AIMS

- Be able to define and explain differences between taxonomy, classification, identification
- Define the concept of numerical taxonomy
- List features used in classification systems
- Understand the rules for nomenclature
- Understand differences between identification systems
“Taxonomy is written by taxonomists in this form the subject is so dull that few non-taxonomists are tempted to read it and even fewer are tempted to try it!

It is the most subjective branch of biological science and is more an art than a science”
DEFINITIONS

- **Taxonomy**
  - study of the biological relationships between organisms

- **Classification**
  - the orderly arrangement of units

- **Nomenclature**
  - naming or labelling of these groups to distinguish one from the other
DEFINITIONS

◆ Identification
- assigning a name to an unknown by comparison to characteristics of known organism in a previously made classification system
HISTORY of CLASSIFICATION

- 1700 Carl Linnaeus divided all living organisms into TWO KINGDOMS
  - Plantae and Animalia
  - no allowance for single celled organisms such as bacteria, fungi, algae
- 1866 Ernst Haeckel proposed THREE
  - Plantae, Animalia and Protista
- 1966 Whittaker proposed FIVE KINGDOMS
  - Plantae, Animalia, Protista, Fungi, Monera
FIGURE 1.4
FIVE-KINGDOM CLASSIFICATION

- Fungi
- Animalia
- Plantae
- Protista
- Monera
A classification system divides a larger group into 2 or more smaller groups

Hierarchical type classification tree

- Enterobacteriaceae
  - Escherichia
    - E coli
    - E hermanii
  - Klebsiella
    - K pneumoniae
    - K oxytoca
KEY ELEMENTS OF CLASSIFICATION SYSTEMS

- Morphological (phenotaxonomic)
- Physiological (phenotaxonomic)
- Cultural
- Biochemical (chemotaxonomic)
- Chemical
- Genetic (genotypic)
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Morphological (phenotaxonomic)
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MORPHOLOGICAL FEATURES

- Shape and size
- Cellular arrangement
- Motility
  - number and distribution of flagella
- Endospore production
  - size, shape, location
- Capsule
- Staining properties
Figure 1.2.
Schematic diagram illustrating bacterial cell structure. The text indicates which of the structures are common to some bacteria and which are common to all bacteria.
PHYSIOLOGICAL GROWTH REQUIREMENTS

- Oxygen/CO2 requirements
  - aerobes, anaerobes, microaerophils
  - obligate, facultative

- Temperature
  - psychrophilic, mesophilic, thermophilic
  - minimum, maximum, optimal

- Specialised nutritional growth factors
CULTURAL APPEARANCE

- Colonial morphology
  - size and shape of colony (umbonate, rhizoid, raised, swarming)
- Growth in liquid media
  - surface, puffball
- Pigment production
- Extracellular products
  - haemolysins, slime, lipases
BIOCHEMICAL ACTIVITY
(Phenotypic Characteristics)

- Respiratory Function
  - catalase, oxidase, nitrate reduction
- Carbohydrate (CHO) utilisation
  - method (O/F) and range of CHO
- Activity on nitrogenous compounds
  - decarboxylation, urease, deaminases
- Hydrolysis of complex biochemicals
  - proteases, lipases, DNAase
Rapid pre-formed enzyme detection - 4 hours

Minaturised biochemical from a single colony
Limitations

- **API20E/Commercial Systems**
  - same biochemical substrates 1975-2003
  - best test not on panel $\Rightarrow$ misidentification
  - new organisms “need to fit” existing databases
  - work well where large number of strains can be compared $\Rightarrow$ accurate database
CHEMICAL COMPOSITION

- Cell wall amino acids
  - gram positive genera
- Cell wall sugars
  - speciation within species (ß haem Strep)
- Lipids - mycolic acids
  - Mycobacterium, Nocardia
- Proteins
  - electrophoretic patterns species specific
CHEMICAL COMPOSITION

❖ Problems
  ▼ Labour intensive
  ▼ Time consuming
  ▼ Technically demanding
  ▼ Relevant to select groups of organisms only
GENETIC CHARACTERISTICS (genotype)

- Genome size
- G+C Ratio (1960s)
  - constant within species but not unique
- Whole genome DNA-DNA homology (Brenner)
- 16sRNA (mid 1990s)
- Sequencing DNA (automated PRISM)
G+C RATIOS

- G+C Ratio
  - bacterial genome is dsDNA (circular)
  - G+C ratio determines the $T_m$

  **denaturation temperature**
  dsDNA $\leftrightarrow$ ssDNA

  - high G+C $\Leftrightarrow$ high $T_m$
  - determined by HPLC of DNA hydrolysed by nuclease P1

- G+C ratio constant for a particular species
- Range 24-76% (3% within species, 10% within genus)
- different genera may have same G+C
DNA-DNA hybridization (1)

- total genome comparison
- measures the degree of DNA similarity
- variety of methods available
  - labelled DNA - radioactive, biotin
  - thermal stability of annealed strands
- >70% relatedness \rightarrow same species
  - values vary with method (55-75% range)
  - expensive, labour intensive, technically complex
DNA-DNA hybridization (2)

