Scientific Practice Notes

Imports

import ../../Modules/Biology/Viruses as Viruses

import ../../Modules/Biology/BiologicalMolecules as BiologicalMolecules

import ../../Modules/Biology/Solutions as Solutions

import ../../Modules/Biology/ScientificReasoning as ScientificReasoning

import ../../Modules/Biology/AcidsandBases as AcidsandBases

import ../../Modules/Biology/Steriochemistry as Steriochemistry

import ../../Modules/Biology/Electrophoresis as Electrophoresis

import ../../Modules/Biology/Immunology as Immunology

import ../../Modules/Biology/Microorganisms as Microorganisms

Scientific practice

Microorganisms

Microorganism is a general term used to describe all organisms too small to be seen with the naked eye. There are six main types of microorganisms namely: fungi, bacteria, viruses, protozoa, archaea and algae.

Viruses

Background

All viruses are acellular obligate parasites. Some viruses can survive for a long period outside host cells, but no viruses can replicate independently of a host cell. This is because the translation system of the cell is required for viral replication.

> NOTE: Viruses should always be referred to as replicating and not reproducing, to differentiate replications as a externally dependant process.

Virion Constituents

Matrix

The matrix is an additional protein structure between the envelope and the capsid.

> NOTE: not all viruses contain a matrix

Capsid

Capsids are made of of multiple protein/ capsomer subunits

SYNONYM: The viral capsid is also referred to as a protein coat.

Icosahedral Capsid

The polypeptides making up the coat pack very specifically into the triangular faces of the icosahedron.

Subunits

Each basic triangular subunit may consist of up to 3 different polypeptides, one at each vertex.

Evolution

The Icosahedral protein capsid has evolved on many independent occasion in evolutionary history. The evolutionary advantage is that the icosahedron is a lowest energy structure, so is very stable.

Icosahedron Structure

An Icosahedron is a 3D structure with 30 identical equilateral triangular faces. Five faces form the top cap, five form the bottom cap and 5 form the middle ring. The 20 faces are connected by 12 vertices.

Symmetry

An icosahedron has five fol symmetry when viewed axially, and 3 fold symmetry when viewed equatorially.

Subunits

Subunits can be made up of several smaller identical or non identical polypeptides.

Envelope

The envelope consists of host derived bilipid membrane containing host and viral proteins/glycoproteins. Some viruses contain protein spikes which protrude from the membrane.

Function

The envelope helps the virus evade the immune system as it appears similar to host cells.

Function

The viral capsid functions to protect the viral genome, from physical and chemical damage or enzymatic degradation

Nucleic Acid (Genome)

The nucleic acid genome may be DNA or RNA, single stranded or double stranded and linear or circular.

Multipartate Viruses

Viruses with more than one nucleic acid molecule are called segmented/multipartate. These molecules are non redundant each containing different and vital parts of the viral genome.

> NOTE: Most viruses contain only one nucleic acid molecule.
>
> NOTE: multipartate viruses can swap nucleic acid molecules leading to recombination.

Virion Structure

Size

Viruses have a very large size range, from about $50nm - 1\mu m$ in length/diameter. For example rabies virus is $70 - 170nm$, whereas Ebola is about 970nm.

> NOTE: This size range implies that viruses are 10-100 times smaller than bacterial cells, and $10^3 - 10^4$ times smaller than eukaryotic cells.

Icosahedral Viruses

As the structure of an icosahedral virus is very set, size can only be increased by increasing the size of triangular constituents, (while maintaining their equilateral shape), or by the formation of larger (equilateral) triangular subunits from four smaller one. Increasing size of the basic triangular subunits implies increasing the size of the polypeptide(s) which they consist of.

Shape

Overall virion shape is quite diverse, but the majority of virion have an icosahedral shape.

Enveloped viruses

Virions which posses an outer envelope are polymorphic due to the fluid nature of the bilipid layer.

Icosahedral

Icosahedral viruses contain an icosahedral capsid, that is a capsid consisting of 20 equilateral triangular faces, fitted together to form an icosahedron.

Helical

Helical viruses contain a helical/rod shaped capsid, This capsid may be long and thin or short and thick. Furthermore they may be flexible folding into loops and coils.

NOTE: Most helical viruses are ssRNA Viruses.

Internal Organisation

All virions contain a capsid. Some virions, additionally contain an envelope which surrounds the capsid. Within the protein coat, and often associated with it s the nucleic acid molecule(s) of the virus.

Nucleic acid

The nucleic acid forms has a helical secondary structure with a ball like tertiary structure. An electrostatic interactions between the nucleic acid backbone and the capsid/matrix protein hold maintains this tertiary structure and holds the nucleic acid molecule in place.

Viral Evolution

Viruses have evolved on many time independently. Viruses evolve co-evolve with their hosts, when a host species becomes extinct so will its viruses. For example HIV evolved to recognise a receptor on human cells, by the adaption the cell receptor, Tetherin. Viruses may also evolve in their host for a long time before becoming infective, again human infection by HIV is an example.

