Project 4 - Secretion system T6SS

The transport of proteins across the bacterial cell envelope is a basic function found in all groups of bacteria. The analyses of a large number of bacterial genomes indicate that up to 17% of Proteobacteria genomes encode for proteins with signal sequences for the general secretory pathway (GSP), with many additional secretion systems and substrate proteins being present in most species. Secreted proteins have various functions in processes such as the biogenesis of the cell envelope, the acquisition of nutrients, motility, intercellular communication and many more. Aggressive bacterial virulence factors that enable a progressive colonization of host organisms are commonly secreted proteins, with toxins and translocated effector proteins as well-studied examples. The GSP and its components are found in all three kingdoms of life: in bacteria, archaea and eukaryotic organelles (chloroplasts but also in the endoplasmic reticulum) and provides a generic mechanism for the transport of proteins across the cytoplasm or organelle membrane. While this transport process is sufficient for the secretion of proteins in Gram-positive species, Gram-negative species are posed with a specific problem, the transport across a second membrane system, the outer membrane (OM).

Gram-negative bacteria have evolved a remarkable array of mechanisms for the secretion of proteins across the cell envelope. Secretion of proteins is one means by which microbes influence their extracellular milieu, which, in the case of pathogenic microbes, includes target host cells. During pathogenesis, bacteria can affect host cells through the export of toxins and enzymes that alter cellular function or through the assembly of extracellular structures such as pili and adhesins that promote adherence to host cell surfaces. Bacteria have also evolved specialized surface structures that deliver proteins and nucleic acids directly into target cells, an export process referred to as translocation. Gram-negative bacterial pathogens use at least six distinct extracellular protein secretion systems (referred to as T1SS–T6SS) to export proteins through their multilayer cell envelope and, in some cases, into the host target cells. These secretion systems are distinguished in part by the conserved structural components that define them but also by the characteristics of their substrates and the path that these substrates take during the export process. Very little is known about the mechanism of protein transport by the newly described T6SS. T6SS gene clusters seems to be highly conserved and are present in one or more copies in many other pathogenic Gram-negative bacterial species, including *Vibrio cholerae, Pseudomonas aeruginosa*, *Yersinia pestis, Escherichia coli, Salmonella enterica, Agrobacterium tumefaciens, Rhizobium leguminosarum, Francisella tularensis, Burkholderia mallei*, and *Edwardsiella* species.

The T6SS gene cluster mediates the extracellular secretion of proteins like Hcp and VgrG. In *Vibrio cholerae* mutations in the *hcp* or *vgrG-2* genes attenuate cytotoxicity and block secretion of the other T6SS protein substrates. In *Vibrio salmoniocida* two *vgrG* and three *hcp* genes has been identified (see sequences below).

In this project you have to use all available tools to gather as much information as possible of the vgrG and hcp proteins:

1) Find background research articles pertaining to bacterial vgrG´s and hcp´s – what is known about the proteins?
2) Collect all sequences of vgrG and hcp using BLAST (use the sequences below from *Vibrio salmonicida* as a starting point).
3) Create alignments of VgrG´s and hcp´s by using ClustalX.
4) Create a phylogeny tree of vgrG and hcp by using MEGA
5) Use different protein analyzing tools to look for repeats, low complexity sequences etc (e.g. SAPs at ExPASy Proteomics tools).

