大葉大學 100 學年度 研究所博士班 招生考試試題紙					
系所別	組別	考試科目 (中文名稱)	考試 日期	節次	備註
生物科技暨資源學院博士班	甲組	專業英文論文閱讀能力測驗	5 月 28 日	第 節	第 1 頁 共 4 頁

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1. Truffles are among the most valuable gourmet mushrooms on the market. By taking Chinese truffle *Tuber sinense* as a typical example, a submerged fermentation process for the production of mycelia and *Tuber* polysaccharides was developed for the first time. Significances of inoculation density, carbon source and its initial concentration were studied in details. For inoculation density within the range of 160–653 mg dry weight (DW)/L, a maximal biomass of 15.59 ± 0.59 g DW/L was obtained at its lowest level of 160 mg DW/L, while the maximal extracellular polysaccharides (EPSs) production of 1.97 ± 0.08 g/L was attained at its highest level of 653 mg DW/L. The maximal intracellular polysaccharides (IPSs) production of 1.40 ± 0.10 g/L was obtained at its level of 487 mg DW/L. The carbon sources examined were glucose, maltose, sucrose and lactose, and sucrose was suitable for the cell growth and IPS production. Lactose was beneficial for EPS production although the cell could not grow well. There was no b-galactosidase activity when T. sinense grew in lactose, which was the reason why lactose was not favorable for the cell growth. Initial sucrose concentration within the range of 20–125 g/L significantly affected the process. At sucrose 125 g/L, both biomass (i.e., 24.07 ± 1.94 g/L) and EPS production (i.e., 2.85 ± 0.04 g/L) reached their peak values. The maximal IPS production of 2.92 ± 0.20 g/L was obtained at sucrose 80 g/L. This work demonstrated the submerged fermentation of Chinese truffle *T. sinense* is a potential alternative for the efficient production of mycelia and Tuber polysaccharides.

2. *Mycoplasma genitalium* has the smallest genome of any organism that can be grown in pure culture. It has a minimal metabolism and little genomic redundancy. Consequently, its genome is expected to be a close approximation to the minimal set of genes needed to sustain bacterial life. Using global transposon mutagenesis, we isolated and characterized gene disruption mutants for 100 different nonessential protein-coding genes. None of the 43 RNA-coding genes were disrupted. Herein, we identify 382 of the 482 *M. genitalium* protein-coding genes as essential, plus five sets of disrupted genes that encode proteins with potentially redundant essential functions, such as phosphate transport. Genes encoding proteins of unknown function constitute 28% of the essential protein-coding genes set. Disruption of some genes accelerated *M. genitalium* growth.

3. *Inonotus obliquus* (Fr.) Pilat is a white rot fungus belonging to the family Hymenochaetaceae in the Basidiomycota. In nature, this fungus rarely forms a fruiting body but usually an irregular shape of sclerotial conk called 'Chaga'. Characteristically, *I. obliquus* produces massive melanins released to the surface of Chaga. As early as in the sixteenth century, Chaga was used as an effective folk medicine in Russia and Northern Europe to treat several human malicious tumors and other diseases in the absence of any unacceptable toxic side effects. Chemical investigations show that *I. obliquus* produces a diverse range of secondary metabolites including phenolic compounds, melanins, and lanostane-type triterpenoids. Among these are the active components for antioxidant, antitumoral, and antiviral activities and for improving human immunity against infection of pathogenic microbes. Geographically, however, this fungus is restricted to very cold habitats and grows very slowly, suggesting that Chaga is not a reliable source of these bioactive compounds. Attempts for culturing this fungus axenically all resulted in a reduced production of bioactive metabolites. This review examines the current progress in the discovery of chemical diversity of Chaga and their biological activities and the strategies to modulate the expression of desired pathways to diversify and up-regulate the production of bioactive metabolites by the fungus grown in submerged cultures for possible drug discovery.

