大葉大學 97 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
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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）

一、英翻中，每題 20 分
由以下 10 題任選 5 題作答，將各題翻成中文，依作答順序計分，超出 5 題時超出部份不予計分

1. The conjugated linoleic acid (CLA) isomers present in milk fat haw a high health amelioration potential. Their high prevalence in fat of ruminants and in milk and dairy products has been described and confirmed over many years. The CLA isomers are formed during biohydrogenation of linoleic acid in the rumen and also through conversion of vaccein acid in the mammary gland. In addition, several strains of Lactobacillus, Propionibacterium, Bifidobacterium and Enterococcus are able to form CLA from linoleic acid and thus could be used to increase the CLA level in fermented dairy products such as yoghurt and cheese. It appears likely that lactic acid bacteria and especially propionibacteria can form CLA during cheese ripening because free linoleic acid is formed in the ripening proems. However, for the time being the reviewed data allow no final conclusion on whether these increased levels of CLA are mainly due to formation by microorganisms, or due to cattle feed or breed. Further studies including all these parameters will benecessary to elucidate the potential role of starter cultures to achieve physiologically relevant CLA levels in dairy products. It appears that contribution of presently used dairy starter bacteria to increased CLA content in cheese is relatively minor.

2. **Cloning and characterization of the constitutively expressed chitinase C gene from a marine bacterium, *Salinivibrio costicola* strain 5SM-1.**
The chitinase C gene (chiC) encoding chitinase C (ChiC) from Salinivibrio costicola 5SM-1 was cloned and the nucleotide sequence was determined. S. costicola ChiC was expressed constitutively and repressed by glucose. A single operon composed of two complete open reading frames organized in the order of chiB, chiC and one partial open reading frame of chiA was found in the same transcriptional direction. chiC was composed of 2610 bp encoding for 870 amino acids with a calculated molecular mass of 94 kDa including a signal peptide. Analysis of the deduced amino acid sequence alignment revealed a domain structure consisting of an N-terminal catalytic domain, followed by a putative cadherin-like domain and two type 3 chitin-binding domains located at the C terminus. Mutation of three highly conserved amino acid residues, two aspartic acids (Asp-313 and Asp-315) and one glutamic acid (Glu-317) resulted in a complete loss of chitinase activity against colloidal chitin substrate. This suggests that these amino acid residues which reside in the putative catalytic domain play an important role in catalysis. chiB classified as a chitin-binding protein with C-terminal type 3 chitin-binding domain was composed of 390 amino acids with the molecular mass of 43 kDa and does not have any detectable chitinase activity. Chitinase C was identified as an exo-type chitinase releasing chitobiose as a major product from colloidal chitin hydrolysis.

3. **Compositional study on rice bran oil after lipase-catalyzed glycerolysis and solvent fractionations.**
(Source: J. Food Science 72:C163, 2007.
Rice bran oil (RBO) was modified through lipase-catalyzed glycerolysis. After 48 h reaction, the reactant (RBO-G, solved in hexane) containing 0.14 mg/mL of MAG, 0.19 mg/mL of DAG, and 0.93 mg/mL of TAG was obtained. Extending the reaction to 72 h resulted in 0.37 mg/mL of DAG with concomitant reduction in TAG (0.68 mg/mL). Two solvent fractionation methods, independent and sequential fractionation, were performed with acetone and hexane at 0, -8, -14, or -35℃. The fraction with most unsaturated fatty acids (ΣUFA) was liquid fraction from independent fractionation at -35℃(-35In) from hexane, showing 88.3% ΣUFA content. Nevertheless, when yield (wt%) was considered, the highest amount of UFA was obtained from 0In (liquid fraction from independent fractionation at 0℃) with hexane, resulting in 82.3% ΣUFA with 97.9 wt% recovery. Normal-phase HPLC was conducted for the compositional study of RBO-G. Overall, solid fractions from sequential fractionation at 0℃ (0SeSo) and independent fractionation at -35℃ (-35InSo) with hexane contained the high concentration of total MAG and DAG, ranging from 0.94 to 1.35 (mg/mL).

