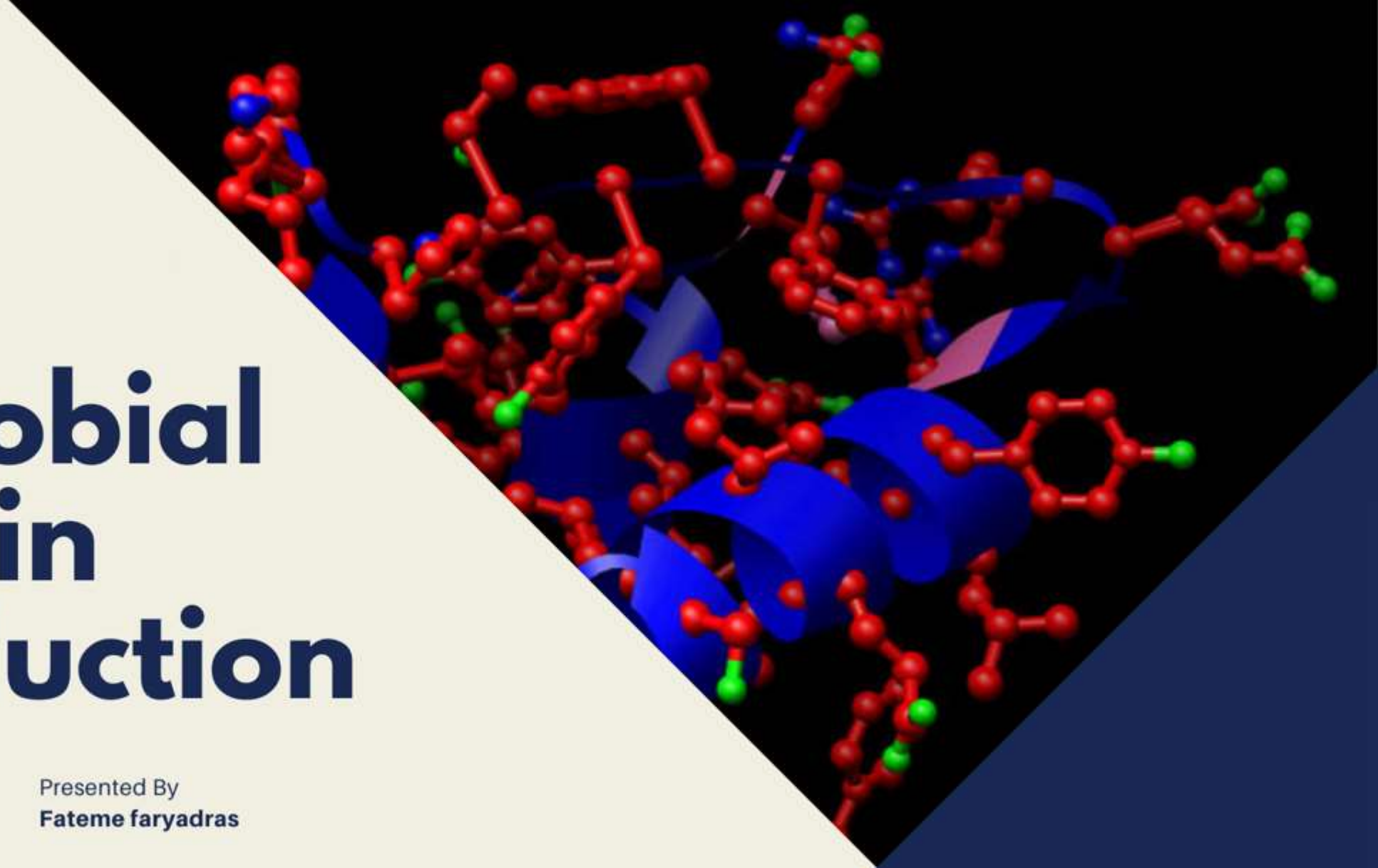
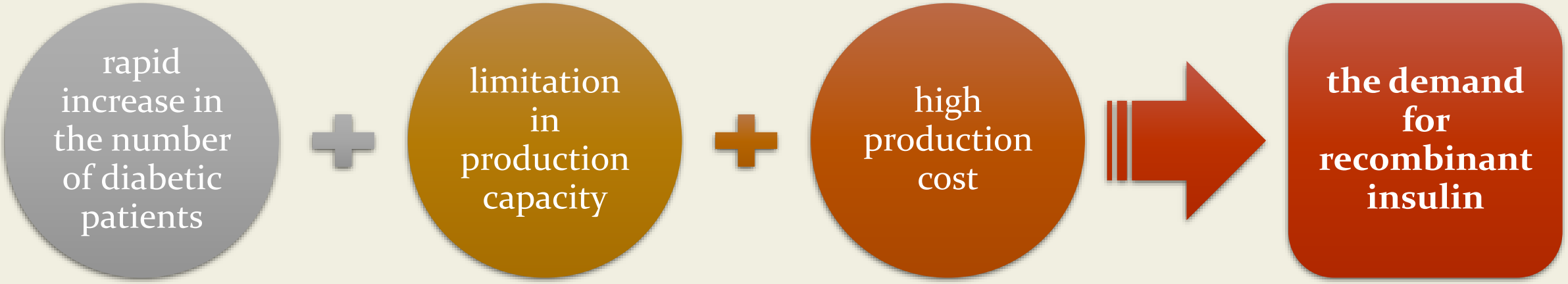


Microbial Insulin Production

Presented By
Fateme faryadras

contents

Introduction



Recombinant human insulin has been produced predominantly using *E. coli* and *Saccharomyces cerevisiae* for therapeutic use in human.