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4. *Ganoderma lucidum* (GL) is known as a valuable herb in the search for anticancer lead compounds because it has been used for thousands of years as a traditional medication. Triterpenoids are the principal active components in this fungus. To examine the bioactivity of triterpene compounds, Ganoderic acid T (GA-T) was isolated from *G. lucidum* by high performance liquid chromatography (HPLC). In this study, we investigated the anti-invasion and antimetastasis mechanisms of GA-T in vitro. GA-T dose-dependently inhibited 95-D cells migration by wound healing assay. Results of cell aggregation and adhesion assay showed that GA-T could promote cell aggregation and simultaneously inhibit cell from adhering to extracellular matrix (ECM). In addition, GA-T suppressed MMP-2 and MMP-9 gene expression, hence, decreased protein level of MMP-2 and MMP-9 in 95-D cells, according to RT-PCR assay and Western blot assay. These results demonstrate that GA-T effectively inhibits tumor metastasis in vitro. According to the result of Western blot, the suppression of NF-B activation likely abrogates the expression of MMP-2 and MMP-9, thus prevents tumor metastasis. These results suggest that GA-T has therapeutic potential against high metastatic lung carcinoma as a new agent.

5. Research on gold nanoparticles has been given much attention recently. The physicochemical property of gold is changed after it is processed through nanotechnology, and its derivative applications include the catalysis, DNA sequence recognition, drug delivery, and electrochemical applications. Gold nanoparticles can be produced by means of laser ablation and inert gas condensation. Their advantage is that they can acquire gold nanoparticles with narrow particle size distribution, but the shortcoming is the need for expensive equipment. Additionally, emulsification can also obtain gold nanoparticles but with a wide distribution of particle diameter. Gold nanocomposites are manufactured by using a reductant to reduce the gold ions and a dispersant in order to avoid excessive gold agglomeration. A unique shape of gold nanoboxes and silver nanocubes can be achieved by self-assembly through Sun and Xia's utilization of poly(vinyl pyrrolidone) as a dispersing agent. When sodium borohydride is taken as the reductant, the dispersants used in the preparation of gold nanocomposites are poly(amidoammine) dendrimers, poly- (propyleneimine) dendrimers , horse spleen apoferritin, and tryptophan. Meanwhile, when sodium citrate is taken as the reductant, the dispersants used are L-α-dipalmitoyl-phosphatidylcholine, 1,4,7,10,13,16,21,24-octaaza-bicyclo[8.8.8]hexacosane (azacryptand). The alkylamine -stabilized gold nanocomposite is prepared by using ethanol reduction through microwave irradiation. Some of the above manufacturing processes bring harm to the environment due to the toxic chemicals used.

Chitosan, an *N*-deacetylation products of chitin, which is a nontoxic biopolymer was applied on the reclamation of metal cations, such as Mg^{+2}, Cr^{+3}, Fe^{+2}, Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, and Pb^{+2}, where chelates of chitosan molecules and metal cations are precipitated. Relevant theses have been found about the utilization of chitosan and its effects on the preparation of precious metal (Pd, Ag, Pt, and Au) nanocomposites, in which chitosan acts as the dispersant to avoid the occurrence of metal particles agglomeration. In our previous report, silver nanoparticle was prepared by using alkaline chitosan suspension as a dispersant and as a reductant in the absence of other chemicals. Silver nanoparticles were obtained by annealing silver/chitosan composites. In this study, utilization of chitosan suspensions to manufacture gold nanopowders and nanoparticles by means of pyrolysis and acid hydrolysis is described.

6. The electrical charge of the membrane's functional groups can depend on the feed composition and on the pH in general. Most of the membranes hold a positive electrical charge at low pH and a negative charge at basic pH. A minimum rejection of salts is found when the feed pH is close to the membrane's isoelectric point. Manipulating the feed pH or selecting a membrane with an appropriate isoelectric point is a strategy that can be used to achieve and optimize the separation of compounds whose electrical charge varies according to the pH. The NF of amino acids has been studied extensively using membranes that have different MWCO and compositions (organic and ceramic), by either filtrating an individual amino acid or a mixture of them. These studies have revealed the viability of amino acid separation. They have also shown that pH is the variable that has the most effect on the amino acid rejection. The influence of pH on the NF of peptides and pharmaceutical products has also been analyzed.

