

八十八學年度 生命科學系 生物技術所 0704 系(所) 甲 組碩士班研究生招生考試
 目 微生物學 科號 1004 共 3 頁第 1 頁 *請在試卷【答案卷】內作答

- I. Suppose the following instructions were given to you by your advisor. Explain the underlined terms briefly (40%).

This recombinant *E. coli* strain contains a non-structural protein (a) gene of a flavivirus (b). Grow it in one liter of Luria Broth together with 50 mg of ampicillin (c). Grow the cells until OD₆₀₀ (d) reaches 0.6, add a little bit IPTG (e), and continue growing the cells for another hour. You can collect the cells using the Beckman centrifuge, resuspend the pellet with 50 ml of binding buffer, and proceed to sonication (f). Take a small aliquot of the lysate for SDS-PAGE (g) and use the rest for chromatography on an affinity column to purify the His-Tag fusion protein (h). As soon as the protein is ready, mix 200 µg of the purified protein with 100 µl of adjuvant (i) and inject a Balb/c mouse once a week by the intraperitoneal (j) route. After three injections, sacrifice the immunized mouse, fused the splenocytes with myeloma cells, and select hybridomas with the HAT medium (k). The presence of the right monoclonal antibodies should be verified by western blot (l) analysis. If you were lucky enough to get a couple of clones, I would suggest that you test the neutralizing activity (m) of the antibody against the virus. You may also test whether they can be used in immunofluorescence assay (n). If you do not know how to do the experiments, talk to Professor May-Ling Chang. She is an expert on developing adenovirus-based gene therapy vector (o) and DNA vaccines (p). What! We don't have any ampicillin left? Try Professor Yu-Chyl Lai's lab. They use transposon (q) to study bacteria so they must have some. OK. Get these wastes to autoclave (r) and go home to prepare your journal meeting presentation. Yes, the relationship between perforin (s) of cytotoxic T cells and viral infections is a very good topic. Otherwise, find a paper deals with dendritic cells. They are very potent APC (t). By the way, you'd better prepare to stay in lab all weekend. I want the preliminary results by next Monday.

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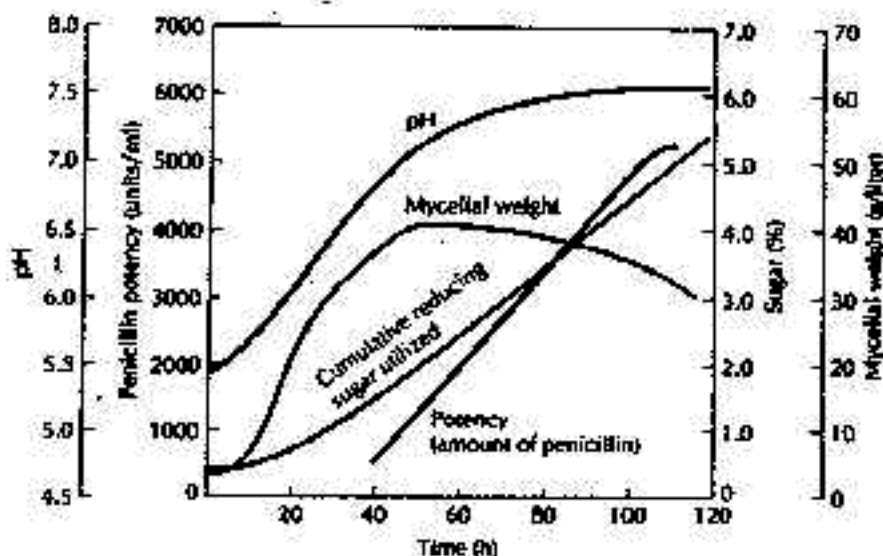
(Choose any **SIX** questions from II to VIII) 每題 10 分，問答題部份共 60 分。

II. Professor Shu-Li Young has recently discovered a new gene in a pathogenic bacterium of humans (or tomato). Please design an experimental strategy to help her find out whether the gene plays a role in the virulence of the bacterium. (10%)

III. When infected into the host cell, the genome of some viruses (such as lambda phage and certain animal tumor viruses) will integrate into the host chromosome. Dr. Hsin-Wen Fan suspected that her newly-discovered virus may do the similar thing. What experiments do you think she should perform to prove it? (10%)

IV. Outline an experimental procedure (including the use of the enrichment culture technique) design to isolate a microorganism that can decompose a chemical insecticide, which we will call compound X. If you have isolated such a bacterial strain, what methods will you use to identify the species of the microorganism? How are you going to improve the compound X degradation capability of the microorganism? (10%)