History

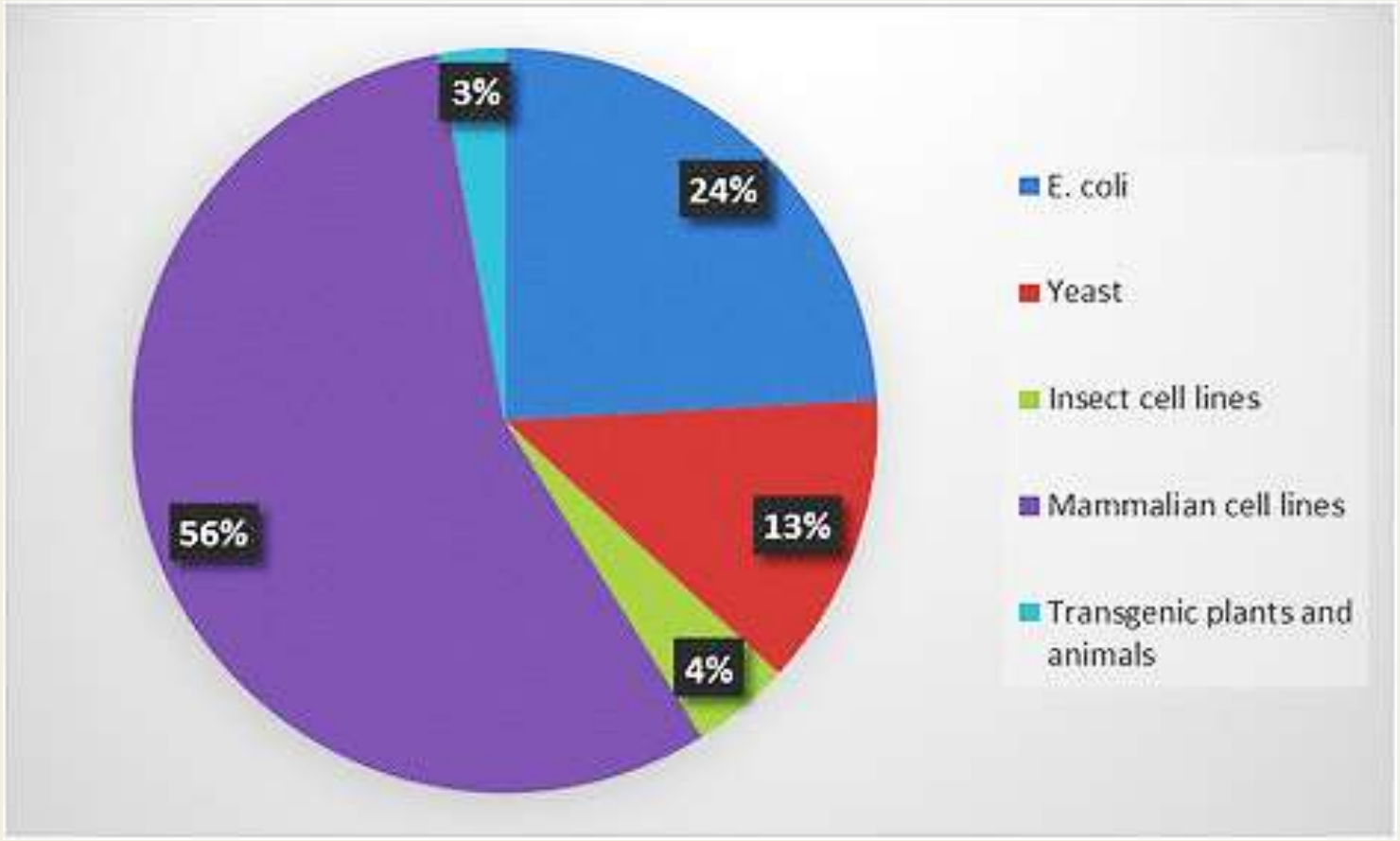
Stanley N. Cohen , who received the Nobel Prize in Medicine in 1986 for his work on discoveries of growth factors.

Stanley N. Cohen (1935–) (top) and Herbert Boyer (1936–) (bottom), who constructed the first recombinant DNA using bacterial DNA and plasmids.