7. Essential oils are isolated using a number of methods such as steam distillation (SD), hydrodistillation (HD), organic solvent extraction, microwave assisted distillation (MAD), microwave hydrodiffusion and gravity (MHG), high pressure solvent extraction (HPSE), supercritical CO2 extraction (SCE), ultrasonic extraction (UE) and solvent free microwave extraction (SFME). However, the properties of the essential oils extracted through these methods have been found to vary depending on the method used.

Rosemary (R. officinalis L.) is a perennial herb that belongs to the Lamiaceae family. It is used as a food flavouring agent and known medicinally for its powerful antimutagenic properties, antibacterial and as a chemopreventive agent. The plant is also known for its powerful antioxidant activity. The essential oil of R. officinalis has usually been isolated by traditional hydrodistillation, steam distillation or organic solvent extraction. Losses and degradation of some volatile compounds due to long extraction times, degradation of unsaturated or ester compounds through thermal or hydrolytic effects are the principal disadvantages of these extraction methods. For example, monoterpenes are well known to be vulnerable to chemical changes under steam distillation conditions and even conventional solvent extraction is likely to involve losses of more volatile compounds during the removal of the solvent. Recently, the supercritical fluid extraction of rosemary with CO_2 has been a subject of a lot of research and has become a valid alternative to the conventional extraction procedures mainly because the dissolving power of the extracting medium can be adjusted by regulating the pressure and temperature conditions. Today, an alternative method for extracting natural products by using microwave energy has been developed. Solvent free microwave extraction (SFME) is based on the combination of low microwave heating and distillation is performed at atmospheric pressure. Solvent free microwave extraction (SFME) appears to be particularly attractive for the isolation of essential oil from rosemary. Some of the advantages of this method over HD includes, rapidity in attaining the extraction temperature of 100 °C for the first essential oil droplet, high yield of essential oil, lower energy requirement and high purity of the oil extracted using SFME.

8. Metallo-β-lactamases (MBLs) are metalloenzymes of Ambler class B and are clavulanic acid-resistant enzymes. They require divalent cations of zinc as co-factors for enzymatic activity and are universally inhibited by ethylenediamine tetra-acetic acid (EDTA), as well as other chelating agents of divalent cations. The first plasmid-mediated MBL was reported in *Pseudomonas aeruginosa* in Japan in 1991. Since then many reports from all over the world are available regarding the prevalence of MBLs. As MBLs will hydrolyze virtually all classes of β-lactamase, their continued spread will be a clinical catastrophe. With the global increase in the types of MBLs, early detection is crucial. Over the last decade, most of the studies were on different methods of MBL detection in clinical species. Apart from being imipenem resistant, MBLs were resistant to important groups of antibiotics tested, including the third-generation cephalosporins, aminoglycosides and quinolones – a characteristic feature of MBL producers. For MBLs, limited treatment options are available and the only therapeutic option may be polymyxins, but it should not be used as monotherapy. It can be combined with an appropriate aminoglycoside. Aztreonam is the drug of choice for MBL producing *Pseudomonas aeruginosa.* Combination therapy is often employed in treatment of multidrug-resistant *Acinetobacter baumanii.* Imipenem or meropenem combined with ampicillin-sulbactam is active against carbapenem-resistant as well as MBL-positive strains of *Acinetobacter* species. Emergence of MBL producing *P.aeruginosa* and *Acinetobacter* species in intensive care units (ICUs) is alarming and reflects excessive use of carbapenems. Intensity of selection pressure for usage of broad spectrum antibiotics is high in ICUs, resulting in eradication of competitive flora and selection of multidrug-resistant strains. Therefore a strict antibiotic policy should be followed in intensive care areas to prevent further spread of MBLs. Clinicians should prescribe antibiotics judiciously. Resistance to β-lactams and other antibiotics in the *Enterobacteriaceae* is frequently associated with plasmidic resistance determinants that are easily transferred among species. β-Lactamase-mediated resistance is increasingly associated with plasmid-encoded extended-spectrum β-lactamases (ESBLs) and carbapenemases,. Although clonal dispersion of resistant isolates was seen initially, more diverse genetic platforms are being observed as variations of mobile elements are transferred worldwide. These enzymes are now appearing in multiple combinations of ESBLs and carbapenemases, thereby conferring resistance to virtually all β-lactam antibiotics.
(**note:** if you are not familiar with the names of antibiotics & strain names, leave them as they are)