Viruses can evolve very fast due to their very short life cycle and high replication number.

Receptor Binding Proteins

Viral membrane/capsid proteins mutate particularly fast, partly because of selective pressure supplied by the host immune system, and partly because of the evolutionary advantage conferred by more efficient entry into host cells.

Important Viruses

Ebola Virus

Origin

Ebola Virus originated in bats, although it took a long time to trace its source.

Constituents

Envelope

Ebola is an enveloped virus

Influenza

Constituents

Nucleic Acid

Influenza is a multipartate RNA virus, with 7-8 non redundant RNA molecules.

> NOTE: All 7-8 RNA molecules are still packaged together within the viral capsid.

Structure

Influenza is an enveloped virus.

Host

Influenza is limited to respiratory tissue but can replicate in pigs, ducks horses and birds.

> NOTE: Influenza has killed more people in the history of humanity than HIV has.

Herpes Virus

Size

Herpes is a very large virus

Constituents

Matrix

Herpes contains a protein matrix

Envelope

Herpes is an envelopes virus.

Rabies Virus

Hosts

Rabies virus can replicate in multiple host species, including humans and dogs.

HIV Virus

Constituents

Matrix

The HIV virus contains a protein matrix.

Host

HIV can replicate in many different host species.

Origin

HIV derived from Zoonosis from primates, which share 98% of their genome with humans. For example HIV evolved to recognise a receptor on human cells, by the adaption the cell receptor tetherin.

HIV first emerged as a major disease in 1950s, ,though it was present in the human genome from far earlier, just in a less virulent form.

Subtypes

There are many different strains of HIV.

Subtype C

Subtype C is prevalent in South Africa.

Classification

Bacteria are not classified on the tree of life, but are still classified into family (written in italics, with a capital and ending in viridae).

Viral species.

A group of viruses sharing the same genetic information and ecological niche (i.e. the host). Common names are used for species, subspecies are designated by a number. Do not reproduction, and have no phylogenetic trees (a tree showing evolutionary relationships based on homology of nucleic acids and amino acids).

Viral species are host specific so are not related to each other.

NOTE: every living host currently on the planet has a associated virus.

For example HIV, is a species, there is HIVI and HIVII which both infect humans but are completely different species, within a species there are different strains/subspecies/subtypes within a species,which have amino acid or genomic variation.

NOTE: viruses cannot be classified by morphology either. Also as they have arisen many times during evolution there is not common ancestor.

For example, *Herpesviridae* is the family, *Herpesvirus* is the genus, Human herpes virus (is the species), subtypes HHV-1 HHV-2, HHV-3. *Retroviridae*, *Lentivirus*, human immunodeficiency virs, HIVI (lost of subtypes), HIVII (constrained to particular location in west Africa).

HIV is a very fast mutating virus, (variation of about 18%), different host immune systems will but different evolutionary pressure on the virus to evolve.

The amount of similarity required for viruses to be classified as a different species, depends on the virus itself.

NOTE: Classification can also be based on pathogenic character.

Characteristics used for classification

Viruses are devided into different taxonomic groups based on several features

Genomics

80% RNA 20% DNA. classified by type of nucleic acids RNA/DNA, and doulbe/single stranded.

Double stranded DNA

Tend to be very large. The largest virus is small pox.

Single stranded

Very small

Single stranded RNA

There are two types

Megaviruses, gyruses.

Discovered about 15 years ago, (should they be viruses or a whole new domain, they contain dsDNA,)

dsDNA

with a RNA intermediate, copied back to DNA. COntain hepatisis virus.

Retroviruses

Single stranded RNA, copies itself into dsDNA using reverse transcriptase, which then integrates into the DNA of the cell, (which makes it very hard to recognise.

Host

Shape

Presence of envelope

Transcription mechanism in the host

NOTE: dsDNA replicates the same way as in cells.

There are seven groups, ssDNA, dsDNA, dsRNA, + sense ssRNA, -ve sense RNA ssRNA reverse transcribing virus with DNA intermediates, dsDNA with RNA intermediates.

NOTE: virus is classified based on the nucleic acid within the virion.

Baltimore Classification Scheme

COPY IMAGE.

NOTE: remember RNA viruses have genes, some can translate directly, some require some form of intermediate. NOTE: viruses tend not have introns.

Summary

viruses are composed of portiens nucelic acids and sometimes a lipid envelope, there are two basic symetries helical and icosohedral (polyherdal, for larger vieuses) Viral caposids are mode up of portien subunits or capsomere s which fold to form specific structures. Genomes within viruses capos are lieat or circular RNA/DNA which are folded and interact with the capsid proteins through electronstatic or hydrophobic interactinos. Viable virus particles or viruses are infectious.Viruses have multiple origins

and co-evolved with their hosts. viruses are classified according to the expression (mRNA) of their genes.

Life cycle.