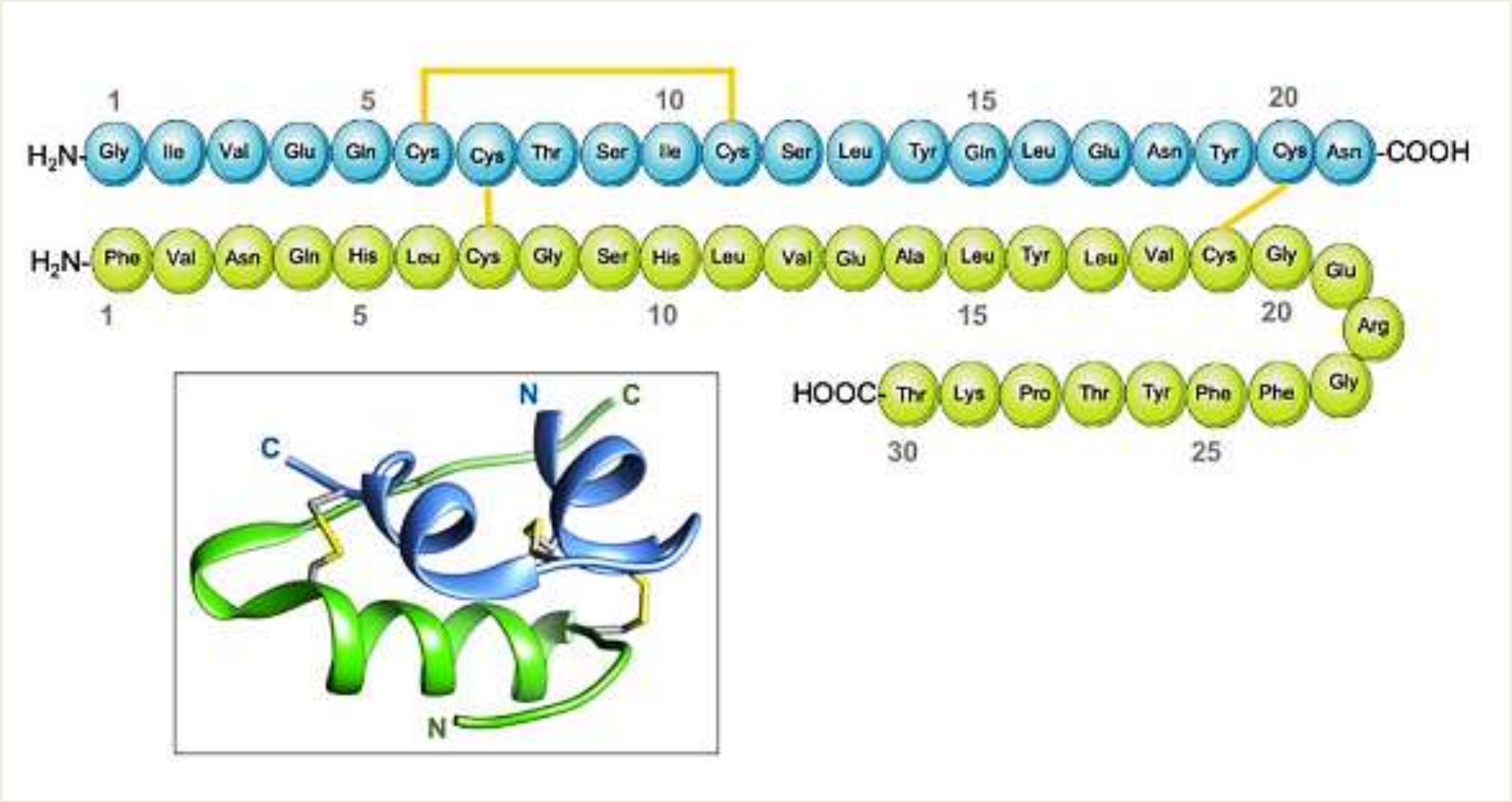
Genentech

Biopharmaceutical products

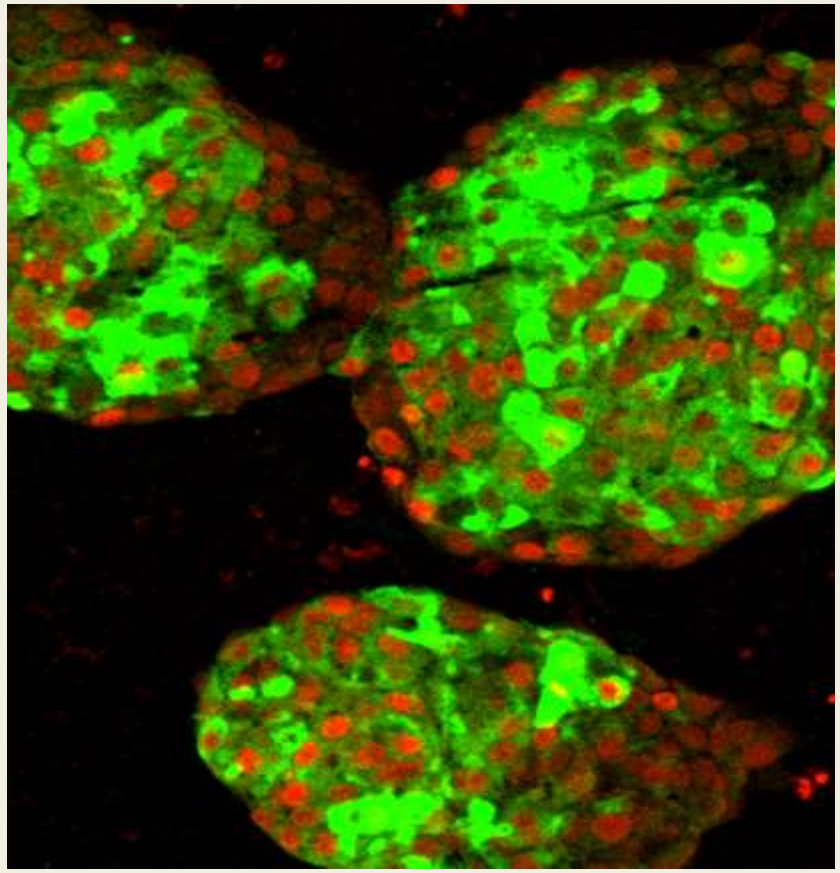
- Therapeutic monoclonal antibodies
- the hormones
- growth factors

