Area: Microbial Genetics

Microorganism: *Vibrio vulnificus*

Reference: McDougald, D, Simpson, M. L., Oliver J. D., and Hudson M. C. 1994. Transformation of *Vibrio vulnificus* by electroporation. Current Microbiology 28: 289-291.

*Vibrio vulnificus* is a Gram-negative pathogenic bacterium found in the ocean. Most cases of *V. vulnificus* infection result from consumption of raw oysters or severe wounds that have been exposed to sea water. The bacteria multiply outside of the gastrointestinal tract and produce several extracellular protein toxins. *V. vulnificus* produces compounds that bind iron and can grow by fermentation of lactose. To study the genetics of this microorganism in more detail, McDougald et al. (Current Microbiol. 28: 289-291, 1994) attempted to introduce plasmid DNA into the bacteria by transformation.

1. Based on the information given, *V. vulnificus* exerts its primary pathogenic effects through the formation of:

a. endotoxins.

b. exotoxins.

c. proteolytic enzymes.

d. hypersensitivity reactions.

2. From the information given, it appears that *V. vulnificus* can grow:

a. only anaerobically.

b. only aerobically.

c. both aerobically and anaerobically.

d. photosynthetically.

To test for transformation, McDougald et al. first extracted plasmid DNAs carrying genes for resistance to tetracycline or kanamycin from several strains of *E. coli*. When the plasmid DNAs were mixed with *V. vulnificus* and the bacteria spread on agar plates containing tetracycline or kanamycin, no antibiotic resistant colonies were found. McDougald et al. then tried a technique called electroporation, in which the plasmid DNA/bacteria mixture was exposed to a series of high-voltage electrical shocks before plating. In this case, antibiotic-resistant colonies were recovered upon plating.

3. These results indicate that *V. vulnificus* is susceptible to:

a. conjugation

b. both natural transformation and artificial

c. only natural transformation

d. only artificial transformation

McDougald et al. then determined the number of antibiotic- resistant transformants as a function of DNA concentration for two strains of *V. vulnificus*. The results are shown below.

Table

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4. These results suggest that:

a. the efficiency of transformation is not affected by the DNA concentration.

b. the efficiency of transformation decreases with DNA concentration.

c. the efficiency of transformation increases equally with DNA concentration for both strains.

d. the efficiency of transformation increases with DNA concentration for both strains but reaches saturation sooner with MO6.

To determine if the efficiency of transformation is affected by the composition of the growth medium, McDougald et al. compared the efficiency of transformation after growth in a rich medium (HI) or in a minimal medium (BSM) supplemented with glucose and glycine betaine, an osmotically compatible solute. The results are shown in the following table.

Diagram

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5. These results suggest that transformation is most efficient in the presence of:

a. BSM medium.

b. BSM medium + glucose.

c. BSM medium + glucose + glycine betaine

d. BSM medium + glycine betaine

McDougald et al. found that *V. vulnificus* has two distinct colony types depending on the amount of extracellular polysaccharide formed: opaque colonies, which have a lot of polysaccharides, and translucent colonies, which have little polysaccharide. They then made two observations: 1) addition of glucose to the BSM medium caused the bacteria to form translucent colonies, and 2) translucent colonies were transformed much more efficiently than opaque colonies.

6. These observations suggest that:

a. extracellular capsules promote DNA uptake and transformation.

b. extracellular capsules have no effect on DNA uptake and transformation.

c. extracellular capsules inhibit DNA uptake and transformation.

d. extracellular capsules make the bacteria resistant to antibiotics.

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