Molecular Microbiology

Glossary

quorum Minimum number of organisms required to be present in order to conduct a specific activity. If a bacterial community reaches the quorum, new genes can, for example, be switched on.
biofilm formation Transition from planktonic (loner) to stationary, community based activities. Biofilm formation evokes profound changes in physiology, drug and biocide resistance, cell surface characteristics, UV resistance, biodegradative capacity and secondary metabolism.
phylogeny An organism's evolutionary history recorded by accumulation of mutations in a certain gene.
metagenomics A community of organisms present lifestyle.
lipopolysaccharides LPS are composed of a lipid A and a polysaccharide. The polysaccharide itself comprises a very variable O-specific chain made of a small, repeated tri- tetra- or pentasaccharides and, between this chain and the lipid, a conserved core. The O-specific chain constitutes the somatic antigen.
peptidoglycan Peptidoglycan (matrix) is like an exoskeleton, giving both Gram-pos. and Gram-neg. bacteria their shape and containing the osmotic pressure (5-20atm). GlcNAc-MurNAc-PP are the building blocks forming peptidoglycan through β-1,4 linkage.
GlcNAc N-acetyl glucosamine
MurNAc N-acetylmuramic acid
Braun's lipoproteins Anchor in E. coli the outer membrane to the peptidoglycan (Gram-neg.). Attachment is mediated by a peptide bridge between a Lys residue and a Meso-DAP residue from the peptidoglycan.
β-lactamases Enzymes that open β-lactame rings. They evolved from transpeptidases.
fimbria Short, filamentous structures on a bacterial cell; although flagella-like in structure, generally present in many copies and not involved in motility. Plays a role in adherence to surfaces and in the formation of pellicles.
pilus A fimbria-like structure that is present on fertile cells, both Hfr and F^+, and is involved in DNA transfer during conjugation. Sometimes called a sex pilus.

Concepts

Symbiosis

Bacterial symbiosis with the squid (10-armed cephalopod): Vibrio fischeri provides light (lux system) as a consequence of bacterial quorum sensing, the squid provides food.

Buchnera is an essential bacterial symbiont for aphids. Their congruent pylogenetic trees sustain the thesis that they evolved together. The bacteria live within bacteriocytes, special organs of aphids, containing about 10'000 Buchneras each.
Buchnera lacks genes for different essential functions:

- Synthesis of certain amino acids provided by the aphid.
- Synthesis of cell surface components, including lipopolysaccharides and phospholipids.
- Regulator genes and defensive genes (radiation, drugs, etc.).

Aphids lack essential biosynthetic genes for:

- Leucine and tryptophan synthesis. This is why aphids can be killed using antibiotics killing the symbiotic Buchneras.

Wolbachia induces speciation in insects by controlling their ability to reproduce and inducing cytoplasmic incompatibility. Different Wolbachia species induce different cytoplasmic incompatibilities.

An other example of symbiosis is the tripartite symbiotic system involving the luminescent bacteria Photorhabdus, the nematode Heterorhabditis, and diverse insect larvae. The bacteria provide the nematodes with food, insect toxins, inhibitors of insect immune system, antibacterials and fungicides. The nematodes are the delivery system. Insects are ultimate source of nutrition for both nematodes and bacteria.

Biofilms
Bacteria convey their presence to one another by releasing chemical signalling molecules that allows them to behave as multicellular organisms facilitating coordinated activities such as:

- symbiosis, requiring communication with each other and higher organisms
- swarming (invading new territories in large groups)
- virulence
- antibiotic biosynthesis, especially in soil bacteria; ¾ of all antibiotics are produced by bacteria (biological warfare)
- biofilm synthesis (living together in communities where different individual organisms take on specific tasks in order to optimise the overall competitiveness of the community e.g. antibiotic resistance)

Gram negative bacteria use homoserinelactone (HSL) derived molecules as signalling substances. Gram positive bacteria mostly use specific peptide molecules. Despite this, there are a few universal signalling molecules used by all kinds of bacteria. One example is borate furan AI-2.

Biofilm development is initiated by loose attachment triggering developmental signals, followed by irreversible attachment accompanied by extracellular polysaccharide (EPS) production. Through this process, the early biofilm begins to take shape. Maturation of the architecture follows. As a last stage the biofilm might again disperse, a process mediated by detachment signals.
Functions required for biofilm formation are:

- Nutritional signals
- Ability to attach to a solid substrate (flagella) and to move on it (pili)
- Capability of sensing cell densities and producing multidimensional structures (EPS)

Channels within a biofilm allow circulation of nutrients and quorum-sensing signalling compounds. The extracellular polysaccharides (EPSs) provide structural stability, a controlled internal environment, and protection.

