

大葉大學 96 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
生物醫學技術博士班	甲	專業英文閱讀測驗	6月12日	第一節	共三頁 P.3-1

註：考生可否攜帶計算機或其他資料作答，請在備註欄註明（如未註明，一律不准攜帶）

英翻中：請將下列各題翻譯成中文(題目共 10 題請任選 5 題作答，每題 20 分共 100 分，請務必書寫題號，題號寫錯該題不予計分，答題超過 5 題時依書寫順序之前 5 題加以計分)

第 1 題: Leaf extract of *Stevia rebaudiana* promotes effects on certain physiological systems such as the cardiovascular and renal and influences hypertension and hyperglycemia. Since these activities may be correlated with the presence of antioxidant compounds, leaf and callus extracts of *Stevia rebaudiana* were evaluated for their total phenols, flavonoids content and total antioxidant capacity. Total phenols and flavonoids were analyzed according to the Folin–Ciocalteu method and total antioxidant activity of water and methanolic extracts of stevia leaves and callus was assessed by ferric reducing/antioxidant power (FRAP) assay as well as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The total phenolic compounds were found to be 25.18 mg/g for stevia leaves and 35.86 mg/g for callus on dry weight basis. The flavonoids content was found to be 21.73 and 31.99 mg/g in the leaf and callus, respectively. The total antioxidant activity was expressed as mg equivalent of gallic acid, ascorbic acid, BHA and trolox per-gram on dry weight basis. Total antioxidant activity found was ranged from 9.66 to 38.24 mg and 11.03 to 36.40 mg equivalent to different standards in water and methanolic extract of stevia leaves, respectively.

第 2 題: Spelling Bee contest opens

The third National Spelling Bee, an annual English spelling competition, has started taking applications for semi-final participants from the winners of preliminary rounds held in elementary, junior high, senior high and vocational schools, a competition organizer said yesterday. Ho Neng-yu, chief executive of the Sayling Wen Cultural and Educational Foundation, said the spelling competition is modeled on spelling bees in the US and aims to improve Taiwanese students' English competence. Applications for the semi-finals will be accepted until Sunday, Ho said. The semi-finals will be held in Taoyuan on June 2, in Taichung on June 3, in Kaohsiung and Taipei on June 9 and in the newly added location of Hualien on June 10, Ho said.

第 3 題: The 54 kDa protein that was suggested to be processed from the 65 kDa and 88 kDa

chitinases of *Bombyx mori* was purified and proved to be a third chitinase (EC 3.2.1.14). This chitinase was purified from the fifth larval instar of *B. mori* by chromatography on DEAE-Cellulofine A-500, hydroxylapatite, Butyl-Toyopearl 650M, and Fractogel EMD DEAE 650(M) columns. The apparent molecular mass was confirmed to be 54 kDa by SDS-PAGE. Its optimum pH was 6.0 toward a short substrate, N-acetylchitopentaose (GlcNAc<sub>5</sub>), while in its reaction with a longer substrate, glycolchitin, the enzyme showed a wide pH-range between 4.0 and 10. Kinetic parameters for the chitinase could be obtained in the hydrolysis of glycolchitin but not in that of N-acetylchitooligosaccharides (GlcNAc<sub>n</sub>, n=2-6) because of substrate inhibition. The chitinase hydrolyzed N-acetylchitooligosaccharides except for dimer as follows: trimer to monomer plus dimer, tetramer to two molecules of dimer, pentamer to dimer plus trimer, and hexamer to dimer plus tetramer as well as two molecules of trimer. These results suggest that the 54 kDa chitinase is an endo-type hydrolase and preferred the longer-chain N-acetylchitooligosaccharides. Moreover, the anomeric forms of N-acetylchitooligosaccharides were analyzed in the reaction with the 54-kDa chitinase. It was revealed that this enzyme cleaves the substrate to produce the anomeric product.

大葉大學 96 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
生物科學技術博士班	甲	專業論文閱讀能力測驗	6月12日	第一節	共三頁 P3-2

註：考生可否攜帶計算機或其他資料作答，請在備註欄註明（如未註明，一律不准攜帶）

第 4 題：The regulation of the molar fraction of 4-hydroxybutyrate (4-HB) in the poly(3-hydroxybutyrate-4-hydroxybutyrate) [P(3HB-4HB)] biosynthesis by *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) was attempted by the supplemental addition of propionate. The molar fraction of 4-HB in P(3HB-4HB) was increased significantly from 12.3 to 51.8 mol% by the addition of a small amount of propionate along with gamma-butyrolactone commonly used as a precursor for the biosynthesis of P(3HB-4HB). The mechanism of regulation by propionate was investigated by measuring the variation of enzyme activities related to the biosynthesis of P(3HB-4HB) and the level of intermediate metabolite acetyl-CoA. PHB synthase activity was induced significantly by propionate, and the acetyl-CoA concentration also increased significantly due to the additional supply of propionate. The overflowing acetyl-CoA seems to cause an inhibitory effect on the ketolysis reaction catalysing the lysis of 4-hydroxybutyryl-CoA to two molecules of acetyl-CoA; consequently, the 4-HB fraction available for polymerization increased. Accordingly, the molar fraction of 4-HB in P(3HB-4HB) biosynthesis seems to be regulated by both an increased 4-HB fraction and an activated PHB synthase due to the supplemental addition of propionate as a stimulator.

