Microbiology I
BIOL211-CAD Lab

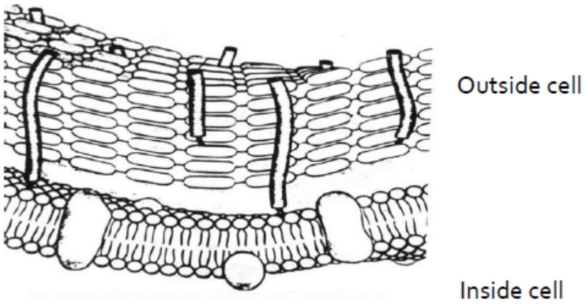
This activity introduces BLAST (Basic Local Alignment Search Tool), a valuable tool for analyzing nucleic acid and protein sequence data. In addition, this activity highlights some important differences between the cell envelopes of Gram positive and Gram negative bacteria.

A) Cell Envelope Review: Look at the diagrams below of Gram (+) and Gram (-) type cell envelopes.

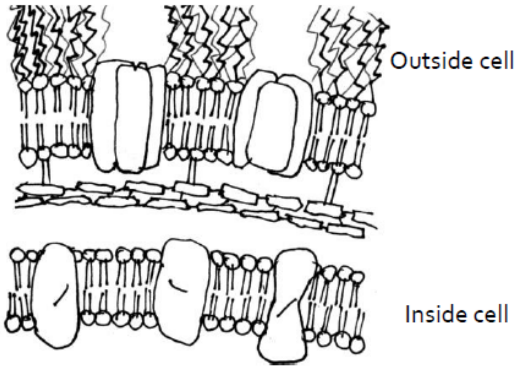
i) Label each cell envelope as being from a Gram (+) or Gram (-) cell type
ii) Label the components of each cell envelope using the list below

Note: not all cell-types have all the structures.
☐ cytoplasmic membrane
☐ outer membrane
☐ membrane-bound proteins
☐ peptidoglycan layer
☐ periplasmic space
☐ porins

Cell wall type ____________



Cell wall type ______________



B) BLAST review: One of the bioinformatic tools that you will use is **BLAST** (**Basic Local Alignment Search Tool**), that can be found at the National Center for Biotechnology Information site: http://blast.ncbi.nlm.nih.gov/.
As the name implies, BLAST makes alignments between sequences. Alignment is the process (or result) of matching up the nucleotide or amino acid residues of two or more biological sequences to achieve the best possible match. BLAST identifies sequences similar to your query sequence in the NCBI database by making alignments and assessing how well the sequences match.

Please view the *BLAST Video Tutorial* ((https://www.youtube.com/watch?v=x_dAyY5-VNc) **if you need additional help in answering the questions below.**

Below is the amino acid sequence of a protein associated with some bacterial cell envelopes. Use a protein BLAST (**BLASTP**) search to obtain information about it, and answer the questions below.
MKLKNTLGVVIGSLVAASAMNAFAQGQNSVEIEAFGKRYFTDSVRNMKNADLYGGSIGYFLTD
DVELALSYGEYHDVRGTYETGNKKVHGNLTSLDAIYHFGTPGVGLRPYVSAGLAHQNITNINSD
SQGRQQMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMAGLGVGFNFGGSK
AAPAPEPVADVCSDSDNDGVCDNVDKCPDTPANVTVDANGCPAVAEVVRVQLDVKFDFDKSK
VKENSYADIKNLADFMKQYPSTSTTVEGHTDSVGTDAYNQKLSERRANAVRDVLVNEYGVEGG
RVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK
Questions:

Identify the Top BLAST hit and fill in the box to answer questions 1-3.
1) What kind of protein does this sequence encode, based on the name given (annotation)?
2) From what organism did it come?
3) What is the BLAST % query cover, E value and % Max Identity for the top hit? **Top BLAST hit for the sequence from your isolate**

Protein	Organism	% Query Coverage	E-value	% Max Identity

4) What is the function of this kind of protein?

5) Based on what this protein does and where it is found, do you think this organism is a Gram positive or Gram negative bacterium? Explain your logic.

6) Look at the BLAST tutorial (or look at the glossary section in the BLAST website at http://www.ncbi.nlm.nih.gov/books/NBK62051/) and fill in these definitions:

E-value:

% Max Identity: