Chapter 5 –II - Microbial Nutrition

When facilitated diffusion mechanisms are not adequate for transport of molecules across the cell membrane, there are two very and more important transport processes available:

1. ***Active transport***
2. ***Group translocation***

Active Transport Systems

- *Active transport systems facilitate the movement of a compound to a higher concentration or against a concentration gradient with the use of metabolic energy.*
  - ***ENEGRY DEPENDENT PROCESS***

- ***Involves Protein Carrier (premeases)***
  - *Premeases has a great specificity for binding the transport molecule.*
  - *Similar molecules can compete for the same carrier protein in both AT and FD.*
  - *AT – carrier saturation effect at high concentrations.*

Active Transport differ from Facilitated Diffusion:

- *AT requires metabolic energy input*
- *Has ability to concentrate substances*
- *Metabolic inhibitors that block energy production will inhibit AT but will not effect facilitated diffusion*

ABC Transporters

- *Binding protein transport system*
  (*Active in Bacteria, Archea, and Eucaryote*
- *Consist of two (2) hydrophobic membrane-spanning domains associated on their cytoplasmic surfaces with two nucleotide-binding domains. (figure 5.3)*
  - *forming a* ***pore*** *in the membrane*
  - *nucleotide binding domains* ***bind and hydrolyze ATP to drive uptake***

- *ABC transporters employ special substrate **binding proteins***
  - *In gram negative bacteria: in perplasmic space*
  - *In gram positive bacteria: attached to membrane lipids on the external face of the plasma membrane*
  - *These binding molecules – bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecules inside the cell. (E.coli: sugars & amino acids).*
  - *Many transport proteins use the energy of proton and sodium gradients to move molecules across the membrane (coupled transport).*
    - ***Symport:*** *transport of two substances in the same direction*
    - ***Antiport:*** *Transported substances move in the opposite direction.*

Group Translocation

- *Molecules are transported into the cell while being chemically altered and metabolic energy is used.*
  - ***Phosphenolpyruvate: sugar phosphotranseferase system (PTS)***
    Transports a variety of sugars into procaryotic cells while phosphorylating them using phosphoenolpyruvate (PEP)- ***a high energy phosohate*** *as the phosphate donor.*
    PEP + sugar (outside the cell) → pyruvate + sugar---P (inside the cell)

- *PTS are widely distributed in prokaryotes*
- *Aerobic bacteria lack PTS – except for species like Bacillus that have glycolysis and PTS*
- *Escherchia, Salmonella, Staphylococcus and other Facultatively Anaerobic have PTS.*
- *Clostridium: obligately anaerobic have PTS.*

Iron Uptake

Siderophores.

- *Under aerobic conditions iron assumes the Fe^{3+} form and is highly insoluble.*
- *In order to facilitate its uptake many bacteria synthesize compounds called* ***siderophores (iron bearers)*** *which are secreted from the cells and chelate the iron ion.*

- *This process solublizes the iron. The iron-siderophore complex is transported into the cell by a mechanism that remains unclear.*
- *In E. coli the siderophore is called **enterochelin***

Culture Media

- *Solid or liquid preparation used to grow, transport, and store MO.*
- *Medium must contain all necessary nutrients the MO requires for growth.*
- *Medium is used to select and grow specific Mo to help ID a particular species.*

- ***Synthetic or Defined Media***
  Medium in which all of the components are known

- ***Complex Media***
  Media that contain some ingredients of unknown chemical components
  - *Rich and complete to meet the nutritional requirements of many MO*
  - *Used b/c nutritional requirements are unknown (used for fastidious MO)*
  - *Contain components like **peptones, meat extracts and yeast extract**:*
    Peptones:** protein hydrolysates prepared by partial digestion of meat, casein, soya, meal, gelatin, and other protein sources, serving as **C, energy and N sources.
    - ***Beef extract** contain AA, peptides, nucleotides, organic acids, vitamins, and minerals*
    - ***Yeast Extract:** source of B vitamins, nitrogen, and carbon compounds.*
    - *Three commonly complex media: Nutrient broth/agar Tryptic broth/agar, MacConkey agar*

- ***Broth can be solidified with the addition of agar (1.5%)***
  - ***Agar** is a polysaccharide (long chain of monosaccharides linked by glycosidic bonds.*
  - Agar: extracted from red algae
  - Solidifying agent b/c most bacteria cannot degrade it.
  - Melt at temp above 80º Cand solidify at temp below 42º C.