Attachment

The virus has to attach to the host

Inject DNA

Transcribe

Make viral proteins important for replication. These proteins interact with the host cell, dirrecting it to replicate the virus, transcribe it and package it. Each protein can have many functions.

Replication

Late transcription.

Packaging

Maturation phase

Exit

Lysis

bursts the host cell open

Exocytosis

bud out of the membrane by exocytosis.

Some insert into host DNA.

NOTE prac is on the one step growth curve.

NOTE: there is a difference between generalised and specialised transduction,

Generalised transduction.

normally beneficial to the bacteria recieving the new bacterial DNA.

Culture of viruses.

because viruses need a living host, a living host is required, this can be a tissue or a cell culture. (cells separated from tissue and placed in a growth medium).

dedifferentiated cell lines are immortalised, there is an immortalised cell line, hela (henriety lax) cells, taken from ovary tissue. had several children and eveloped the most virulent form of overian cancer ever seen, lead to a debate over who has control over other people cells.

Growing viruses. viruses kept in living cells, which form monolayer. plant cells require removing the cell wall, to form protoplasts, or the cells can be separated with still their cell walls by dissolving the middle lamella.

normally cows or horses are used, and cells separated by physical and enzymatic treatment, the cells are then placed in the culture medium.

Fungi

Protozoa

Bacteria

Algae

Archaea

Background

Nomenclature.

The Linnaeus system of classification is used. Any given organism is described by its genus, in combination with a specific epithet, i.e. it's species name. For example *Staphylocuccus Aureus*

Ecological Niche

Microorganisms can be producers or decomposers.

Human impact.

Food Spoilage

Microorganisms can lead to food spoilage. When humans eat food contaminated with microorganisms they are t risk of severe sickness of even death, either because the microorganism themselves are toxic or because some product of their metabolism, eg a waste product is. Closely related is the risk of infection from ingesting a pathogenic micro-organism.

Direct uses

Microorganisms Industrial Applications

The two major applications of microorganisms are in production of fermented foods and beverages (for example beer/ethanol in any alcohol and yogurt) and industrial chemicals for example insulin. Another example is a pair of jeans. The bleach used derrives from peroxidase in mushrooms, the indigo die is derrived from *Escherichia coli*. Even the (plastic) buttons can also be made by bacteria which can be manipulated to produce poly–toante, by manipulation (?) (or is in maniputed to produce plastic)

Laboratory Work

Lab Safety

Lab safety

If agar plates are cultivated with sucessive imprints of a persons fingers, normally by the sixth plate there are still enough microorganism transferred that significant growth is observed.

Aseptic techniques.

Aseptic technique are used to avoid contamination of microorganism samples under study in the laboritory. One major source of contamination is the air itself. Millions of bacteria fall on each m^2 of the earths surface per day.

Agar plates

An agar plate consists of the agar medium, a gelly like substance extracted from seaweed which is used to form a regular support matrix to which food sources, such as sugars and proteins are added.

Nomenclature.

The linnaeus system of classification is used. Any given organism is described by its genus, in combination with a specific epithet, i.e. it's species name, For example *Staphylocuccus Aureus*

NOTE: Genus and species names should always be italisized, (or underlined when hand written). Furthermore the first time a microorgansim is reffered to in a text its full name should be given, after which the genus name can be abbreviated to an initial.

Bacteria

Cellular consituents.

Bacteria are prokaryotes, so lack a nucleus and associated nuclear membrane.

Cell wall

All bacteria have a cell wall, and these cell walls contain peptidoglycans.

Replication

Bacteria replicate most commonly by binary fission.

Binary fission

Binary fission results in two approximately equally sized daughter cells, which are genetically identical. This form of reproduction is assexual.

Energy Source.

Bacteria can oxidise organic or inorganic chemicals, to produce energy. Some are also capable of photosynthesis.

NOTE: The ability to produce energy by oxidation of inorganic chemicals is relatilvely unique to bacteria.

Archaea

NOTE: Archae are often covered in less detail not because they are far less widespread or numberous than other microorganisms but becuase they are relatively unstudied. The lack of study is explained by their extreme habitats making them harder to sample/collect and grow in labs, and the related fact that they seldom interact with humans and do not act as human pathogens.

Cellular Components.

Archaea are prokaryotes.

Cell walls

Not all archae possess cell walls and those that do, do not contain peptidoglycans.

Habitat

Archaea live in a diversity of 'extreme' envirments.

Methalogens

Methalogens live in environments which contain lots of methane producing bacteria (methane is a waste product of their metabolism).

(Extreme) Halophiles

Live in very high salt concentrations.

(Extreme) Thermophiles.

Live in very high temperature environments. ($> 115°C$ easily)

NOTE: The combined biomass of all bacteria under the surface of the earth is greater than the combined biomass of all plants and animals on the earth.

Fungi

Cellular consituents.

Fungi are eukaryotic.

Cell wall

All Fungi posses cell walls made of chitin.

Energy source.

Fungi metabolised organic molecules for an enery source.