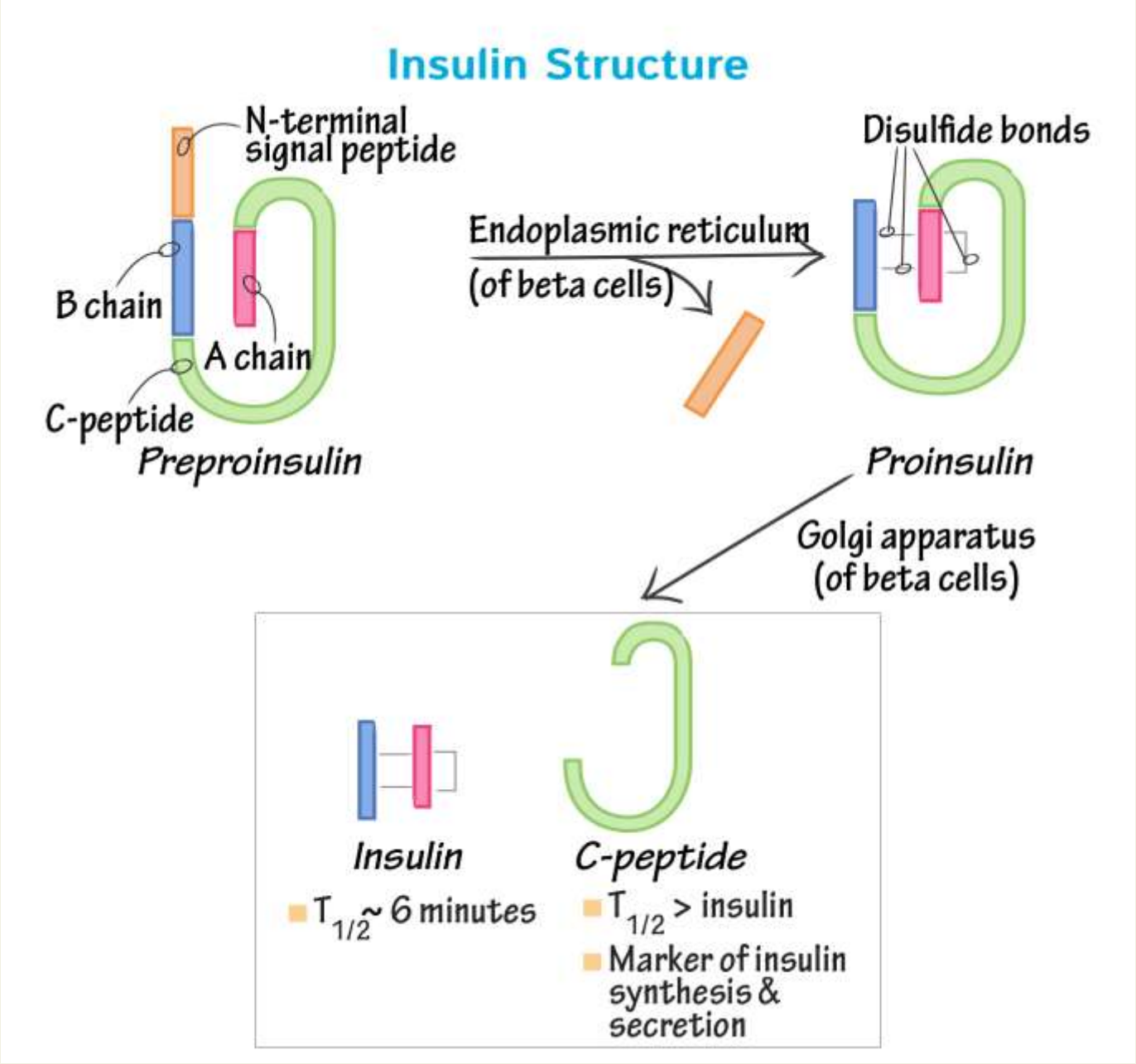


Structure and function of insulin

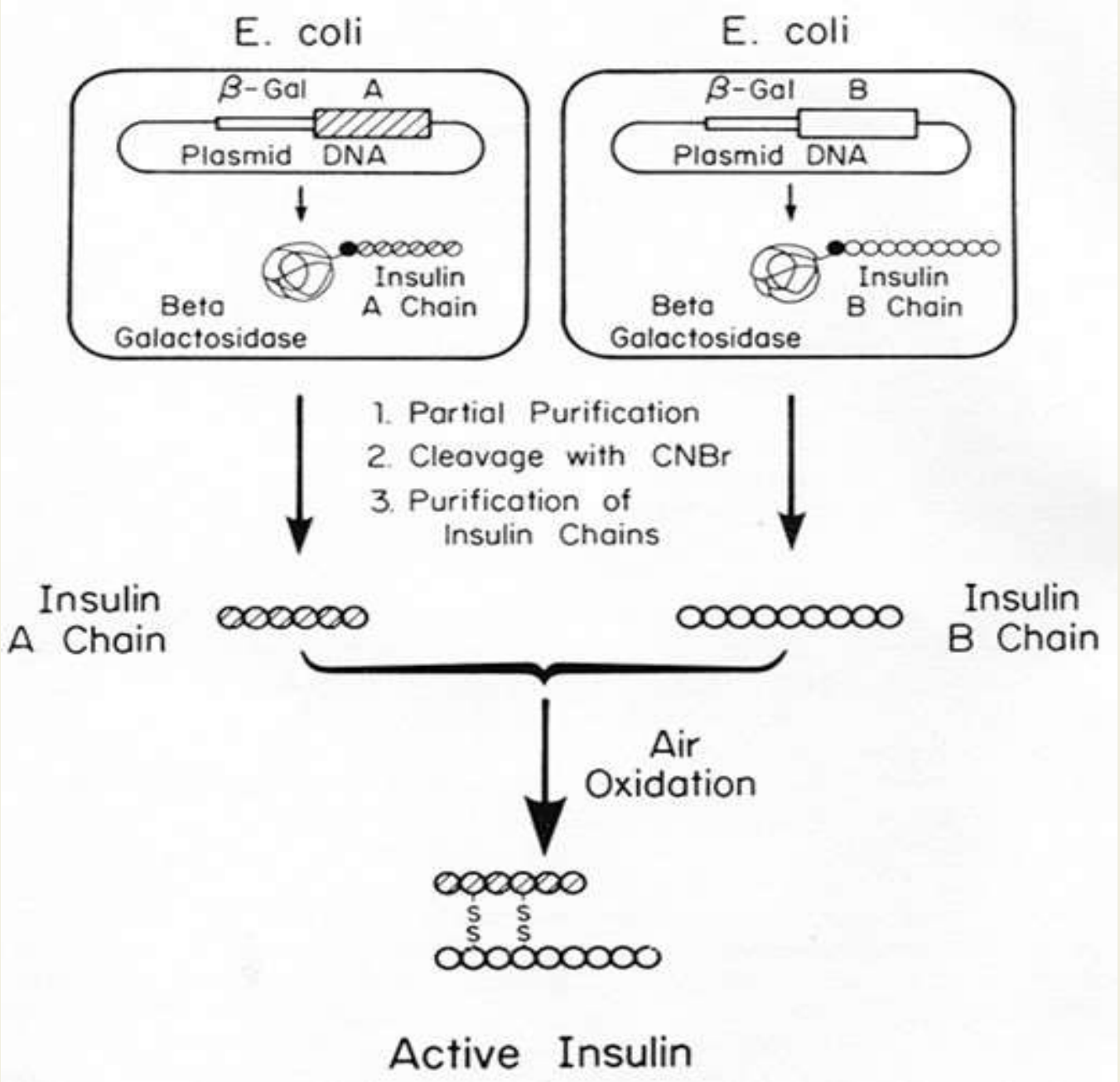


Insulin synthesis