Types of Media:

- ***General Purpose Media**: support the growth of many microorganisms.*
  Example: Tryptic soy broth ot Tryptic soy agar

- ***Enriched Media:** Blood or other special nutrients are added to general purpose media to encourage the growth of fastidious heterotrophs.*
  Example: Blood agar

Selective Media:

- A medium that is specific and favors the growth or isolation of a particular MO.
  Examples:
  Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram – by inhibiting the growth of gram +.

  Endo agar, eosin methylene blue agar, and MacConkey agar used for the detection of E.coli

Differential Media:

- Media that distinguishes between different groups of bacteria.
  -- *media that incorporates different ingredients that cause certain organisms to develop a different appearance from other microbes growing on the same medium.*

  Example: *Blood agar is both a differential medium and enriched.*
  It distinguished between hemolytic and nonhemolytic bacteria.
  It allows you to determine if a bacterial colony has produced the enzyme hemolysin and whether the resulting hemolysis is partial (alpha), complete (beta), or gamma (no hemolysis).

Isolation of Pure Cultures

In nature, MO usually grow in a complex mixed populations containing several species, therefore it is necessary to isolate the bacteria into single species for study.

Pure Culture:

- *a population of cells arising from a single culture cell characterize an individual species.*
- *A culture containing a single type of MO*

Spread Plate:

Spread Plate*: a technique where a mixture of cells is spread out on an agar surface so that every cell grows into a completely separate colony.*

Colony: *a macroscopically visible growth or cluster of MO on a solid medium, where each colony represents a pure colony.*

- *A small volume no more than 0.1 ml is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-rod glass.*
- *The dispersed cells develop into an isolated colonies*

- *The number of colonies should equal the number of viable organisms in the sample*
- *Used to count the microbial population*

Viable Plate Count contains 30-300 bacterial colonies
TMTC - more than 300 colonies on the plate
TFTC – less than 30.

Streak Plate:

Streak Plate: *Technique used to isolate pure colonies.*
Using an inoculating loop or swab, obtain bacteria, transfer to the edge of the agar plate and then streaked out over the surface in one or several patterns. Single cells drop of the loop at it is rubbed along the surface of the agar and develop into separate colonies

- *Successful isolation depends on spatial separation of single cells inboth streak and spread plate..*

The Pour Plate:

Pour Plate: *original sample is diluted several tined to reduce microbial population to obtain separate colonies when plating.*

- *Small volume of several diluted samples are mixed with liquid agar (cooled to 45º C)*
- *Mixtures are poured immediately in sterile culture Petri dishes*
- *After agar is solidified, each cell is fixed in place and forms an individual colony.*

Colony Morphology and Growth:

- *Form of the colony and shape of the edge or margin can be determined by looking down at the top of the colony.*
- *Elevation of the colony when viewed from the side as the plate is held at eye level.*

*The most rapid cell growth occurs at the **colony edge**:*
- *At colony edge, oxygen and nutrients are plentiful.*

*Slower growth takes place at the **colony center**:*
- *Much thicker growth than at the edge.*
- *Cells autolysis takes place in the older central portions of some colonies.*
- *Oxygen does not diffuse readily into the center, toxic metabolic products cannot be eliminated quickly, and growth in the colony center is slowed or stopped.*

(Differences in growth are due to gradients of oxygen, nutrients, and toxic products within the colony).