ADVANCED ENZYME TECHNOLOGY

PRODUCTION OF ENZYMES

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Chapter Description

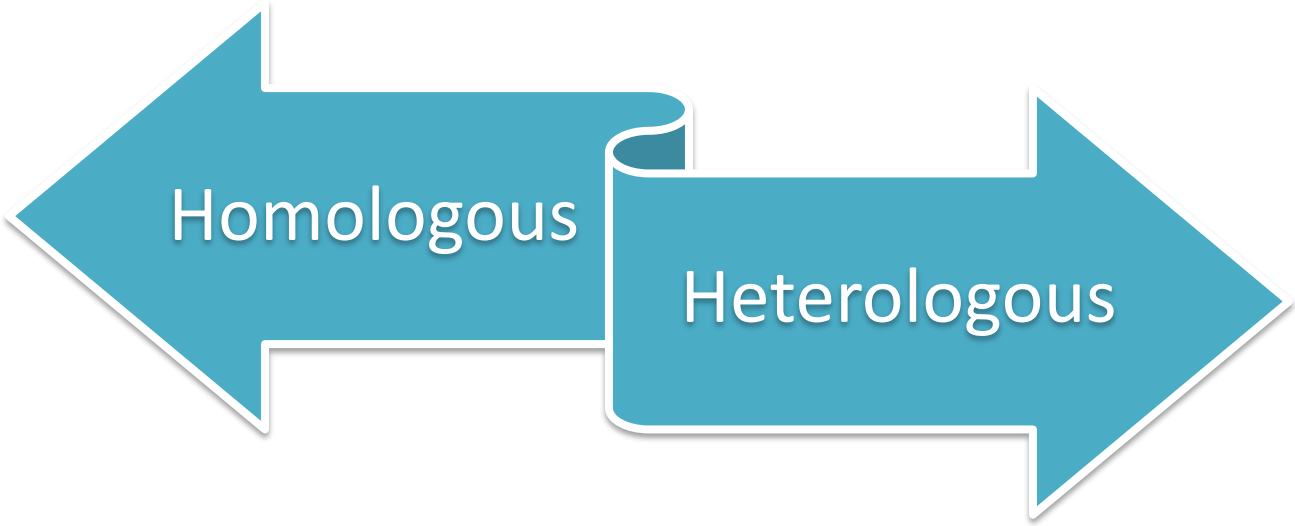
- Aims
  - At the end of this chapter we hope that students able to explain the principles of enzyme production and can make comparisons between the production systems including homologous and heterologous expression systems. In addition we also hope that students can differentiate between the intracellular and extracellular expression types of enzymes.

- Expected Outcomes
  - To explain why it is more efficient to use heterologous enzymes than homologous enzymes.
  - To compare the heterologous systems used for enzyme production.
  - To compare intracellular and extracellular expression of enzymes

OUTLINE

- Introduction to enzyme production
- Homologous versus heterologous production of enzymes
- Applicable heterologous expression systems for enzymes production
- Intracellular and extracellular expression of enzymes

Types of enzymes production



HOMOLOGOUS PRODUCTION

- The production in a system from where it originates or productions in their native host.
- Simple and guarantees the authenticity of target proteins.
- Usually challenge by the low production yield and the time consuming.

Study case

- A homologous expression of a−glucosidase from *Leucosporidium antarcticum* in submerged culture at 5°C reaches approximately 1.5 mg of enzymes per liter of culture medium and corresponds to 0.2 nanounits activity per single yeast cell after 12 to 14 days submerged cultures of the strain.

  Source: Turkiewicz et al., 2005. Polish Polar Research. 26(2):125-136

  - Low yields of production
  - Time consuming (nature of comologous - antarctic microorganism)

HETEROLOGOUS PRODUCTION

Recombinant DNA technology

- provided an **alternate route in enzymes production**:
  - basic research
  - industrial applications
- enable **exploitation of the biotechnological applications of enzymes**.
- as the **simplest genetic approach.**
- promise unrestricted source of uncommon, high-value proteins that are not reachable using **conventional protein** isolation techniques.
- Enable **high yield of soluble and correctly folded target enzymes**.

HETEROLOGOUS PRODUCTION

- The enzyme of interest was produced or expressed in either a different species or cell type.

- Generally used for the production of proteins which can be quite difficult to be produced in its native host (eg: low production, time-consuming, specific requirement).

Study case

- A heterologous expression of α−glucosidase from *Leucosporidium antarcticum* in *E. coli* expression system at 25°C reaches approximately 13 mg of enzymes per liter of culture medium after 2 days.
  - High yield
  - Short time

Example of enzymes which have been produced in bulk quantities and their industrial applications.

Enzymes	Industrial applications
Protease	(i) Inclusion in detergent preparations (ii) Cheese making (iii) Brewing/backing industries (iv) Meat/leather industries (v) Animal/human digestive aids
Amylase	(i) Starch processing industry (ii) Fermentation industry
Cellulases/ hemicellulases	(i) Brewing industry (ii) Fruit juice production (iii) Animal feed industry
Pectinases	(i) Fruit juice processing industry
Lipases	(i) Dairy industry (ii) Vegetable oil industry (iii) Leather industry
Glucose isomerase	(i) Production of high-fructose syrups
Lactase	(i) Hydrolysis of milk lactose (ii) Digestive aid
Cyclodextrin glycosyltransferase	(i) Production of cyclodextrins for pharmaceuticals and other industries
Penicillin acylase	(i) Production of synthetic penicillin
Carbohydratases	(i) Baking, brewing, confectionary, and fruit industries

TYPES OF EXPRESSION SYSTEM FOR ENZYMES PRODUCTION

- Recombinant enzymes technology has been **employed in various prokaryotic and eukaryotic organisms** to yield important recombinant products for both academic and industrial field.