Phylogeny
Less than 1% of bacteria detected by their distinct rRNA sequence can be grown in culture. In total about 4500 species have been characterized. They inhabit virtually all terrestrial and marine environments. The population density of bacteria is about 10^9/g of soil.

Requirements a gene has to fulfil in order to be used for phylogeny:

- The gene must be present in all organisms of interest.
- The gene cannot be subject to transfer between species (lateral transfer).
- The gene must be easy to align with its distant relatives.
- The gene has to be sufficiently large to contain a record of the historical information.
- The gene must display an appropriate level of sequence conservation for the divergences of interest.
- The rate of mutagenic change over long periods of time must be rather constant.

The most widely used genes for phylogeny are rRNA. Ribosomes are a very complicated machine, so that ribosomal genes can not move from one species to an other because the machine would then not work.

Nitrogen fixation
Nitrogen fixation, carried out by Rhizobium, takes place in the nodules of leguminous plants. Since the nitrogenase located in the bacteroid is an oxygen-sensitive enzyme, leghemoglobin in the plant cell around the bacteroid binds nearly all oxygen molecules ensuring an anoxic environment.
Rhizobia attachment to the root hair is mediated by flavonoid chemoattractants secreted by leguminous roots. These flavonoids induce nod gene transcription that leads to root hair curling and establishment of an infection thread caused by nod factors. Following this, Rhizobia penetrate the root hair and multiply within an infection thread growing toward root cells. Invaded cells and those nearby are stimulated to divide and the formation of a bacteroid state within these plant cells takes place, leading to the characteristic nodule. While the nodule grows vascular tissue connecting it to the plant's xylem and phloem develops.
Nitrogen fixation symbiosis are specific between certain species of rhizobia and leguminous plants, having different nod factors and flavonoids each.

Crown gall
Wounded plants release phenols (e.g. Acetosyringon), stimulating Agrobacterium tumefaciens to infect them. A. tumefaciens attaches to a plant cell and injects its T-DNA which will consecutively be inserted into the plant's genome. T-DNA induces an increase in growth hormones / metabolites synthesis, making the plant produce tumors and synthesize opines (nopaline - nutrients used almost exclusively by A. tumefaciens).
The transfer of the T-DNA is accomplished by induction of vir genes through phenolic compounds.

Pili
Pilus extension occurs when pilin subunits assemble into a helical fiber at the bacterial inner membrane. The fiber is conducted across the outer membrane through a secretin "bushing" made of PilQ subunits. Pilus retraction occurs when the fiber disassembles back into the inner membrane.

Gram-positive (simplest eubacteria)
Gram-positive bacteria are surrounded by a cell wall made of the polymer peptidoglycan. Dispersed in this network are proteins, teichoic acids and lipoteichoic acids. The cell wall measures 20 to 80nm.

Gram-negative
The peptidoglycan cell wall of Gram-negative bacteria measures only 5 to 10nm. The outer membrane is an asymmetric lipid bilayer composed of a phospholipid inner layer and an outer layer made of phospholipids and lipopolysaccharides (LPS). Between the cytoplasmic and the outer membrane lays the periplasm that has special control functions. The cell wall is part of the periplasm.

Peptidoglycan biosynthesis
Crosslinking is accomplished between a penultimate D-Ala and a Meso-DAP (Diaminopimelic acid) of another pentapeptide. The last D-Ala is cleaved in order to gain energy that is needed for the crosslinking since there is no ATP readily available outside the cell.
The building blocks of peptidoglycan are GlcNAc-MurNAc-PP. Activation of sugars is achieved by UDP in the cytosol. During reticulation the carrier (undecaprenyl-PP) is phosphorylated in each polymerisation cycle and has to be dephosphorylated after each round in order to be recycled. The enzyme responsible for this transglycosylation is located on the outer side of the inner membrane.
Bacitracin inhibits the dephosphorylation enzyme of the carrier (undecaprenyl-PP) and therefore prevents recycling of the carrier.

β-lactame antibiotics
β-lactame antibiotics function as structure analoga of the acyl D-Ala D-Ala group. They engage irreversibly in the first step of the transpeptidation reaction and block the transpeptidase as a penicilloyl-enzyme. The first β-lactame antibiotics were only active against Gram-positive bacteria because they couldn't pass the outer membrane of Gram-negative bacteria. The main family of antibiotics with β-lactame ring comprise penicillins, cephalosporines, carbapenems and monobactams.