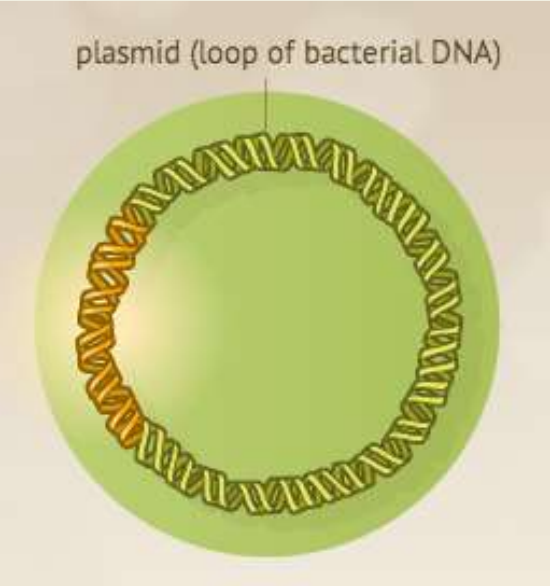


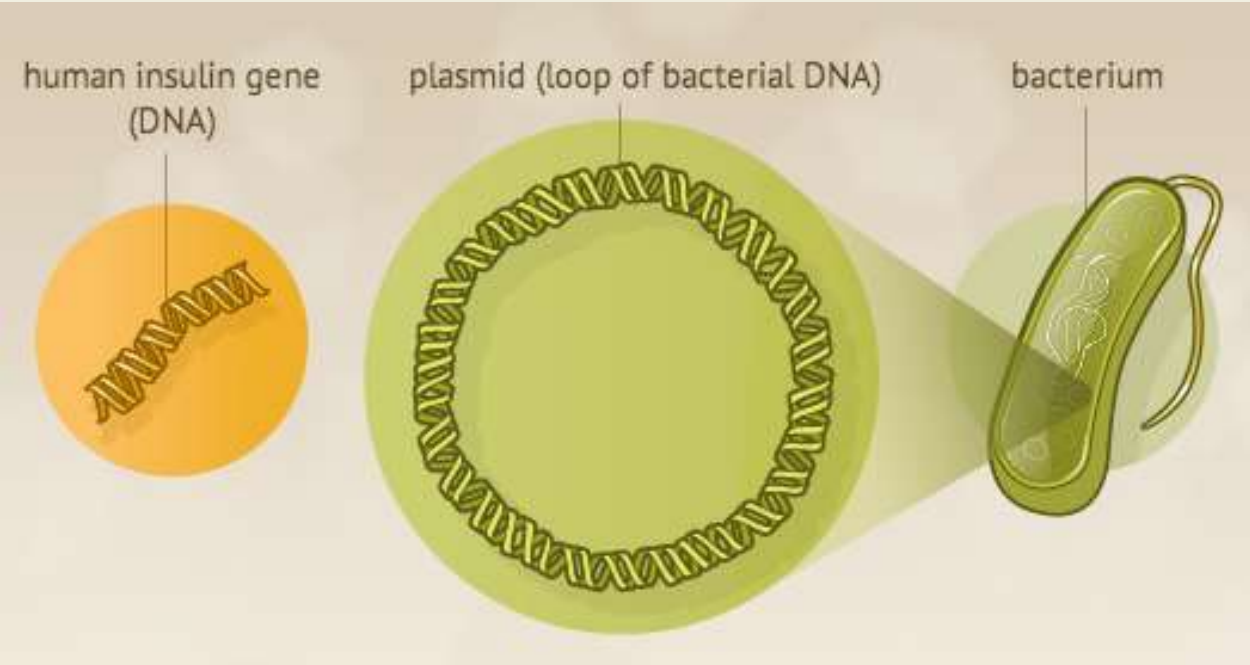
Insulin production by Recombinant DNA technology: Two-chain method