4. Our results suggested that both neighboring amino groups and sequence affected the formation of volatiles and Amadori products. Among the four peptides, γ-glu-cys significantly accelerated the production of sulfury, onion-like, and coffee-like aroma such as 3,6-dimethyl-1,2,4,5-tetrathiane, 3,5-dimethyl-1,2,4-trithiolane (syn-, anti- isomers), 2-acetyl-thiophene, 5-Methyl-2-thiophene carboxaldehyde and 2-thienyl disulfide. The neighboring amino group before cysteine such as glutamyl and glycyl group increased the formation of 3,6-dimethyl-1,2,4,5-tetrathiane and 3,5-dimethyl-1,2,4-trithiolane and also had a positive impact on the formation of the Amadori cysteine (1-deoxy-fructosyl-cysteine). The cys-gly was found generated more alkylpyrazines than gly-cys peptide. We also tentatively identified an intermediate product -pyroglutamic acid in the heated glutathione by LC-MS. The research allows us to improve the flavor generation with better control and assists us in understanding the degradation pathways of flavor precursors. Moreover, since there is no well-targeted approach to generate Maillard reaction flavors with enzymatic protein hydrolysates, this research may provide guidance for enzymatic protein hydrolysis in flavor production.

5. The important key technologies required for the successful biological conversion of lignocellulosic biomass to ethanol have been extensively reviewed. The biological process of ethanol fuel production utilizing lignocellulose as substrate requires: (1) delignification to liberate cellulose and hemicellulose from their complex with lignin, (2) depolymerization of the carbohydrate polymers (cellulose and hemicellulose) to produce free sugars, and (3) fermentation of mixed hexose and pentose sugars to produce ethanol. The development of the feasible biological delignification process should be possible if lignin-degrading microorganisms, their ecophysiological requirements, and optimal bioreactor design are effectively coordinated. Some thermophilic anaerobes and recently-developed recombinant bacteria have advantageous features for direct microbial conversion of cellulose to ethanol, i.e. the simultaneous depolymerization of cellulosic carbohydrate polymers with ethanol production. The new fermentation technology converting xylose to ethanol needs also to be developed to make the overall conversion process more cost-effective. The bioconversion process of lignocellulosics to ethanol could be successfully developed and optimized by aggressively applying the related novel science and technologies to solve the known key problems of conversion process.

6. Sugarcane bagasse, consisting of cellulose, xylan, and lignin, was chemically treated to generate bagasse samples with continuously decreasing content of lignin. These bagasse samples were hydrolyzed by cellulase and xylanase enzymes, produced earlier by Penicillium janthinellum NCIM 1171 in the same bagasse polysaccharides production medium. The hydrolysis was carried out by using different concentrations of the enzymes at two different temperatures, 30 and 50 8C, taking hydrolysis of Avicel as control. It was found that while the maximum hydrolysis for Avicel was 70% that of some of the bagasse polysaccharides was as high as 95%. The products of hydrolysis were glucose, xylose, and arabinose, as confirmed by high pressure ion chromatography (HPIC). It is interesting to note that arabinose, which constitutes about 10% of the weight of

bagasse xylan, could also be released easily by the enzymes. Also, the initial rates of hydrolysis was found to be much higher for the bagasse polysaccharides, and in some cases about 90% of the hydrolysis occurred within 20 h. Amongst all bagasse samples, the sample with (Kappa no. 1.2, lignin content 0.18%) gave the highest degree of hydrolysis at 50 °C. Even the bagasse polysaccharide with Kappa no. 16.8 (lignin content 2.5%) underwent greater extent of hydrolysis than Avicel. Apparently, the delignified bagasse medium appears to be a facile medium for the combined hydrolytic action of the cellulase and xylanase enzymes. Considering that sugarcane bagasse is a waste biomass material available in abundance annually, this methodology can be used to value-add to this biomass to produce sugars, which can be fermented to produce biofuels like ethanol.

7. Microencapsulation technology is a convenient method to alter and regulate cell product formation. In order to probe the metabolic response of different osmo-sensitive *Sacchromyces cerevisiae* to ACA microcapsule, the hyper-osmo-sensitive type *S. cerevisiae* (Y02724) and wild type *S. cerevisiae* (BY4741) were encapsulated into liquid core ACA microcapsules. The behavior of cell growth, glucose consumption, ethanol production and the yields of glycerol and organic acids were determined. Free cell culture was used as control. The enzyme activities of $NADP^+$-glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT) on microencapsulation cells and free cultured cells were measured too. The results demonstrated that the growth of Y02724 in both aerobic and anaerobic conditions was seriously inhibited by ACA microcapsule, while the ethanol and acetatic acid yield of microencapsulation Y02724 in anaerobic condition were significantly higher than that of suspended cultivation. For Y02724, the microencapsulation cultivation significantly increased the GS and GOGAT activities and decreased the GDH activity in comparison with control group. ACA microcapsules did not significantly change the growth behavior and metabolic performance of BY4741, but decreased the GS activity. In conclusion, microcapsules microenvironment significantly changes the metabolism behavior of hyper-osmo-sensitive type *S. cerevisiae* (Y02724), but nearly had no effect on BY4741.