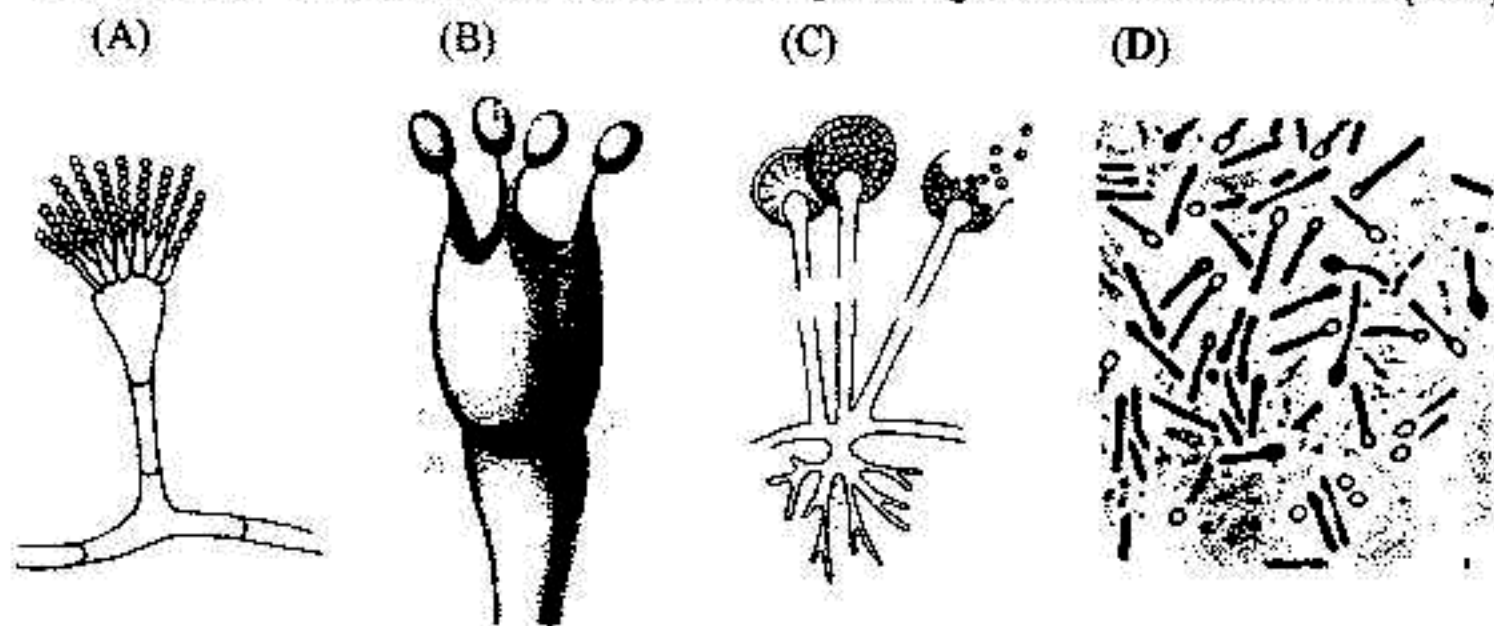
V. This figure shows biochemical changes that occur in the fermenter during production of penicillin by *Penicillium chrysogenum*. At the end of this experiment, how much sugar was consumed (a), and how many units of penicillin were produced (b)? How much of the sugar consumed was used to synthesize mycelium(c)? Does the fungus produce a lot of acids (d)? (10%)



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VI. Figure A -D show the spores (and fruiting body, if any) of four microorganisms. Can you tell which is *Rhizopus* sp., *Clostridium* sp., *Agaricus* sp. (the common mushroom), or *Aspergillus* sp.? Which figure shows sporangiospores, conidia, endospores, or basidiospores? Which kind of spore has the highest degree of resistance to heat? (10%)



VII. What are: polyclonal antibody, monoclonal antibody, humanized antibody and single-chain antibody? Describe their differences in structure, methods of generation, and possible applications. (10%)

VIII. Food-borne diseases are serious threat to humans. Please give the names of one bacterium, one virus and one protozoa that are frequently found as the causative agent of food-borne diseases. Choose one of the organisms and describe anything you know about it (such as ecology, molecular biology, infectious cycle, epidemiology, vaccine, treatment, etc). (10%)