⑨. Lung cancer is the most common cause of cancer death and accounts for 17% of the total deaths from cancer. The overall 5-year survival rate for these patients is less than 15%. Non–small-cell lung carcinoma (NSCLC) is the predominant type of lung cancer. Approximately 30% of patients with NSCLC are diagnosed at an early stage of the disease and receive curative surgery. However, disease will recur within 5 years in approximately 40% of these patients. The current clinical staging systems for lung cancer may have reached their limits in providing critical information that may influence patient management strategies. It is therefore important to identify patients who are at high risk of recurrence or of treatment failure after surgical resection. Identification of the specific genes involved and understanding the molecular pathogenesis in these high-risk patients are urgently needed. Gene expression profiles have been used to identify possible associations with lung cancer behavior or clinical outcome to better predict patient prognosis. In a previous study, we screened lung cancer cell lines with various invasive abilities using microarray analysis of 9600 genes and identified a panel of 589 (6.1%) genes whose expression was associated with invasion and metastasis. We used information from functional databases and hierarchical clustering analyses to categorize many of these genes by function. We then chose 30 candidate genes of unknown function for further characterization, investigated their mechanisms of action, and verified their functions in cancer patients. Here, we focused on one of these genes, the HLJ1 gene (DnaJ-like heat shock protein, also known as DNAJB4), whose expression was inversely associated with invasive ability in our previous analysis. HLJ1 was recently cloned and classified as a member of the heat shock protein 40 family (Hsp40/DnaJ).

⑩. The concerns of skin aging are increasing important to the fields of dermatology and cosmetic medicine. Skin aging is a complicated progress caused in part by the reduction of ECM components including laminin, fibronectin, collagen, etc. Twenty types of collagen are known to exist and are the major component of the ECM in humans. Type I collagen is the most abundant type of collagen and functions in structural integrity, cell adhesion and migration, tissue remodeling, and wound healing. These characteristics have led researchers to focus on the importance of collagen in the aging process. In 1993, Kou Katayama et al. identified that a pentapeptide (Lys-Thr-Thr-Lys-Ser) from C-peptides of type I procollagen has the ability to promote collagen biosynthesis. This group suggested that precursor molecules containing NH_2 and COOH propeptides that were cleaved off extracellularly by specific peptidases may be involved in the regulation of collagen biosynthesis. It is well known that L-ascorbic acid also functions in promoting the biosynthesis of collagen, a key structural protein of the skin. The addition of ascorbic acid to cultured human dermal fibroblasts not only remarkably increased the effects of the collagen biosynthesis but also reduced skin damage from free radicals. When ascorbic acid is properly delivered to skin cells, damaged skin may be improved and strengthened during skin regeneration. The stability of ascorbic acid is so low, however, that it is difficult to deliver and does not remain in skin tissue for extended periods of time. According to previous reports, ascorbic acid is rapidly oxidized by air in aqueous solutions. Historically, retinoic acid, also known as a potent inducer of the collagen biosynthesis, has been used as a key cosmetic material commercially. Unfortunately, overdoses of retinoic acids, a family of lipid-soluble vitamins, are known to have negative side effects in pregnant women and children.