

Biological Transformation

to help with writing the lab report, read p. 392-400 in text

transformation is taking in and expressing free DNA

- some organisms can do this naturally

 - some, not all, bacteria can do this

 - bacteria have surface proteins that can bind to free DNA in the environment and transport it inside themselves

 - once inside, enzymes read the new DNA & compare it to their own DNA

 - if it is similar enough, the new DNA can be substituted

 - this is recombination

 - why would an organism do this? may help survival

 - may help organism evade the immune system of a host

 - think about the rough & smooth strains of pneumococcus

artificial transformation

- tinkering with cells to allow them to take-up DNA in environment

 - is called competent

- to make cells competent, must change the permeability of the cell membrane

competent

can change permeability by heating cells in presence of Ca^+ (positive ions)

cells do not want to take-up DNA that is not similar (not same species)

we get around this problem by using plasmids

plasmids are small, circular pieces of DNA that contain an origin of replication

b/c there are origins of replication (sequence of bases that signal start), the host cell will copy the DNA

plasmids occur naturally in bacteria and yeast

in order to transform bacteria, we must overcome 2 problems

1-there has to be an advantage to the host cell to take-up the DNA

copying the plasmid DNA takes E, when cells are grown in a mixed culture, some containing plasmids, some not, those without plasmids grow faster

2-we have to be able to tell who took up the DNA, we need a marker

in a typical transformation exp, only 1 in 1000 bacteria take up the plasmid DNA

when we grow bacteria on normal agar----->should see lots of colonies
a lawn of bacteria

when we grow bact on agar w/ampicillin (antibiotic that kills bacteria)
should see no growth

if we give bacteria a plasmid with a gene for ampicillin resistance, and
the bacteria took up the plasmid, we should see growth on plate

some plasmids contain a second gene that has a marker

we used beta-galactosidase, a gene that makes a protein that splits
a disaccharide into 2 monosaccharides

also splits a substance called X-gal, when it does this it makes a blue
color

if we grow bact that make B-galactosidase on X-gal, colonies will be blue

if bact do not make B-galact, colonies will be white

the color marker was used to see if new plasmid DNA was incorporated
into bacteria, plasmid grown on X-gal shows bact that are blue