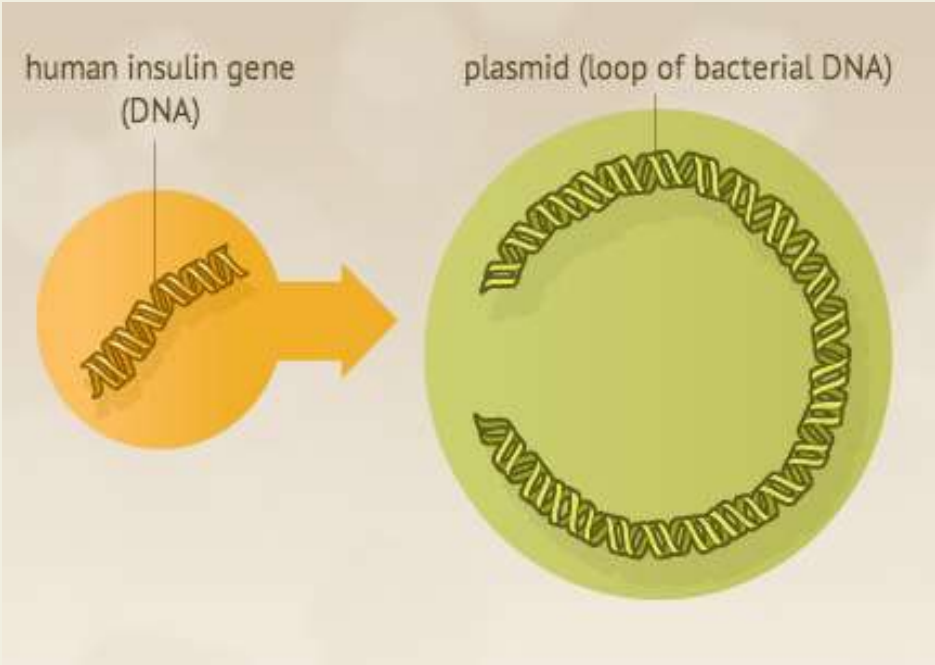


Bio-production Steps

1. Gene Isolation
2. Insertion into the Plasmid

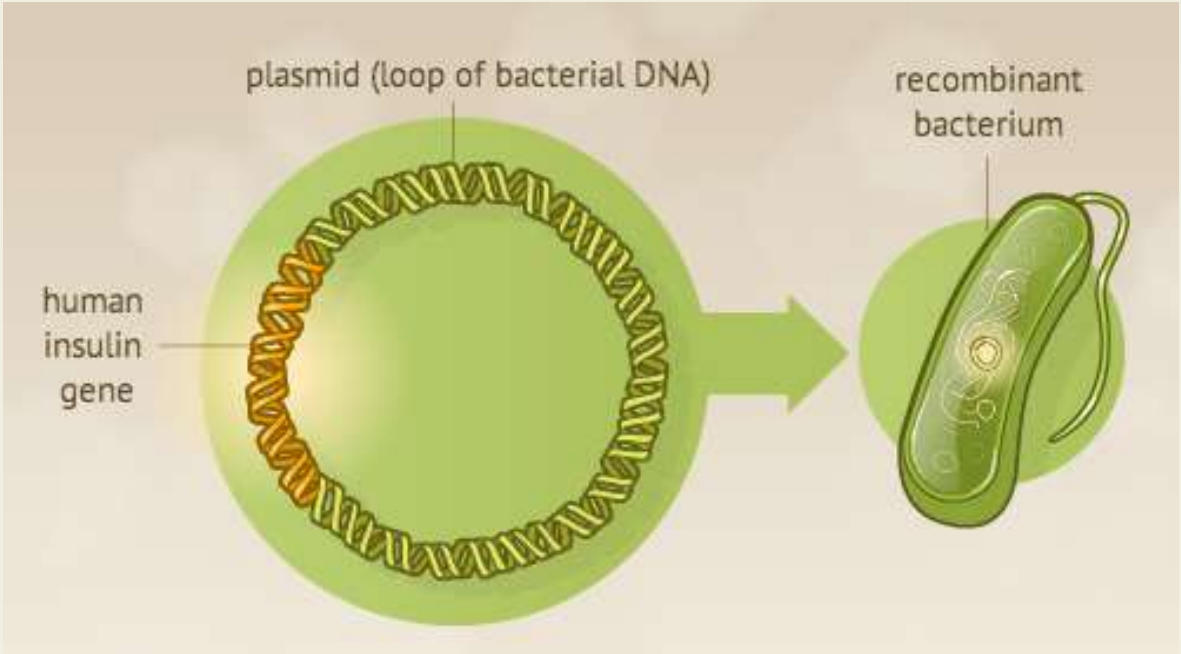






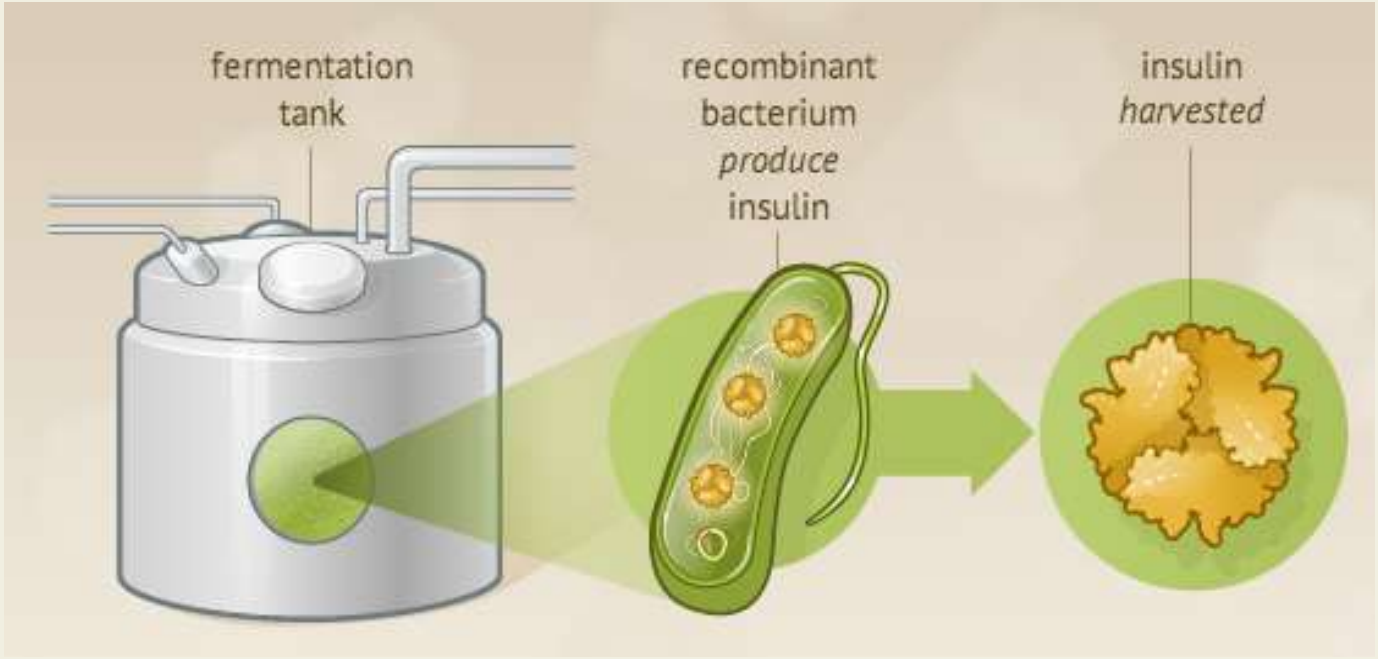
Bio-production Steps

