

University of Guelph

Department of Chemistry and Biochemistry
Chem*4570 Applied Biochemistry

Midterm examination, October 26 1999

Answer FIVE of the questions 1-7 (7 marks each, total 35 marks) Time allowed, 2 hours

1.
 - a) Investigators looking for the genes for erythromycin **synthesis** first isolated the erythromycin **resistance** gene *ermE*. Why was this considered a good method to locate the synthesis genes?
 - b) The genomic DNA of *S. erythraea*, about 4×10^6 was cut by **limited** digestion with the enzyme BamHI. What were the benefits of incomplete cleavage of the DNA ?
 - c) The DNA fragments were cloned in a **cosmid**. How does a **cosmid** differ from an ordinary **plasmid**? Why is this an advantage?

2.
 - a) What two **mutational defects** result in *C. glutamicum* overproducing glutamate?
 - b) What **additional enzymes or pathways** must be **active** to sustain glutamate production?

3.
 - a) A bacterial strain is described as a **homoserine auxotroph**. What does this term mean?
 - b) Explain the method for selecting an auxotrophic strain.
 - c) Why is the biotin⁻ auxotroph useful in strains that overproduce amino acids?

4.
 - a) *Clostridium* species contain a **pyruvate dehydrogenase** that is coupled to **hydrogenase**. What are the products of this enzyme?
 - b) Show the pathway leading from **pyruvate to butanol**. How is NAD^+/NADH balance maintained? How does this lead to generation of extra ATP?

5.
 - a) What are roles of the components of **pUC18** labelled in the diagram?
 - b) *E. coli* strain 13002 is **defective** in the enzyme **DAP decarboxylase**. How is this used to obtain lysine overproduction?
 - c) If you insert a **functional** copy of DAP decarboxylase at the polylinker of pUC18, the gene can be **expressed** only when it is induced by isopropylthiogalactoside. How could this be exploited to create a more effective lysine overproducer in *E. coli* ?