8. Apoptosis, or programmed cell death, is an important physiological process in the normal development and homeostasis of multicellular organisms. Derangements of apoptosis can have deleterious consequences as exemplified by several human disease states, including acquired immunodeficiency syndrome, neurodegenerative disorders, and cancer. Apoptosis, which was originally described for its role in normal homeostatic cell turnover in healthy adult tissues, is a form of cell death associated with nuclear and cytosolic condensation and the formation of apoptotic bodies. Apoptosis-associated nuclear condensation is usually accompanied by oligonucleosomal DNA fragmentation into oligomers of _180 base pairs. Apoptosis is induced by some stimuli such as oxidants, various xenobiotics, growth factor withdrawal, glucocorticoid therapy, irradiation, and heat shock. Nevertheless, cells undergoing apoptosis exhibit a similar morphology, suggesting that these divergent apoptotic stimuli converge to trigger a common pathway of cell death. The common pathway involves a family of proteases known as the caspases, which are activated in a proteolytic cascade to cleave specific substrates. Their substrates include fordin, lamin and poly-ADP-ribose polymerase (PARP). Activated caspase-3 is found only in cells undergoing apoptosis and consists of p18 and p12 subunits that are derived from a 32 kDa proenzyme by cleavage at multiple aspartic acid sites. Protease caspase-3 can cleave and inactivate PARP, an enzyme that is used for DNA repair [23]. Cleavage of PARP can be used as a convenient marker of apoptosis.

9. **N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: endocytosis, exocytosis and drug release.**

Abstract

Nano-sized vesicular systems (nanoparticles), ranging from 10 nm to 1000 nm in size, have potential applications as drug delivery systems. Successful clinical applications require the efficient intracellular delivery of drug-loaded nanoparticles. Here we describe N-acetyl histidine-conjugated glycol chitosan (NAcHis-GC) self-assembled nanoparticles as a promising system for intracytoplasmic delivery of drugs. Because N-acetyl histidine (NAcHis) is hydrophobic at neutral pH, the conjugates formed self-assembled nanoparticles with mean diameters of 150-250 nm. In slightly acidic environments, such as those in endosomes, the nanoparticles were disassembled due to breakdown of the hydrophilic/hydrophobic balance by the protonation of the imidazole group of NAcHis. Cellular internalization and drug release of the pH-sensitive self-assembled nanoparticles were investigated by flow cytometry and confocal microscopy. NAcHis-GC nanoparticles internalized by adsorptive endocytosis were exocytosed or localized in endosomes. The amount of exocytosed nanoparticles was dependent on the pre-incubation time prior to removal of free nanoparticles from the culture media. Flow cytometry and confocal microscopy showed that NAcHis-GC nanoparticles released drugs into the cytosol and cell cycle analysis demonstrated that paclitaxel-incorporated NAcHis-GC nanoparticles were effective in inducing arrest of cell growth.

10. DNA microarrays represent a powerful technology whose use has been hampered by the uncertainty of whether the same principles, established on a scale typical for membrane hybridizations, apply when using the smaller, rigid support of microarrays. Our goal was to understand how the number and position of base pair mismatches, probe length and their G+C content affect the intensity and specificity of the hybridization signal. One set of oligonucleotides (50-mers) based on three regions of the *Bacillus thuringiensis cry1Aa1* gene possessing 30%, 42%, and 56% G+C content, a second set with similar G+C content (37% to 40%) but different lengths (30 to 100 bases), and finally amplicon probes (101 to 3000 base pairs) with G+C contents of 37% to 39%, were used. Probes with mismatches distributed over their entire length were the most specific, while those with mismatches grouped at either the 3' or 5'-end were the least specific. Hybridizations done at 8 to 13°C below the calculated T_m of perfectly matched probes, as compared to the widely used lower temperatures of 20 to 25°C, enhanced probe discrimination. Longer probes produced higher fluorescent hybridization signals than shorter ones. These results should help to optimize the design of oligonucleotide-based DNA microarrays.