella flexneri] | Shigella flexneri | 2406 | 3608 | 81% | 0.0 | 68.14% | 1947 | WP_144038553. |
| 1 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2405 | 2405 | 81% | 0.0 | 68.21% | 1928 | EFW4131607.1 |
| 1 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.16% | 1928 | EFV6700637.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1928 | EFZ8507385.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 3605 | 81% | 0.0 | 68.09% | 1956 | RIE49906.1 |
| 1 | TPA: inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1928 | HAY5349584.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EGD9838738.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1928 | EFV7480487.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EFX2207962.1 |
| 1 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EFY0858302.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EAA3114360.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EGD7431760.1 |
| 1 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2404 | 2404 | 81% | 0.0 | 68.11% | 1937 | EGD7187710.1 |
| 1 | Ig-like domain-containing protein [Shigella flexneri] | Shigella flexneri | 2403 | 3603 | 81% | 0.0 | 68.09% | 1947 | WP_133298076. |
| 2 | TPA: inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2403 | 2403 | 81% | 0.0 | 68.11% | 1928 | HAY5337492.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2402 | 2402 | 81% | 0.0 | 68.11% | 1928 | EFZ3494640.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2402 | 2402 | 81% | 0.0 | 68.06% | 1937 | EFX2216494.1 |
| 2 | inverse autotransporter adhesin-like protein YeeJ [Shigella flexneri] | Shigella flexneri | 2402 | 2402 | 81% | 0.0 | 68.06% | 1937 | EAA1459946.1 |
| • | | | | | | | | | |

In Uniprot (Slower)

Blast vs UniprotKB, only for those organisms.



Don't use the "reference proteomes" because if your protein is not in one of them, you will end with the wrong conclusions:





If you use uniprot blast vs UniprotKB we just click on the UP accession code of the first result (https://www.uniprot.org/uniprotkb/A0A3T2V007/entry) and we see in in Function->GO Annotations->Biological process: Cell adhesion

if you use ncbi blast

We get the best 40 NCBI IDs from the last column in the the ncbi blast results (Download -> csv, open with excel, copy the accession column) and map them to uniprot:

https://www.uniprot.org/id-mapping

From EMBL/GeneBank/DDBJ CDS to UniprotKB

and we get:

https://www.uniprot.org/uniprotkb/A0A3T2V007/entry mapped from EAA0483991.1 (the one previously mentioned with the uniprotKB blast search)

in the function section we see a GO code for "cell adhesion"

Question 6

Correct

Mark 1.00 out of 1.00

Question 6. What can we learn from our protein by searching at the <u>PROSITE</u> database?

Select one:

- a. It contains one sequence fragment (domain) seems to play a role in a recombinational process of DNA repair. And several big1 domains
- b. It contains several sequence fragments (domains) seem to play a role in a recombinational process of DNA repair.
- c. It contains one sequence fragment (domain) that recognizes polysaccharides (carbohydrates) in the bacterial cell wall. And several big1 domains
- Od. It contains several sequence fragments (domains) that recognize polysaccharides in the bacterial cell wall.
- oe. Don't know/no answer (without penalty).

https://prosite.expasy.org/

and we paste P76347 sequence or this Uniprot accession and we pres scan

The correct answer is: It contains one sequence fragment (domain) that recognizes polysaccharides (carbohydrates) in the bacterial cell wall. And several big1 domains

Correct

Mark 1.00 out of 1.00

Question 7. Which of these Pfam domain architectures does our protein exhibit? Select one: PF11924 - PF02369 - PF02369 - PF02369 - PF02369 - PF02369 - PF02369 O a. PF11924 PF02369 There are 4975 proteins with this architecture (represented by P36943) O b. PF11924 PF11924 PF11924 - PF09134 - PF02369 - PF0236 c. PF11924 PF09134 PF02369 Don't know/no answer (without penalty). There are 93 proteins with this architecture (represented by Q8XB95): O e. PF11924 - PF09134 - PF02369 - PF02369 - PF02369 - PF02369 - PF02369 - PF02369 - PF09134 - PF09134 PF11924 PF09134 PF02369

From Uniprot jumpt to pfam.

you will be redirected to interpro

https://www.ebi.ac.uk/interpro/protein/UniProt/P76347/

pay atantion only to the pfam domains (PF...)

you will see one PF11924 folleowed by one PF09134 followed by thirteen PF02369

The correct answer is:

PF11924 - PF09134 - PF02369 - PF02

Question 8

Correct

Mark 1.00 out of 1.00

Question 8. Analyzing our protein at <u>InterPro</u> it seems that it contains much more domains or important protein signatures when compared with the result of Pfam. Why?

