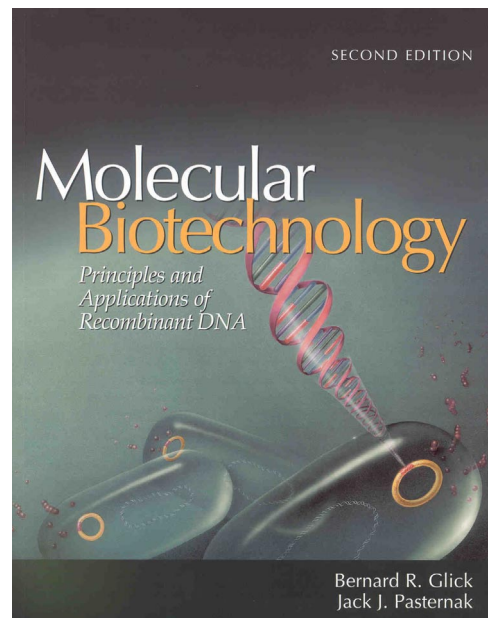


Molecular Biotechnology
Uses of Genetically-Engineered Microorganisms

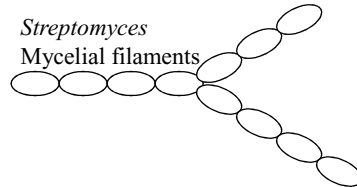
David R. Shonnard
CM4710 Biochemical Processes
December 13, 2000



Improving Antibiotic Production

Problem Statement:

- *Streptomyces* spp. are the predominant microorganisms
- Large-scale production of antibiotics is often limited by O₂ availability

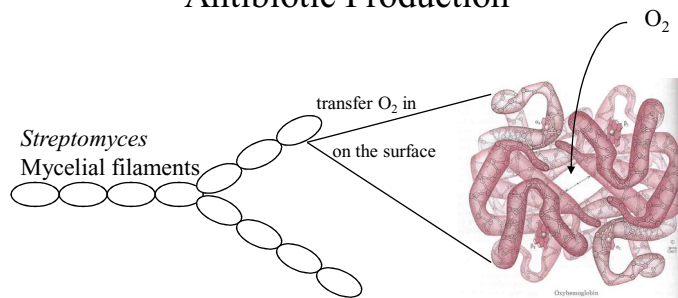


Highly dense culture
limits O₂ transfer

Problem Solution Strategy:

- Make *Streptomyces* spp. synthesize a hemoglobin-like molecule to sequester O₂
- *Vitreoscilla* sp. produces a heme protein similar to eukaryotic hemoglobin
- The gene for *Vitreoscilla* hemoglobin was isolated and cloned into *Streptomyces coelicolor*

Results from Recombinant DNA Antibiotic Production



Results:

- Transformed cells produced 10 times more antibiotic at low O₂ levels than the untransformed cells

Vitreoscilla
hemoglobin
expressed in
Streptomyces

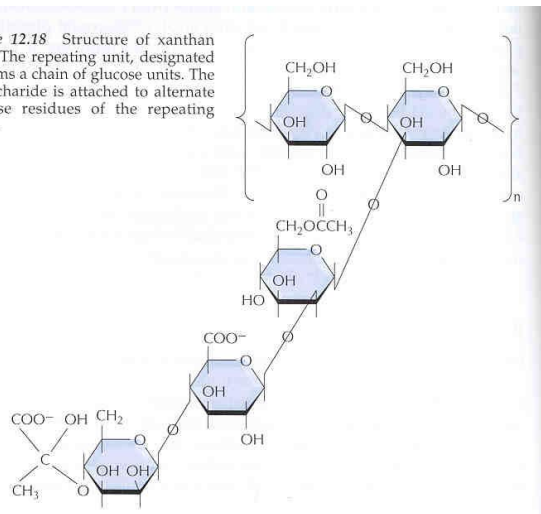
Engineering *Xanthomonas camperstris* for Inexpensive Xanthan Gum Production

Xanthan Gum:

- a high molecular exopolysaccharide byproduct of metabolism
- properties similar to a plastic
- used as a stabilizing agent, emulsifying agent, thickening agent
- wild type *Xanthomonas camperstris* can utilize glucose, sucrose, and starch, but these are too expensive
- Whey is a waste byproduct of cheese-making consisting of
 - 3 - 4% lactose
 - 94 - 95% water
 - small amounts of protein, minerals, and low molecular weight organic compounds
- Disposal of whey is expensive and causes oxygen depletion in rivers

Structure of Xanthan Gum

Figure 12.18 Structure of xanthan gum. The repeating unit, designated n , forms a chain of glucose units. The trisaccharide is attached to alternate glucose residues of the repeating chain.



Genetic Engineering of *Xanthomonas campestris* using the *E. coli lacZY* genes

- *E. coli lacZY* genes encode for the enzymes β -galactosidase and lastose permease
- *E. coli lacZY* genes were cloned into a plasmid
- DNA construct (plasmid) was introduced into *E. coli*
- Plasmid (DNA construct) was transferred from *E. coli* to *X. camp.*
- β -galactosidase and lastose permease were expressed in high levels within the transformed cells

Results from Genetic Engineering of *Xanthomonas campestris* using the *E. coli lacZY* genes

Table 12.4 Production of xanthan gum by wild-type and transformed *X. campestris*

<i>X. campestris</i>	Amount of xanthan gum produced ($\mu\text{g/mL}$) with:		
	0.4% Glucose	0.4% Lactose	10% Whey
Wild type	3,530	245	224
Transformant	3,711	3,608	4,241

Adapted from Fu and Tseng, *Appl. Environ. Microbiol.* 56:919–923, 1990.

The amount of product is expressed as micrograms per milliliter of culture grown on a minimal medium with either 0.4% glucose or 0.4% lactose added or on diluted whey (10%) which contains approximately 0.4% lactose. The transformant carries the *E. coli lacZY* genes on a plasmid.