PROGRAMME Rapid Microbiological Analysis Techniques

Academic course: 2018-2019

Identification and characteristics of the subject					
Code	502238			Créditos ECTS	6
Name (Spanish)	**Técnicas Rápidas de Análisis Microbiológico**				
Name (English)	**Rapid Microbiological Analysis Techniques**				
Degree	Food Science and Technology Degree				
Center	Agricultural Engineering School				
Semester	8º	Type	Elective		
Module	Elective				
Subject	Food hygiene				
Language	Spanish/English				
Professor/s					
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Field of knowledge	Nutrition and Bromatology				
Departament	Animal Production and Food Science				
Coordinator (if there is more than one professor)	**Mª José Benito Bernáldez**				

Lessons and contents

Syllabus

LESSON 1: GENERAL ASPECTS.
1.1. INTRODUCTION. Systems, methods, importance of new detection techniques. General concepts.
LESSON 2: DETECTION TECHNIQUES OF MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY MOLECULAR BIOLOGY METHODS
2.1. NUCLEIC ACIDS. Physical and chemical structure of DNA. Renaturalization. Structure of RNA.
2.2. RECOMBINANT DNA TECHNOLOGY I. DNA extraction: extraction. Visualization of DNA. DNA fragmentation: restriction enzymes. Union of DNA molecules.
2.3. RECOMBINANT DNA TECHNOLOGY II. Nature of vectors: plasmids and phage vectors.

Genes synthesized in the laboratory: complementary DNA. Cloning: stages.
2.4. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS I. Hybridization: Definition of probe. Marking of the probe. Advantages of the probes. Sensitivity and specificity. Fragment hybridization techniques: Southern Blotting and Northem Blotting.
2.5. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS II. Sequencing, visualization and types. DNA digestion with restriction enzymes (REN). Amplification of DNA fragments by the polymerase chain reaction: limitations and efficiency; uses and applications. Study of C + G values. Complementarity of DNA.
2.6. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS III. Studies of genetic polymorphisms I. Karyotyping. Restriction Fragment Analysis (RFLPs), rDNA Study. Study of non-ribosomal DNA and RNA (RT-PCR).
2.7. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS VI. Studies of genetic polymorphisms II. DNA fingerprinting or fingerprinting, Random PCR or RAPD, PCR fingerprinting or PCR fingerprinting, polymorphisms of amplified DNA fragments or AFLP.

LESSON 3: TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY PHYSICAL, CHEMICAL AND IMMUNE METHODS
3.1. PHYSICAL METHODS: Impedance, microcalorimetry and flow cytometry. Turbidimetry.
3.2. CHEMICAL METHODS: determination of adenosine triphosphate (ATP), direct Epifluorescence (DEFT). Radiometry. Fluorogenic and chromogenic substrates. API Galleries. Thermostable nuclease. Limulus lysate for endotoxin screening (LAL).
3.3. CHROMATOGRAPHIC METHODS: classification of chromatographic methods. Analysis and detection.
3.4. IMMUNOLOGICAL METHODS I: Precipitation. A) in liquid medium: quantitative and qualitative. B) in solid medium: double immunodiffusion, radial immunodiffusion and immunoelectrophoresis. Agglutination: agglutination in port, sero-agglutination in tube and direct hemagglutination in microplate.
3.5. IMMUNOLOGICAL METHODS II: Immunofuorescence: direct and indirect. Radioimmunoassay: solid phase; Direct and indirect. ELISA: Fundamnetos and types. Direct ELISA. Indirect ELISA. Double ELISA antibody sandwich. Indirect ELISA double antibody sandwich. ELISA competition.
3.7. BIOSENSORS. Definition. Components of a biosensor. Characteristics of biosensors. Future of biosensors.

LESSON 4: TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOOD THROUGH BIOASSAYS AND RELATED METHODS
4.1. TESTS IN LIVE ANIMALS. Lethality in mouse, lactating mouse, rabbit and mouse diarrhea, kitten test, skin tests in rabbit and guinea pig.
4.2. MODELS REQUIRING SURGICAL TECHNIQUES. Ligament bowel ligation techniques. RITARD method
4.3. SYSTEMS OF CELL CULTURES. Human cells of mucous epithelium. Guinea pig intestinal cells. Vero cells

PRACTICAL SYLLABUS

PRACTICAL LESSON 1: Contents of Lesson: Identification of microbial toxins by nucleic acid techniques (PCR). Bacterial DNA extraction, real-time PCR

PRACTICAL SESSION 2. Contents of Lesson: Visualization of toxins of a protein nature by polyacrylamide gel electrophoresis. Identification of different microorganisms by protein profiles in polyacrylamide gel electrophoresis.

PRACTICAL SESSION 3. Contents of Lesson: Identification of microorganisms using immunological techniques ELISA, TECRA UNIQUE and VIDAS.

PRACTICAL SESSION 4. Contents of Lesson: Use of other rapid methods for detection of microorganisms index indicators such as VIP (E.coli EHEC), SIMPLATE (coliforms and E. coli).

PRACTICAL SESSION 5. Contents of Lesson: Rapid biochemical methods: API GALLERIES.

PRACTICAL SESSION 6. Contents of Lesson: Physical-chemical methods (Chromatographies).