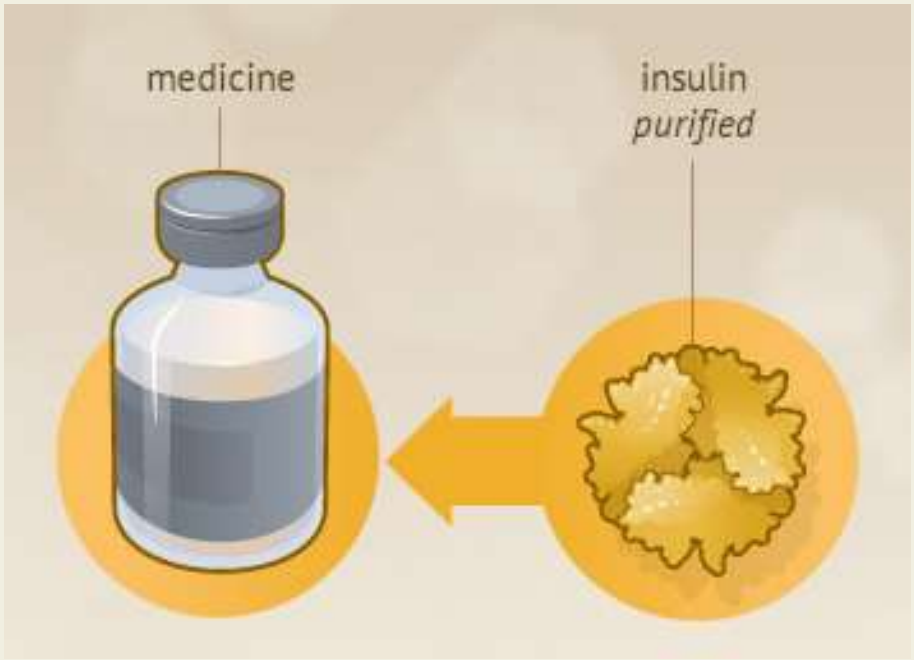
3. Transfection
4. Medium Preparation
5. Bioreactor Fermentation



Bio-production Steps

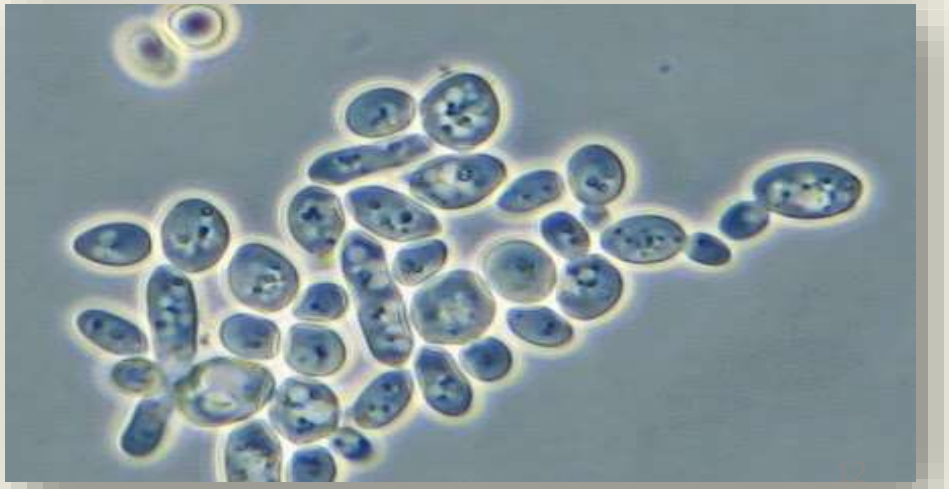
6. Crude Product Isolation
7. Purification
9. Chain Joining