- problems
  - many bacteria have sequences in common
  - annealing of strands when up to 15% base sequence is different
  - *Escherichia coli* versus *Shigella sp*
- additional criterion - $\Delta T_m < 5^\circ C$
  - difference between $T_m$ of known:known strain against $T_m$ of unknown:known
- International definition of genomospecies
Ribosomal RNA

- All living cell contain rRNA (highly conserved)
- bacteria possess
  - 16S, 23S and 5S genes (1-11 copies)
- most useful method is rRNA sequencing
- 16S rRNA best due to size
  - 1,500 base that can be sequenced in 1 day
  - 23S (3,000), 5S (150)
- International databases GENBANK
- Useful for non-cultivatable bacteria
SEQUENCING

- Amplification of 16S RNA gene by PCR
- Sequence PCR product (ABI PRISM)
  - ss DNA add primer + DNA polymerase
  - add deoxyribonucleotides (dA,dT,dC,dG)
  - add labelled didoxyribonucleotides individually (limit one eg ddA)
  - reaction stops when ddA are incorporated
  - separate on gel
  - determine sequence from position of band
Limitation of Molecular Characterisation

- Survey only small part of genome
- Ribosomal genes highly conserved
  - level of similarity required for species 97%
  - level of difference strain not species
- Databases derived from 1-2 strains
  - not reflect diversity of species
  - related to biochemical differences (laboratory level detection)
Evolution of Classification Systems

- Pre 1900s - based on disease process
- Morphology, size, motility (Cohn)
- In vitro agar culture ➔ first biochemical tests
- 1920s - first heirarchial systems
  - Bergey 1923, Topley and Wilson 1929
- Limited test types ➔ duplication of names
  - using only a small number of easily measured tests
  - different tests given more weight than others
Exercise 1
Shape
Colour - yellow
NUMERICAL TAXONOMY (1)

- Computer analysis proposed by Sneath 1959
- Principle - all phenotypic characters are weighted equally
- Uses 100 or more phenotypic characteristics
- 300-400 organisms compared simultaneously
- Entered into computer database as + or -
- Determine the coefficient of similarity
- Develop dendogram (phenogram)
  - joins individual strains into groups
NUMERICAL TAXONOMY (2)

◆ Problems
  ▼ what % similarity = genus (70%)
  ▼ what % similarity = species (80%)
  ▼ what type of tests to include
    phenotypic diversity ✗ genotypic diversity
  ▼ how many of each different test type
  ▼ is a negative a true negative
EXERCISE 2

A
B
C
D
E

60  70  80  90
Polyphasic Species Concept

- Developed by Colwell 1970
- Integrates all available phenotypic, genotypic and phylogenetic information to achieve a classification scheme that facilitates identification

E.g. Bordetella
- 3 species (pertussis, parapertussis, bronchiseptica)
- all > 80% DNA homology (?single species)
- are 3 distinct species as they differ in numerous phenotypic and chemotaxonomic tests.
NOMENCLATURE

- System of naming organisms
- Name defines the organism without listing individual characteristics
- Names traditionally taken from Latin
- Binomial
  - genus with a capital letter
  - species with no capital letter
- Italicised or underlined
International Codes of Nomenclature

- International Code for naming organism
  - must be fully characterised (pheno/geno)
  - must be a type strain in recognised collection - NCTC, ATCC and UQCC
  - “priority of publication” applies

- Approved List of Bacterial Names 1980

- Valid names published annually in IJSB
  - only recognised publication
Bacteriological Code (1990)

- Lists the rules of bacterial nomenclature
  - outline rules for generation of new names to ensure continuity between disciplines
  - avoid names that create error of confusion
  - revision (new species 5-10 strains from geographically unrelated areas)
  - revision (new species independently confirmed)
  - set procedures for handling disputes

- ICBS Judicial Committee presides over disputes
Why Names Change?

- New genetic data
  - Pseudomonas has 5 distinct groups
  - New genera created (Burkholderia, Comamonas, Acidovorax, Brevundimonas, Stenotrophomonas)

- Single strain genera
  - genetic and phenotypic variation from other known genera
  - e.g. Stenotrophomonas (formerly Pseudomonas, Xanthomonas)
Identification Systems

- Practical application of taxonomy
- Evolution of ID systems
  - Dichotomous Key
  - Flowchart
  - Diagnostic Tables
  - Probability Tables
Keys and Flowcharts

- Keys use a series of characters in turn
- Limitations
  - assumes all strains will be either positive or negative
- Flowcharts use a series of characters in a set order
  - allow for some variation within species
  - species appear more than once in the chart
Diagnostic Table

- list a series of characters in any order
- each result is categorised
  - ▼ + = >85% positive
  - ▼ V = 16-84% positive
  - ▼ w = weak reaction
  - ▼ () = delayed reaction
- time consuming
- manual application only
Probability Tables

- % positivity determined for each test
- ideal for computerisation
- provides for % certainty of ID
- limitations
  - test large numbers of same species
  - test from different geographical locations
  - test human, veterinary and environmental
  - needs to be an ongoing process
Ideal Identification System

- Combination
  - phenotypic, chemotaxonomic, molecular
- Not practical for all situations
  - New species ➔ ALL
  - Clinical laboratory ➔ phenotypic, chemo
  - Special situations ➔ molecular backup
- Evolution!