Higher organisation

Fungi can be unicellular in the case of yeasts, or multicellular in the case of molds and mushrooms. Multicelluar fungi consist of a mass of filamentous hyphae which together form a tangled mess known as the mycelium.

Reproduction

Fungi can reporduce both sexually and assexually.

Life cycle

Fungi can be parasitic or free living.

Protozoa

Absorb/ ingest organic materila.

Motility

May be motile, due to the presence of flagella.

Reproduction

Protozoa can reproduce both sexually and assexually.

Life cycle

Protozoa can be parasitic or free living.

Algae

Cellular components.

Algae are eukaryotes,

Cell wall

Algae posses a cellulose cell wall.

Energy Source

Photosynthesis is used to produce (more more accurately store energy) in the form of carbon sugars.

NOTE: photosyntheisis is not necessarily oxygenic, i.e. it does not necessarily involve the formation of oxygen.

Reproduction

Algae reproduce sexually and assexually

Energy source.

Algae can use the oxygen they generate in photosynthesis for their respiration, and in general to produce oxygenic compounds.

Morphology

Strikingly algae can become unusually large for single cellular organisms, eg giant kelp.

Viruses.

Viruses are acelluar.

Genetic material

Viruses can contain either DNA or RNA as their genetic material.

NOTE: All other microorgansims rely predominately on DNA but also contain an RNA component (eg rRNA) so extracting pure DNA or pure RNA is difficult. In the case of viruses however either exclusively DNA or exclusively RNA will be present.

Numerosity and distribution

Viruses may in fact be the most numerous microorganisms. The evidence for this claim lies in the fact that for all studied bacteria at least one bacteriophage specific to that bacterial species has been discovered, and in fact it is currently thought that there are at least two bacteriophages per bacterial species one with a lytic and one with a lysogenic life cycle. Furthermore for each infected bacteria there will be on the order of 10^8 phage, hence as bacteria were suspected to be the most numerous, viruses (in terms of the phage component alone) can be considered to be the most numerous.

Multicelluar animal parasites.

These species are considered to be microorganims in the sense that they have a stage fo their life cycle which is microscopic, this is far from the general classification system however.

Cell constituents.

Multicellular animal parasites are exclusively eukaryotic.

Higher organisation

they are by definintion multicellular.

Jeans example.

Theoretically an entire pair of jeans could be made from microorganism derrived products. The indigo dye used is derrived for *E. coli*. The bleach used is derrived from peroxidase in mushrooms, even the buttons could be made from plastics which bacteria can be manipulated to produce.

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Aseptic techniques.

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Bacterial structure and function

Learning Outcomes.

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Electron micrograph

An electronmicrograph is prepared the following process:

1. bacteria are spun into a liquid gel medium.
2. the cell is solidified and dehydrated with ethanol, and then placed in a block of (liquid?) resin, which is relatively hard but soft enough to be cut by the diamond (or occasionally) glass blade cutter.
3. nm thick sections are sliced off the block and float off onto water from which they are retrieved with the use of a special grid, and fixed onto a slide.

NOTE: literally hundereds of slides would be required to build up a 3D image, which even then would be distorted by the process of dehydration.

Ecological niche

Microorganism are both producers and decomposers, (they produce organic sugars both by photosynthesis and by chemolithotrophic. Microorganisms can be mutualists, some are pathogens. The are also vital in maintaining the geochemistry planet cycle.

Anthropic Appilications.

The two major applications of microorganisms are in production of fermented foods and beverages (for example beer/ethanol in any alcohol and yogurt) and industrial chemicals for example insulin.

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NOTE: The production of plastics is related to the natural pathway for the production of bicarbonates (?).

Anthropic impact.

Microorganisms can lead to food spoilage. When humans eat food contaminated with microorganisms they are at risk of severe sickness of even death, either because the microorganism themselves are toxic or because some product of their metabolism, eg a waste product is. Closely related is the risk of infection from ingesting a pathogenic micro-organism.

Overview of microorganim types.

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Cell constituents.

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Higher organisation

they are by definition multicellular.

Bacterial structure and function

Learning Outcomes.

1. What are bacteria
2. What is the structure of a bacterium
3. What are the sizes and morphologies of bacteria.

Cell constituets.

Bacteria are prokaryotes so the have no true membrane enclosed nucleus (TMEN) ie no nucleus or nuclear membrane.

Genetic material

The genetic material of bacteria in compacted and contained within a region of the cytoplasm known as the nuceloid, however this region is not set or enclosed, it is merely the region in which the genetic material is found.

Mitochondria

bacteria do not contain mitochondria because they are essentially mitochondria (or more accurately they have a common ancestor with the mitochondria of eukarytic cells and can perform a similar respiratory process with their own cell membrane as mitochondria perform with their outer membrane.)

NOTE: generally speaking prokaryotes are less complex than eukaryotes.

Morphology

The most common bacterial shapes are spherical/coccus and rod shaped/bacillus, however these are far from the only possible shapes.

NOTE: Most bacteria are monomorphic, i.e. only take on one shape so shape is a good identifying characteristic.