Vancomycin
Vancomycin is a cyclic glycopeptide essentially active on Gram positive bacteria. It inhibits peptidoglycan synthesis by complexing the terminal D-Ala-D-Ala from the disaccharid-pentapeptides presented at the external face of the plasma membrane. Vancomycin represents the last active drug against some Staphylococci that became resistant to all penicillins and other classes of antibiotics.
Enterococci have become resistant to Vancomycin by replacing the acyl-D-Ala-D-Ala by an acyl-D-Ala-D-lactic acid.

Inner membrane
- Proton gradient generated by the respiratory chain. The proton motive force (PMF) is used to generate ATP
- The inner membrane is full of energy while the outer membrane lacks energy.
- TonB is a inner membrane protein that transports energy from the inner membrane to the outer membrane.
- Positively charged residues are generally in the cytosol (positive inside rule).
- In inner membrane proteins (IMPs) the transmembrane (TM) segments spanning the bilayer are hydrophobic α-helical segments of at least 20 hydrophobic residues.

PhoA
The alkaline phosphatase PhoA is a periplasmic protein that requires disulfide bonds for its activity. This oxidation occurs in the periplasm but not in the cytoplasm. Therefore, through random introduction of PhoA into a protein it can be determined whether a certain domain lays in the periplasm (PhoA active) or in the cytoplasm (PhoA inactive).

Outer membrane
- Integral outer membrane proteins (OMPs) are characterized by anti-parallel β-strands that form barrel-like structure with a hydrophobic outer surface. Because they have to cross the IM to reach the OM, OMPs are always synthesized with a classical cleavable signal sequence.

Sec pathway
The Sec pathway is found in both Gram-pos. and Gram-neg. bacteria. In Gram-neg. bacteria it does not secret proteins, however, it does transport them to the periplasm, from where they might be exported by other systems.
Targeting of proteins to the Sec system is dependent on the presence of an N-terminal signal peptide.
The Sec machinery can only translocate unfolded proteins across the IM. The SecB chaperone and the SecA ATPase are required for translocation.
On the other hand, insertion of inner membrane proteins by the Sec pathway is only co-translational and requires a signal recognition particle (SRP) that acts as chaperone and targeting factor replacing SecB and SecA respectively.

TAT system
The twin-arginine translocation system can even translocate bulky, folded green fluorescent proteins (GFPs) across the IM. The TAT system has also been discovered in chloroplasts. The TAT signal peptide contain a conserved amino acid sequence motif at the n-region / h-region boundary (S-R-R-x-F-L-K where x is normally a polar amino acid) that gave the system its name.
The TAT pathway is energized exclusively by the PMF.

Type II secretion pathway
The type II secretion pathway transports folded proteins through the outer membrane. Most of the components of the T2S apparatus are anchored in the IM rather than in the OM. Additionally, essential component resemble the type IV pilus components and it has been shown, that a pseudopilus is involved in T2S. The current hypothesis is that the pseudopilus serves as a piston pushing proteins through the secretin channel.
The transport of DNA from the extracellular milieu into the cytoplasm by naturally competent bacteria (transformation) is a process which requires proteins that are related to those involved in the assembly of type IV pili and type II secretion system.
Type IV pili and T2S are related as well to filamentous phages, the only difference being that phages contain DNA inside. Single stranded DNA filamentous phages receive their protein coat at the IM and then cross the OM via a protein of the secretin family.

Type V secretion pathway
T5S is an autotransporter, where the C-terminal domain forms a β–barrel in the outer membrane that serves as a channel for the functional N-terminal domain. In between these two domains there is an α–helical plug that closes the channel after translocation. The N-terminal domain might be cleaved by an autoproteolytic function.

Type I secretion pathway
T1S is an one-step transport system of the ABC (ATP-binding cassette) transporter family. It transports only unfolded substrates across the inner- and outer membrane.

Type I pili
Type I pili are assembled via the highly conserved chaperon-usher pathway. The chaperone interacts with pili subunits, emerging from the Sec pathway, in a way to complete their Ig type fold. This semi-correct (parallel) complementation is called donor-strand complementation.
During type I pilus assembly, chaperone-subunit complexes are targeted to the usher, that forms a pore in the outer membrane. At the usher, the N-terminal extension of one subunit complements the Ig type fold of the following subunit in the correct antiparallel way. This procedure is called donor-strand exchange.