- This technology was **lead to a variety of available effective expression systems** which enable researchers to achieve their specific objectives
  - **from high level expression** of recombinant products for large scale production **to subtle expression** for studying protein function in the cell.

TYPES OF EXPRESSION SYSTEM FOR ENZYMES PRODUCTION

- There are **four most commonly** available expression systems for heterologous production of desired recombinant proteins which are ***E. coli*, yeast, mammalian cells and insect cells**.

- When choosing an expression system for desired enzyme production in a heterologous host, several factors should be taken into consideration.
  - **Different expression systems have different characteristics** which may have a large influence on the expressed protein or the use thereof.

Summary of the most commonly available expression systems.

Expression system	Dis-advantages	Advantages	Cell growth	Complexity of growth medium	Expression level	Scale-up potential	Cost-effectiveness
Prokaryotic							
E. Coli	No post-translational modifications	Easy to manipulate	Rapid (30 min)	minimum	high	Very good	Low to moderate
Eukaryotic							
Yeast	Glycosylation profile	Biomass	Rapid (90 min)	minimum	Low – high	Good	Low to moderate
Mammalian cells	Additives	Suitable for complex molecules	slow (24 h)	High	Low - moderate	Good	High to very high
Insect cells	Glycosylation profile	Viral safety	slow (18-24 h)	High	Low – high	Good	High

PRODUCTION IN BACTERIA SYSTEM, *E. Coli*

- **extensively used for heterologous enzyme production due to the following benefits:**
  1. **Simple growth and handling** with simple laboratory equipment;
  2. **Accessibility of a number of vectors and host strains** that have been designed for optimum expression;
  3. **A lot of information regarding** the genetics and physiology of *E. coli*;
  4. **Rapid production can be achieved** Usually less than 2 weeks
  5. **Appropriate fermentation technology** well recognized;
  6. **Capable to produce large scale** of target recombinant protein;
  7. **Low cost required**.

SUCCESSFUL PRODUCTION OF ENZYMES IN *E. Coli*

Enzyme	Source	Reference
β-galactosidase	*Kluverymyces lactis*	Dickson and Markin., 2014
Cellulase and Polygalacturonase	*Pectobacterium carotovorum*	Ibrahim et al., 2013
Amylase	*Pseudoalteromonas sp.*	Tao et al., 2008
L-Asparaginase II	*E. coli MTCC 739*	Vidya et al., 2011
Cellobiohydrolase	*Lentinula edodes*	Taaipakoza et al., 2011

DISADVANTAGES OF USING *E. coli* AS HETEROLOGOUS EXPRESSION SYSTEM

1. Limited ability to **perform post-translational modification** which is common in eukaryotic organism;

2. Incapability to **accomplish wide broad formation of disulfide bond**;

3. Tendency for **insoluble protein formation**;

4. Lead to **protein degradation or insufficient translation**;

5. "**codon bias**" phenomenon- different codon sequence formation in coding DNA.

PRODUCTION IN YEAST SYSTEM, *Pichia pastoris*

- Next to *E. coli*, the methylotrophic yeast ***P. pastoris*** **become the second most popular expression systems** used for the production of enzymes.

- Although a number of expression systems using mammalian cells, insect cells and other bacteria as host have been developed, yeast has received attention as a suitable host for expression of many heterologous proteins due to **many specific characteristics**.

PRODUCTION IN YEAST SYSTEM, *Pichia pastoris*

***P. pastoris* have many advantages over other systems and may be the host of choice for the expression of enzymes because of:**

1. Its ability to **express high level** of heterologous proteins;
2. Can be produced in **high cell densities**;
3. Having an **efficient secretory system**;
4. can **easily be cultured**;
5. comprises a number of tools for **molecular manipulation or genetically manipulated**.

SUCCESSFUL PRODUCTION OF ENZYMES IN *P. pastoris*

Enzyme	Source	Reference
Beta-mannanase, cellobiohydrolase	*Trichoderma reesei*	Mellitzer et al., 2012
Xylanase	*Thermomyces lanuginosus*	Mellitzer et al., 2012
Acetylcholinesterase	*Platichthys flesus*	Sato et al., 2008
Phytase	*Bacillus subtilis*	Guerrero-Olazarán et al., 2010

LIMITATIONS USING *P. pastoris* AS HETEROGENEOUS PROTEIN EXPRESSION HOST

- Usage of **methanol** as inducer is a **safety (fire) hazard** at scale

- Glycosylation still **different to mammalian** cells

INTRACELLULAR versus EXTRACELLULAR ENZYMES PRODUCTION

- **Enzymes can be produced**
  - **intracellular or**
  - **extracellular.**

- **the benefit of extracellular production?**

  - **Target enzyme already secreted outside cell**

  - **Number of secreted protein is limited so easier for isolation**

  - **More robust enzyme thus reduce the tendency to be broken down by heat of chemicals**

- **Limitation of intracellular enzymes compared to extracellular ones**

  - **Enzyme of interest is inside the cell thus the cell has to be broken open followed by the enzyme separation from the mixture of all the cellular contents**

- **Advantages of isolated enzymes than whole cells.**

  - **Isolated enzymes are usually more efficient in biotechnology than whole cells because enzyme concentration is higher and no unwanted enzymes are present**

Thank you