Colonial association

Individual bacteria can associate in a number of different ways, but a given species will (generally) only have one form of colonial association. Common forms include, Paired (Diploid), Clustered and chained.

Diploid

A single division would lead to a paired conformation, provided the bacteria do not separate

Strepto

Repeated binary fission in the same axis would lead to the formation of a chain.

Tetra

Binary fission first along one axis and then along a perpendicular axis would lead to the formation of a square

Sarcinae

Binary fission along three perpedicular axies. would lead to a structure consisting of eight bacteria.

Staph

multiple divisions along multiple axes lead to a disorganized mass of bacteria

NOTE: there is not clear advantage to these association. They are most probably caused by the presence of sticky (stuff?) on the outside of the bacteria's surface.

Multinucleiod

some bacteria can take on a miltinucleoid filamentous structure.

Angular

some bacteria snap back into a specific angle relative to each other after binary fission, probably due to incomplete separation of the cell walls of daughter cells.

Pallisade

Bacteria snap back to lie directly adjacent to each other.

Coccibacillus

Very short rods and can be very hard to distinguish from true cocci bacteria.

Bifurication

Bifurcation can occur to give Y shaped bacteria

Vibrion

bacteria have a distinct curved rod morphology.

Spriillion

bacteria take on a corkscrew shape

Spirochete

bacteria take on a corkscrew shape (more flexible than Spirillion)

Walt's square bacterium

dimensions of about $2\mu m \,\mathrm{x}\, 2 - 4\mu m \,\mathrm{x}\, 0.2\mu m$ taking on the shape of a square or a rectangle.

NOTE: some bacteria are polymorphic changing their shape continuously.

Endospores

There are often endospores within bacteria which can cause swelling changing shape.

Plasiticty

bacteria have the facility to adapt their environment by changing their gene expression patterns.

Size.

The average is about $0.2 - 1.0\mu \mathrm{x} 2 - 8\mu m$ For example E. coli $1.1 - 1.5\mu \mathrm{x} 2 - 6\mu m$

Smallest.

$100 - 300nm$ range, and ultramicrobacteria which are about $50nm$ in size. Very nutrient poor so do not maintain large cytoplasm.

Largest.

Bacteria Epulopiscium fishelsoni

$80\mu m - 600\mu m$ (visible with the naked eye), about 10^6 times larger in volume than most bacteria

reproduction

produces daughter cells inside the mother, which then pop out through a slit.

Addaptions

The key limitation to size is SA:V, as movement of nutrients and gasses are by diffusion. This problem is overcome in part by massive invaginations of the surface membrane. (Additional problem of transporting proteins to membrane as membrane already covered in proteins.).

It was thought that a particular bacterium would have one copy of a bacterium, however in Epulopiscium fishelsoni chromosome number increases with size, also as transcription and translation are linked, if the gene is everywhere in the cell, then a protein can be produced anywhere in the cell and the need for sophisticated transport systems in removed. The number of copies of important genes can be in the hundred thousands, for example ribosomal RNA genes.

Thiomargarita namibiensis

very large volume.

Addaptations

cytoplasm is filled with vacuoles so minimal depth of cytoplasm from any point. Epulopiscium fishelsoni requires nutrient dense environment so lives in fish and floats around to find nutrient dense areas.

The other advantage of size is that it hep avoid (protozoan) predation.

Structure and function

NOTE: Not all structures are found in all bacteria. Some structures are specific to specific bacteria some are specific to the bacterium's environment, ie parasitic only produce a capsule when they encounter a host immune response.

(Copy image of bacteria)

NOTE: cytoplasm is actually packed/ very full of constituents.

Plasma membrane.

they are the main contact point between the cell and its surroundings. functions splits internal and external environment in its capacity as a boundary layer, and allows the exchange of matter and information between the internal and external environment.

NOTE: bacteria are very plastic in their response to the environment improving energy efficiency

Fliud mozaic model

Phospholipids+ integral and poriferal proteins

Membrane associated lipids

Amphiphatic, (hydrophilic and hydrophobic groups associated with them), most of these lipids are phospholipids, they spontaneously arrange with phosphate head by water to form low energy micelles or bilayers.

Phospholipids are diverse and complex groups. Phosphatidylethanolamine (75%) phosphatidylglycerol (20%) cardiolipin (diphosphatidylglycerol, 1-5%)

Important as they confer strength, and spread out overall negative charge of the plasma membrane and cardiolin can play a role are a chaperon, helps lactase permease fold correctly in the membrane.

For a particular species of phospholipid the fluidity is adjusted by saturation/desaturation of tails, to fit the requirements of the external environment.

Lipids can also flip between leaflets of the membrane, there are proteins flipases (out to in), flopases (in to out) require ATP, and scamblases (exchange) do not require ATP. Related to adaptions to environmental changes such as temperature (with season).

NOTE:membrane is packed with proteins, especially in bacteria which require membrane proteins for energy production, this applies especially of bacteria with less efficient respiration processes.