Select one:

- a. InterPro is a primary database and contains information for all known protein structures.
- b. InterPro consists of a collection of annotated multiple sequence alignment models available exclusively as position-specific score matrices (PSSMs).
- o. Don't know/no answer (without penalty).
- d. InterPro is a protein annotation resource for protein domain classifications based exclusively on hidden Markov models (HMMs).
- e. InterPro is an integrated database that combines protein signatures from a number of member databases.

V

https://www.ebi.ac.uk/interpro/about/

The correct answer is: InterPro is an integrated database that combines protein signatures from a number of member databases.

Correct

Mark 1.00 out of 1.00

Question 9. Based on information taken from derived protein databases (Interpro or Pfam), what is the most likely function of the repetitive domains in our protein?

Select one:

| ○ a. | Don't | know/no | answer | without | nenalty). |
|------|-------|-----------|--------|-----------|-----------|
| oa. | Dont | KIIOW/IIO | answer | (WILLIOUT | penany. |

- b. This repetitive unit is a phospho-relay system usually found in regulator proteins. This signal transduction system enables bacterium to sense, respond, and adapt to a wide range of environments, stressors, and growth conditions.
- c. These repetitive units have the capacity to interact with hyaluronic acid, which promotes the stability of the extra-cellular matrix.
- d. Big-1 proteins are surface-expressed proteins that mediate mammalian host cell invasion or
 attachment. The tandem of Ig-like domains appears to form a rod to link the bacterial outer membrane
 anchor to the C-terminal lectin-like domain to interact with their receptors in the host cell membrane
- e. This multi-domain adopts a classic alpha/beta fold and contains an unusual metal ion coordination site at
 its surface. It has been suggested that this site represents a general metal ion-dependent adhesion site for
 binding protein ligands.

The repetitive domains are the "Big-1"

Big-1 domain description in interpro

https://www.ebi.ac.uk/interpro/entry/InterPro/IPR003344/

Take a look also at the description in the DBs that originated this interpro entry:

http://smart.embl-heidelberg.de/smart/do annotation.pl?DOMAIN=SM00634

https://prosite.expasy.org/PDOC51127

Se that it is almost the same.

The correct answer is: Big-1 proteins are surface-expressed proteins that mediate mammalian host cell invasion or attachment. The tandem of Ig-like domains appears to form a rod to link the bacterial outer membrane anchor to the C-terminal lectin-like domain to interact with their receptors in the host cell membrane

Question 10 Question 10. We finally want to know if our protein, or a protein similar to this, has a 3D structure experimentally solved. Mark -0.25 out of 1.00 Select one: a. Don't know/no answer (without penalty). b. Yes, protein P76347 has its 3D structure already solved. ★ c. There is no 3D structure for this protein. However the structures of all its known domains are known in other proteins or organisms. d. Yes, there is a 3D structure for this protein but only for the whole region that contains the repetitive

We can see in interpro that this protein has this 3 pfam domains, and here you can see that these domains' structures have been solved in other proteins:

e. No, there is no 3D structure for this protein or for any of its domains in other proteins or close organisms.

https://www.ebi.ac.uk/interpro/entry/pfam/PF02369/structure/PDB/#table

https://www.ebi.ac.uk/interpro/entry/pfam/PF09134/structure/PDB/#table

https://www.ebi.ac.uk/interpro/entry/pfam/PF11924/structure/PDB/#table

We can also see that it contains 4 CATH structural domains (Gene3D). Since CATH is a DB created from the the structure of proteins experimentally solved we can see other proteins with similar domains

http://www.cathdb.info/version/latest/superfamily/2.60.40.10

domain Big-1.

http://www.cathdb.info/version/latest/superfamily/3.10.100.10

http://www.cathdb.info/version/latest/superfamily/2.40.160.160

http://www.cathdb.info/version/latest/superfamily/2.60.40.1080

The correct answer is: There is no 3D structure for this protein. However the structures of all its known domains are known in other proteins or organisms.