Microbial Biotechnology
Lec. 9
Manipulation of Gene Expression in Prokaryotes

Dr. Marwan Abu-Halaweh
Office 908
Email:mhalaweh@philadelphia.edu.jo
Microbial Biotechnology
Philadelphia University

Removing Selectable Marker Genes

- Integration of selectable marker gene along with the gene of interest is helpful in identification of transformed cells.
- It is a health risk to release a genetically modified organism with antibiotics resistance gene into the environments.
- To solve this problem integration could be directed to the nonessential site within the chromosome by means of a single crossover.
- As a result selectable marker will also integrated.
- An *insertion/removal* system for selective removal of marker genes from host cell chromosome has been developed.

L-Form of Bacteria

- L-form of bacteria are variants of ordinary bacteria that exist in a form that lacks cell wall.
- L-form can arise as
  - A consequence of spontaneous mutation.
  - After treatment with L-form inducer such as penicillin.

L-form strain

- Inducer such as penicillin inhibits the final step in cell wall synthesis, and lysozyme, which hydrolyzes some of the linkage between cell adjacent cell wall saccharides.
- After treatment with an inducer the building unit of the cell (which is peptidoglycan layer) will disappear.
- Unstable variant of L-form bacteria may be maintained in culture by the addition of the inducer to the medium.
- Stable L-form ones can be maintained without inducer.

L-form strain

- Examples of L-form strain application:
- The stable L-form of *P. mirabilis* were found to be 10-fold better producers of bovine pronchymosin or human interferon α1 than the common *E. coli* strain.

L-form strain

- Several disadvantage L-form strain that limits its use:
  1. L-form strain are naturally resistance to antibiotics commonly used, which minimis their use as antibiotics resistance marker in cloning.
  2. Unstable L-form strain have the potential to revert to their native form.
  3. L-form strain grow slower than the native form.

Metabolic Load

- Introduction of foreign DNA into a host organism often change the metabolism of the organism in such way that could affect normal cellular function.
- This is due to the metabolic load of the cell.
- One of the most consequences of the metabolic load is a decrease in the rate of cell growth after introduction of foreign protein.

- How to minimize the impact of metabolic load:
- 1- use low copy number plasmid
- 2-Integration into chromosomal DNA

- Metabolic load can occur as a result of different conditions including:
  1. Increase plasmid copy number which lead to increase the energy needed for plasmid replication.
  2. Limited amount of dissolved oxygen in the medium.
  3. Overproduction of target and marker protein, may depletes the pools of certain aminoacyl tRNA {or even amino acid) (aa)}.
  4. Sometimes exporting of over expressed foreign protein to the cell membrane or the periplasm, may jam the export sites and thereby prevent the proper localization of essential host cell proteins.
  5. Foreign protein may interfere with the function of the host cell, example change an important needed metabolic intermediate into irrelevant compound or toxic substance.

- TO solve metabolic load problems different strategy could be followed such as:
  - Using low-copy-number plasmid, or even integrate the target protein into the host DNA.
  - The use of regulatable promoters is also an effective way.
  - The protein codon is different that the codon usage of the host, may minimize depletion of the aminoacyl-tRNA pools. E.g. in a study streptavidin were 10-fold higher in *E. coli* when expression is directed by a synthetic gene which had a G+C content of 54% compare to the natural gene with G+C content of 69%.

- One problem associated with achieving high level of foreign gene expression and a high cell density at the same time is the accumulation of harmful substances especially acetate which inhibit cell growth and protein production.
- One strategy for reducing acetate accumulation without impairing cell growth entails decreasing the glucose uptake rate of the cells by adding methyl α-glucose (glucose analogue) to the growing cells.
- Or by introducing mutation in *E. coli ptsG* gene which code enzyme II in glucose phosphotransferase system.

Isolation and Screening of Microorganism

- It is important to select the most suitable microorganism(s) to carry out the desired industrial process.
- Isolation and screeniing of microorganism is one of the most important step in the microbial industry process.
- Isolated microorganism either obtained as pure or mixed cultures, after that these microorganism are tested to determine which is suitable for the industrial application.

Characteristics of the ideal microorganism for industrial application (biotechnology processing)

1. The microorganism must be stable genetically.
2. The strain should be pure, free from other microorganism and phages.
3. The strain should grow vigorously and rapidly after inoculation into the medium (fementer medium), strain that react with the equipment should be avoided.
4. The strain should exhibit the desired characteristic(s) or produce the desired product(s) within reasonable time, preferably free of other toxic by-products.

Characteristics of the ideal microorganism for industrial application (biotechnology processing): contuined

5. The strain should protect itself against contamination, if possible self protection can be on the form of lowering pH, ability to be cultured at high temperature or production of antimicrobial agents (antibiotics)
6. Strain should be amenable to genetic manipulation.
7. The strain should be readily maintained for long period of time.
8. The strain should ideally exhibit simple growth requirements. This characteristic would depend on the feedstock available.

Isolation and Screening of Microorganism

- Not all microorganism posses the mentioned criteria.
- Screening is the use of highly selective procedure to detect the microorganism or the metabolites of intrest from large population.

The ideal industrial screening should posses the following

1. Rapid
2. Sensitive
3. Inexpensive
4. Suitable for high-throughput (automation).
5. Specific (e.g. low false positive or false negative)
6. Predictive
7. Simple