Glycolipids

heterogeneous

Functions

1. Help to stabilize the plasma membrane (protective function)
2. Important in cell-cell interactions
   (a) Adhesion. (contact inhibition, muscles, desosomes etc)
   (b) identification (the flip side is that is can be recognized by the host immune system).
   (c) assist in signal transduction.

Transport systems

A transport system is required to move across the plasma membrane, could be for toxins such as bacteriosin which attach other bacteria in the environment, They can also trap food outside the PM to prevent its loss. Furthermore important metabolic functions such as photosynthesis and respiration involve the plasma membrane. Motility is also associated with plasma membrane associated structures, and the synthesis of many important chemicals.

NOTE: bacteria can distinguish which host they are infecting and can adapt accordingly.

Integral membran proteins.

make up about 60-70% of membrane proteins. These proteins can move around the membrane freely by diffusion.

Ease of removal.

tend to be relatively difficult to remove in aqueous environments (as low solubility). Such proteins are usually embedded deeply, some may span the entire membrane. To span the membrane they must be amphiphathic (otherwise would flip into the membrane).

Periferal proteins.

20-30%

Ease of removal

relatively easier to remove, as they are on the outside, and more soluble in aqueous environments.

(Copy image of the plasma membrane.)

NOTE: there is no reason for the tails to align between layers.

S layer

additional very strong outer layer formed on membrane

Cell wall

The majority of bacteria posses a cell wall.

Nucleiod

Gas Vacoules.

Inclusion bodies

Robosomes.

Cell enveope.

contains the outer membrane(where present) the cell wall and the cell membrane

External Structures

Capsule

A capsule is very similar to a slime layer but consists primarily of proteins and exhibits increased organisation and structural integrity. Capsules are also more firmly attached to the bacterium cell wall. A capsule implies a cost as many valuable food substances must be placed outside the cell to constitute it however it also confers several important benefits. A capsule makes the bacterium, more resistant to dessication and phagocytosis, (especially in mammalian hosts), it also facilitates substrate adherence to substrate, increases antibiotic and bacteriophage resistance and acts as a readily accessible store of carbohydrates. Finally it even relieves osmotic pressure acting of the cell.

NOTE: The capsule is very thick compared to the bacterium.

NOTE: Capsules do not take up stain, so they are visualised by using a (positive) primary stain for the bacterium and a (negative) secondary stain for a background. The unstained region remain is then the capsule. Colorless shiny bacterium are likely to be pathogenic.

Slime layer

A slime layer is a layer of material outside of the cell wall, consisting of extracellular, polymeric substances. (EPS) A slime payer consists primarily of polysaccharides , but may also contain proteins. It is not very tightly attached to the bacterium

S layer.

The S layer is always constituted of proteins or glycoproteins, which are set into a regular array. This layer is very hard to pull apart and behaves as a protective layer similar to chainmail. Individual links will compress into each other but only to a point, past this point further compression require very large forces. This protective layer helps to protect against bacterium such as *Del Virbio* which crash into other bacteria at high speeds and then 'drill' into them to live inside them as paracites.
Internal membrane system.

Gram staining.

The gram cell is able to differentiate between two major structure types based on their ability to maintain the primary stain in the presence of the de-coloriser. Gram staining is bar far the most important staining in bacteriology. staining is also related to differences in the lipids.

Process

1. Crystal violet.
   # . Grams iodine. (Helps crystal violet to stick to the cell wall.)
2. alcohol (decolorize), gram negative cells are decolorised, gram positive cells are not.
3. Safranin (red due)

Gram positive have red on purple so remain purple, gram negative have red on colorless so become red/pink.

(Copy image)

NOTE: If decolored too intensely any cell will lose its color.

NOTE: Acetone in involved in eyes dissolving.

Posive

Copy from slides.

Cell wall

Thick homogeneous cell wall containing 50% peptidoglycan by dry weight. The peptidoglycan is about $20 - 80nm$ thick.

Periplasmic space

A area in between the cell wall and cell membrane it is distinct (although so thin that it can often be hard to make out.).

NOTE: The cell wall is the combination of the PG layer and the periplasmic space.

NOTE: Try to draw structures with some sense of relative scale, ie the cell wall must be larger.

Copy image of cell wall from slides.

Function

Maintains the cell integrity against osmotic pressure and maintains the cell shape. cytoskeletal elements are a large part of it, but the peptidoglycan is also significant, and if it is removed the bacteria will usually become spherical

Techoic acids.

(referred to as ? techoic acids), Techoic acids make up a significant proportion of the cell wall. They are covalently attached to the peptidoglycan, which has a mesh-like structure. Techoic acids fill (almost all) of the void spaces in this mesh extending all the way to the outside of the layer.

Lipotechoic acids are embedded in the membrane at one end and the peptidogycan layer at the other. forming a stabilizing link.

Functions

help to maintain the structure of the cell wall. And confer an overall negative charge (to gram positive bacteria).