>VsVgrG1 (pVS840_0036 *Vibrio salmonicida*)
MSKEIEFTFTVFGTAHEFRVESFHVVEELSRPFRVTLSLLSLDADIAFDGLSRKPALLTLMGQGMGNSRQF
HGVVNEVRYLGVGRRFSRYQITLSPQLWFLTQRQDCRIFQDKSVIDIVTEVLDGGAVTDYRFELSAAYPPQ
EYVLQYRETDSHFVQRLLAEQGLWYYFEHSESNHTLVIVDKNDAIPEIISSPENASYMGPFVYQSEGGGNA
DREHVFELSTYHRVRTGHVTQTDYNYEQPRIPLQVEQQRDRDTDLAFFDYPGRYAEASQGQLQAVSLLADN
HLESHQIEAESSIMRLTAGYSFELIRHSRKAINRDYTLLSVSHSGHNPQVHEEESSGLPTTYQNQFICLPR
DVEFRSPTLSAPVVDGPQTAVVVGPAGEEIYTDKLGRIKVQFHWDRYGNNDEYAGCWLRVCQSMAGPTWGA
VYLPRIGHEVVVTFLDGDPDRPLVTGTVYNGVNTPPYPLPENKTKTVFRTQTHKGEGYNEMSFEDEANQEQ
VYFHAQKDMRSKVLNNRFRTIGNDEALTVGRNQENTIKQDRKETIEGNKISITKQTFTETIEQDVKVSYNA
NMEKNVVAKQTLEVHGDRNSTVGKNDELTISGDKSTITMGARSSDISADDTLTVGKLLTVEVGSNASMKSD
GEYTIISADEINATVGSSGFILKNNGTITLYGSNITIDGSGSVALVGGSVAINPGAAASQSSSVSVNPPRT
FSMSPLASTKPVMNSSVYSEMVQEGSIIKELCQCGQGDICKLHS

>VsVgrg2 (TVS1237 vgrG *Vibrio salmonicida*)
MANEVEFKFEVLGTNHEFRVESFNVVEEVSQPFKITLSLLSLDPDIAFDVLSRKPALLTVLGQGMNAGRLF
HGVVNEVRYLGAGRRFSHYQITLVPQLWFLTQRQDCRIFQDKSIVDILTEVLDGASVTDYRFELSNTYPNQ
EYLLQYRETDSHFVQRLLAEHGLWYYFEHTTSNHSMVIIDKNDAIPELISTPLNASYIGPLVYQSEGGGNA
DREHIFELSSYNRVRTGHVIQTDYNYDQPRIPQQVQQQGDRDTDLAFFDYPGRYGESVEGQQRSNYLIADH
QVENHQIEAESNIMRLTAGYSFDLAKHPRNAINRDYTLTAITHSGYNPQVHEEESSGLPTTYQNYFNCIPR
DVGYKAPKLAAPVVDGPQTAVVVGPAGEEIYTDPMGRIKVQFHWDRYGNNDEHSGCWLRVSQSMAAPTWGA
VYLPRIGHEVVVTFLEGDPDRPLVTGAVYNGLHTPPYALPEHKTKTVFRTQTHKGEGYNEMSFEDEANQEE
VYFHAQKDMRTKVLNNRFRTIGNDEELEVGRNQENIINKDRKETIEGHKTSITNQTFSETVEQDVTVKYNA
NIEKSVAVEQTLTIYENRQSTIGKNDELTIKGEQSRITMGASSSDISADDTLTVGQNLTIEVGNNASIKSD
ADHTIISADEITAQVGSSGFILKNNGTITLYGSDITVDGSGSVALVGGNVAINPGAASSQSSSVSANLPRT
FSMSPLASTKPVMNSAAYSEMVEDGALMKELCQCGKEGICKLHS

>Vshcp1
MPTPAYMSIKGETQGDITKDAYSADSVGNTWQEAHIDEFLVQELDHVLTVPRDPQSGQPTGQRVHRPVIVT
KQQDRCSPLLFNSLVSGEKLPECSINFYRTSISGKQEHYYTIKLIDALLVDMQTRMAHCQDAATSDRVTEE
VLKFTYRAIEVTHETCGTAGDDDWRKPREA

>Vshcp2
MPTPAYMSIKGEVQGDITKDAYSADSIGNTWQEAHIDEFLVQELDHVLTVPRDPQSGQPTGQRVHRPVIVT
KQQDRCSPLLFNSLVSGEKLPECSINFYRTSISGKQEHYYTIKLIDALLVDMQTRMAHCQDAATSDRVTEE
VLKFTYRAIEVTHETCGTAGNDDWRAPREA

>Vshcp3
MPTPAYMSIKGEVQGDITKDAYSADSVGNTWQEAHIDQFLVQELDHVLTVPRDPQSGQPTGQRVHRPVIVT
KQQDRCSPLLFNALVSGEKLPECSINFYRTSTSGKQEHYYTIKLIDALLVDVQTRMAHCQDAATSDRVTEE
VLKFTYRAIEVTHETCGTAGDDDWRKPREA