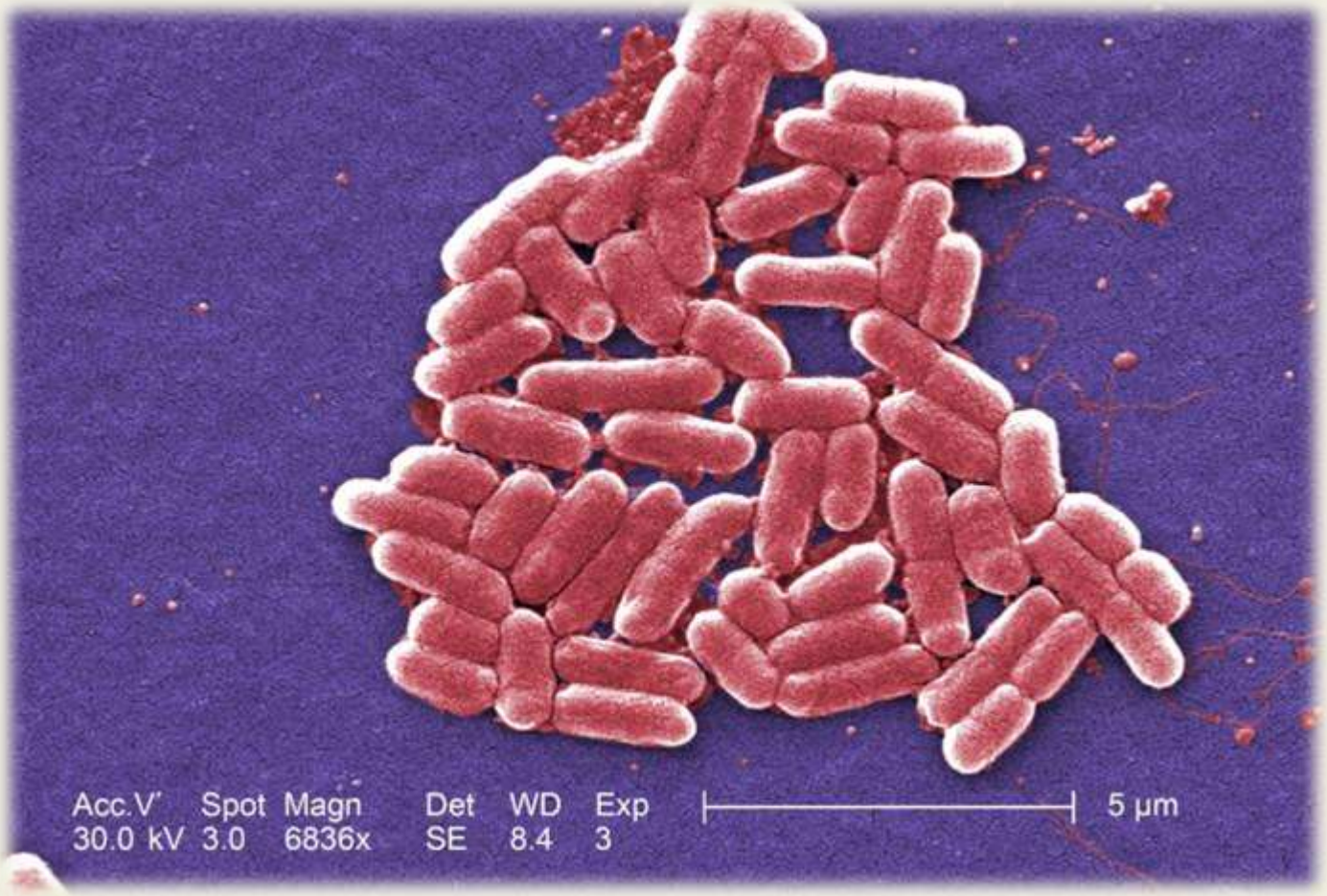
Different Expression Systems of insulin

- *E. coli*
- Yeast:
  - *Saccharomyces cerevisiae*
  - *Pichia pastoris*
- Transgenic Plants:
  - *Arabidopsis thaliana*
  - Tobacco and Lettuce Plants
  - Strawberry Plant



E. coli

- the first expression system used to produce human insulin in 1978
- Advantages
- Disadvantages
- new technologies to solve problems



Yeast

- another commercially-used expression system for heterogeneous protein production with recombinant technology because of its PTMs.
- Advantages
- Disadvantage:

proteins synthesized in yeast have high-mannose N-glycosylation

activates an immune response in humans

shortens the half-life of recombinant proteins *in vivo*

Yeast: *Saccharomyces cerevisiae*

- Produce:
  - Proinsulin
  - Fast-acting insulin
  - Long-acting insulin

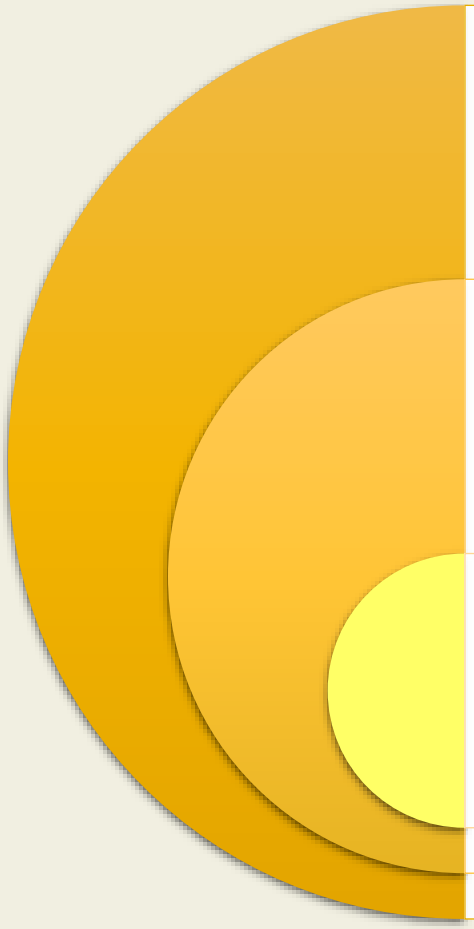
Yeast: *Pichia pastoris*

- presence of the methanol-inducible alcohol oxidase-1 promoter (AOX-1)
- Heterogeneous protein expression attained by *P. pastoris* is approximately 30% of its total cell protein

Transgenic Plants

- Plants do not have human pathogens and because they are eukaryotic, their PTM machinery is more similar to humans than that previously described in yeast.
- *Arabidopsis thaliana*
- Tobacco and Lettuce Plants
- Strawberry Plant

Conclusion



Dietary and lifestyle changes are causing dramatic increase in diabetes incidence all over the world.

Both Type I and Type II diabetic patients use insulin, however late stage Type II diabetes patients require large doses of insulin as they develop insulin resistance.

Current manufacturing technologies will not be able to meet the growing demand of insulin due to limitation in production capacity and high production cost.

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