The acids are also antigenic, (which benefits the host not the bacterium)

Gram negative.

Cell membrane

5-7nm

Peptidoglycan layer

Much thinner $5 - 10\%$ of dry weight of cell negative bacteria. only about 1nm

Periplasmic space

between cell and other membrane, much wider than in gram positive bacteria, can be go up to $70nm$, constituting a significant component of the cell. The Periplasmic space contains lots of proteins and enzymes, in particular those associated with nutrient acquisition such as binding proteins which move out of the cell and bind to nutrients making them easier for the cell to recognise. The Periplasmic space also plays an important role in electron transport and energy production, and contains receptors important in chemotaxis.

Outer membrane.

5-7nm

phospholipid layer (not bilayer) which consists of an single layer of lipopolusaccharides, within which lipoproteins and proteins are found. More permeable/less selective than the cell membrane (?) The outside of gram negative is very wavy as more fluid than a PG layer. The outer membrane functions to prevent the loss of cellular constituents.

NOTE: the outer membrane can be isolated as an intact spherical structure

Lipopolysaccharide '

Lipopolysaccharide is a virulence (disease causing) factor, which consists of three distinct segments, an outer chain a core polysaccharide, and lipid (embedded within the membrane). Lipopolysaccharide is particularly effective in minimizinf the effectiveness of an immune response in a mammalian host.

Outer (O) chain

In *E Coli* alone there are at least 160 different versions of the O chain. The length of the chain often influences the roughness of a colony. Full length outer chains lead to a smoother more hydrophilic surface. Short outer chains lead to a rough more hydrophobic surface. The O side chain is antigenic, often recognised by the host in a immune response. Some bacteria can mutate their O chain by antigen shift to avoid an effective immune response.

Core polysaccharide.

The core poly saccharide is short normally only 8 polysaccharides long. It shows far less variation than the Outer chain, but forms an important link between the outer chain and the membrane embedded A lipid. There is an accumulation of negative charge by the joint between the Core polysaccharide and lipid A which facilitates the intercalation of magnesium ions which help to stabilize the outer membrane, slotting in between the lipids of lipid A. The role of the Mg^{2+} ions is so important that if a strong chelating agent is used to pull these ions out of the membrane that it looses its integrity and starts to break apart. This core component may contain amino acids. The hydrophobicity of the core polysaccharide prevents certain harmful substances from entering the cell.

Lipid A

Lipid A consists of Phosphorylated N-acetylglucosamine and 6-7 fatty acids which are typically saturated, and does not extend past the membrane. It is almost invariably hydrophobic. Lipid A is a major component of the outer membrane and helps to stabilizes and protect cells, forming a permeability barrier which is very efficient at stopping lysozyme and bleaches, and bio-salts from entering and damaging the cell.

Endotoxins.

Endotoxins are bacterial components which are not produced and excreted by bacteria for the express purpose of toxicity but rather substances which play some chemical or structural role in the bacterium, which happen to be toxic to host cells. Endotoxins can nevertheless be extremely toxic, one example is the Botulin toxin (Botox) used in skin treatments. Lipopolysaccharides are also endotoxins which interact with plattlets in the blood decreasing their number to the point where internal bleeding may occur.

Porins

make pores through the outer membrane, consist of three separate units. Porins are transmembrane proteins, which form channels for entrance and exit of certain substances, (they are essentially water filled channels)

NOTE: The presence of Porins indicates the extent of the protective function of the outer layer, in that they show how selective it is in what substance may permeate through it.

Brauns lipoprotein

extends from the GP layer to the outer membrane.

Function

Helps to prevent membrane damage by giving it some form of rigidity, and preventing an indeterminate gap between the outer membrane and the rest of the bacteria, pulling the membrane into the same shape as the bacteria.

Lipopolysachharides. (LPS)

Consist of lipids and carbohydrates. The outer leaflet of the membrane is almost entirely lipopolysacharide, and in genre it is an integral membrane component.

Salmonellatyphimurium in Salmoella can be related to disease.

1. what are bacteria
2. what is the strucure of a bacterium
3. what are the sizes and morpholgies of bacteria.

Defining characteristics.

Cell constituets.

Bacteria are prokaryotes so the have no tru membrane enclosed nucleus (TMEN) ie no nucleus or nuclear membrane.

Genetic material

Major nutritional metabolic types.

Autotrophes get there carbon from CO_2 in the environment. whereas heterotrophes get their carbon from organic compounds. some organisms especially bacteria can switch between different nutritional sources/mechanisms.

NOTE: litho imples the electrons from an inorganic compounds. (electrons or energy) NOTE: autotrophes require electrons for carbon dioxide reduction.

Photolithotrophic autotrophy

energy comes from light, carbon comes from CO_2 in atmosphere, electrons are gained from inorganic substances water in the case of plants. cyanobacteria are an example

Photoorganotrophiic heterotrophy

Photosynthesis is used for energy but electrons and carbon are gained from an organic source, this is a very unusual way to grow and mainly bacteria, very few other organisms. purple (other pigments) and green (chlorophyll) non sulfur bacteria.