第 5 題：Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Lipozyme IM-77, 7.7 Batch Acidolysis Units of Novo (BAUN)/g, water 5.4% w/w) from *R. miehei* supported on macroporous weak anionic resin beads was purchased from Novo Nordisk Bioindustrials, Inc. (Bagsvaerd Denmark). Hexanol (98% pure), lauric acid (99% pure) and glyceryl tributyrate (99% pure) were purchased from Sigma Chemical Co. Louis, MO, USA). Molecular sieve was purchased from Davison Chemical (Baltimore, MD, USA) and n-hexane was obtained from Merck Chemical Co. (Darmstadt Germany). All other chemicals were of analytical reagent grade.

第 6 題：A model of ethanol fermentation by *Zymomonas mobilis* ATCC 10988 on the medium containing glucose and fructose is proposed. This model was developed on the basis of metabolic analysis and many experimental findings. When glucose was used as the substrate, the dependence of the carbon fraction ( $\alpha$ ) assimilating to biomass on the specific growth rate ( $\mu$ ) could be well correlated to  $\alpha = 0.25\mu + 0.012$ . This correlation resulted in a novel equation for specific glucose uptake rate, which could describe the *Z. mobilis* fermentation in both batch and continuous modes. When fructose and glucose were both presented in the liquid medium, the model could predict the uptake of glucose and fructose as well as the formation of biomass, ethanol and sorbitol by *Z. mobilis*. All parameters used in the model were independently evaluated on the basis of various experimental findings. Good agreement was found between the model predictions and data of *Z. mobilis* fermentation on media containing both glucose and fructose. The proposed model could also describe the behavior of ethanol fermentation on sucrose medium supplemented with immobilized invertase.

第 7 題：The valuable pharmaceutical polymer, hyaluronic acid, is produced industrially using the gram-positive bacterium *Streptococcus zooepidemicus*. Synthesis of this polymer is a significant energetic burden upon the microorganism hence the native NADH oxidase gene was cloned and overexpressed to increase the energy yield of catabolism during aerobic cultivation on glucose. Elevated NADH oxidase levels led to a decline in lactic acid generation and prevented ethanol

大葉大學 96 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
生物產業科技系博士班	甲	專英數論文閱讀能力測驗	6月12日	第一節	共三頁 P3-3

註：考生可否攜帶計算機或其他資料作答，請在備註欄註明（如未註明，一律不准攜帶）

formation, leaving acetate as the main fermentation product. Biomass yield increased due to the energy gained from the formation of acetate. Evaluation of the acetate flux control coefficient over a range of NADH oxidase expression levels revealed that acetate production was sensitive to the NADH oxidase level. However, at high NADH oxidase levels, the acetate flux was mainly influenced by another factor. The concomitant excretion of pyruvate at high NADH oxidase levels suggested that the flux through the pyruvate dehydrogenase enzyme complex was limiting the conversion of pyruvate to acetate.

✓ 第 8 題: Modified chitosan such as chitosan alpha-ketoglutaric acid (KCTS) and hydroxamated chitosan alpha-ketoglutaric acid (HKCTS) were successfully prepared. The modified chitosan were employed in the formation of drug-loaded, iron(III)-crosslinked polymeric beads. The produced polymers were characterized by IR, NMR, WXR and DSC measurements. The resulting beads were evaluated in vitro as drug prolonging and potentially orally administered delivery system. Theophylline was used as the loaded model drug. The generated beads proved to be successful in prolonging drug release. The release kinetics was evaluated by fitting the experimental data to standard release equations (zero-, first- and Higuchi equation). The best fit was found with Higuchi model for the polymeric beads.

✓ 第 9 題: Samples were taken at g stages of Processing, from raw milk to cheese aged for 6 mo. Fatty acid distributions, conjugated linoleic acid (CLA), moisture, protein, lipid contents, and titratable acidity were determined. CIA contents were highest after 3 mo, with one type of Cheddar (3.76 mg/g lipid) higher than the other two (3.44 and 3.47 mg/g lipid). Multiple linear regression showed all composition parameters were directly related to CLA content (mg/100 g sample). The content of oleic acid isomer  $C_{18:1\omega7}$  was also directly related to CIA content (mg/g lipid). Thus, content of CIA in Cheddar-type cheeses might be controlled by stage, and conditions of processing. An understanding of the effects of processing on CLA formation in Cheddar-type cheese will be beneficial in designing processing methods to enhance CLA contents.

第 10 題: The phytohormone abscisic acid (ABA) regulates stress-responsive gene expression during vegetative growth. The ABA regulation of many genes is mediated by a subfamily of basic leucine zipper class transcription factors referred to as ABFs (i.e. ABF1-ABF4), whose transcriptional activity is induced by ABA. Here we show that a calcium-dependent protein kinase is involved in the ABA-dependent activation process. We carried out yeast two-hybrid screens to identify regulatory components of ABF4 function and isolated AtCPK32 as an ABF4-interacting protein. AtCPK32 has autophosphorylation activity and can phosphorylate ABF4 in vitro. Mutational analysis indicated that serine-110 of ABF4, which is highly conserved among ABF family members, may be phosphorylated by AtCPK32. The serine-110 residue is essential for ABF4-AtCPK32 interaction, and transient expression assay revealed that it is also required for the normal transcriptional function of ABF4. The expression patterns and subcellular localization of AtCPK32 are similar to those of ABF4. Furthermore, its overexpression affects both ABA sensitivity and the expression of a number of ABF4-regulated genes. Together, our data demonstrate that AtCPK32 is an ABA signaling component that regulates the ABA-responsive gene expression via ABF4.