Chemolithotrophc autotrophy

grow without light and organic carbon electron sources, as well as organic carbon carbon sources, so grow in deep mines etc.

Chemoorganotrophic heterotrophy.

energy from chemical respiration, i.e oxidation of organic compounds, electrons from organic compounds, and carbon from organic compounds.

Classification of microorganisms.

Old model (Universal tree of life)

From monera to protists to plants animals and fungi, and most diversity was thought to reside in the plant, animal and fungi kingdoms.

Three domains Woese

three and a half billion years was the first common ancestor.

Bacteria

Only bacteria for about 2 billion years.

NOTE: the diversity between cyanobacteria and protobacteria (or indeed any other bacterial clade) is greater than that between plants and animals.

Bdellovibrion

Prey on other bacteria, drills through other bacteria, preditor.

Pelagibacter ubique

Dominates in many environments, only discovered by FISH techniques, as could not culture it. Can make up about 20% of all prokaryotes in the oceas, and about 0.5% of all prokaryotes on the planet, and in hot ocean environments can be up to 50% , even though theses environments can be quit harsh, about $2 \cdot 10^{28}$. very small so good SA:V, and so small its not predated on well, $0.4 - 0.9\mu m$ by $0.1 - 0.2\mu m$. very simple, smallest genome known. complexity does not imply more evolutionary success.

Helicobacter.

examples.

Archaea

Eukarya.

Human microbiome.

microbiota all the microbes in a community or that reside in an environmetnal niche.

microbiome: the collective genomes of the microbes in a community or that reside in an environmental niche. (this is useful as many studies do not use cultures but rather molecular techniques.

NOTE: This implies that humans are in fact just a collection of species. furthermore there are no homosapiens with no bacteria, and infact their cells are outnumbered by bacterial cells 10 to 1. so how does one distinguish between them.

NOTE: there are several thousand bacteria species in most distinct areas of the human body, the large intestine in particular has over 30000 species.

Natural childbirth.

Should suzzarians be done, the microbial diversity on a newborn skin for natural vs Cesarean, they are very different. In the viginal tract there are large numbers of lactobacillales , so there are ar more lactobacilli on babies from natural birth. whereas from a Cesarean section is mostly those in the ovaries. The microbiome trains the immune system, if there are not there then diseases such as Exsema can result. moving down the digestional tract you end up with more bacteria.

Obesity

The relationship between obesity and the microbiome. Germ free mice. The germ free mice showed decreased propensity to obesity. in the gut there are firmicutes and Bacteriodetes, so if there are more Firmicutes there is more weight gain, as these bacteria are more effecient at giving them excess nutrient to the human body. Lean mice can be switched into obease and visa versa.

Study in malawe, using twins, same food one mulnurished, diffs microbiome, can be changed back by ingestion of bacterial tables.

Anxiety and fear

depend on the microbacteria. for example *Lactobacillusrhamnosus* if fed to mice makes their anxiety go down, they start being less cautious of open spaces and water in particular. Autism and brain development. with high numbers of chlostridia, the brain produces phenols to kill bacteria , which damage the brain, so sulfur is added, but this may lead to a sulfur defficeincy which in turn may lead to brain damage later on.

Human health

The microbiome cannot be cut out, should be thought of as a collective property of the human-associated microbiota.

Open a window, an ecological perspective, restoration ecology, eat more plants, get your hands dirty, no antibiotics.

AntiBiotics.

switches from antibiotics are not always reversible, and highly unadvised.

Major plays.

Virus identification, can use sequencing next gen/deep.

Life cycles of viruses.

All occurs with a cell. when a virus enters a host.

Stage one

the virus must attach to the cell membrane receptors, combination of general and specific receptors, some viruses will onyl reproduce in specific tissues, some viruses can replicate only in one type of cell

Penetreation by endocytosis or fusion (viruses which have an envelope, the two lipid membranes fuse together). endocytosis, means membrane invaginates around the virus leading to a double membrane around the viruses,

translocation, must get though the membrane, without bursting the membrane, which is added by the fluid nature of the membrane.

injection, in the case of bacteriopahges,

COPY IMAGES

Uncoating

by viral or host enzymes (or both); pH, virus may contain its own enzymes to break down nucleic acids, in this case lipases specifically are used.

Assembly and maturation

biosyntheisi: Production of

Proteins within ther virion are bound to RNA to stabalise them,

some viruses no uncoat completely, partly this is to prevent the cell and its degrading enzymes, especially dsRNA, becuase the celll degrades this in silencing.

NOTE: Cell machinary refernce to enzymes and cofactors.

the virus fuses to the cell membrane leaving all of its envelope material on the outside.

Uncoating of viruses

Occurs via viral or host enzymes or both.

capsid proteins bond together by electrostatic forces,

the capsid protein is always encoded by the virus, only a few genes are for the strucure of the virus the rest are for proteins helping for virus take over of the cell.

Appendix