Code: 1436 **Molecular Diagnostic Methods**
Degree: 2nd cycle – Food Science and Engineering
Stream: Food Safety and Quality
Curricular Year: 1st **Semester Course: 2nd**
Credits: 6 ECTS **Optional**
Language: Portuguese/English
Responsible: Maria Luísa Lopes de Castro e Brito
Other lecturer(s): Arlindo Lima and Manuel José de Carvalho Pimenta Malfeito Ferreira
Web Site: http://www.isa.utl.pt/home/node/3878

1. Contact hours:
Lecture/Practicals 35 **Praticals/Laboratory** 35 **Others** 14 **Total** 84

2. Objectives:

1. To contributed for the development of research in diagnosis and related subjects
2. To learn a range of techniques actually used for identification and detection of food microorganisms and plant pathogen microorganisms based on chemotaxonomic information, immunological principles and nucleic acid-based methods.
3. To know the advantages and limitations of the different techniques used in diagnosis and is level of sensibility, specificity and principal applications.

3. Programme:

Didatic Unit 1-Classical methods used in diagnosis (phenotypic methods)
1. Presentation of contents of the course and activities.
2. Introduction to the detection and identification methods.
3. Evaluation of resistance to biocides.
4. Biolog system.
5. Immunoenzymatic techniques.
6. Evaluation of the virulence potential.

Didatic unit 2 – Molecular methods
A – General methods of molecular biology
Preparation and analysis of DNA. Quantization of DNA. Agarose gel electrophoresis.
B – Molecular techniques of diagnostic.
1. The polymerase chain reaction (PCR) based methods. Principles of the technique. Optimization of PCRs (critical parameters). Primer selection. Other reaction components. The PCR as an instrument of detection and identification of microorganisms. The PCR as an taxonomic instrument.
2. PCR variants: RAPDs,PCR multiplex, rep-PCR, PCR-RFLP, ARDRA.
Principles of the techniques. Primer selection. Advantages and limits. Applications.
3. Methods based on the digestion of DNA with restriction endonucleases. Restriction endonucleases with a high level and low level of frequency of cut. Restriction fragment length polymorphism (RFLP), Amplified fragment length polymorphism PCR (or AFLP-PCR or just AFLP). Pulsed Field Gel Electrophoresis (PFGE).
4. Methods based on DNA hybridation DNA. DNA probes. Southern blot. Colony blot, dot blot (slot blot).
5. Overview of DNA sequencing methods. Dideoxy (Sanger) sequencing. Development in sequencing technology. Computer analysis.

Didatic unit 3 – Numerical taxonomy
1.Application of the numerical analysis method to the results of typing of microorganisms. 2. Use of the NTSYS program in the analysis of the phenotypic and genotypic characters. 3. Utilization of the BLAST software in comparing sequences and to evaluated the relationship between organisms.

4. Bibliography:

Main Bibliography

Ausubel, F.M. et al.. (eds). (1995). Current Protocols in Molecular Biology. John Wiley and Sons, Inc., NY. Vol. I, II.

Harrigan, W. (1998). Laboratory methods in Food Microbiology. Academic Press. San Diego.

Hawksworth, D.L. (ed.) (1994).The identification and characterization of pest organisms. Cab International, UK.

Howe, Christopher (1995). Gene Cloning and Manipulation. Cambridge University Press.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989). Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Smith and Wood, (1991). Molecular Biology and Biotechnology.Chapman & Hall.

Other Bibliography

BioIog, Inc. (eds.) (1992) -MicroStation System. (available at Plant Pathology Lab).

Duncan, J.M. and Torrance, L. (1992). Techniques for the rapid detection of plant pathogens. Blackwell Scientific Publications. Oxford (available at Plant Pathology Lab).

Innis, M.A: et al (eds.)(1990) PCR Protocols. A guide to methods and applications. Academic Press, London.482pp. (available at Plant Pathology Lab).

Ohman, D. E. (1988). Experiments in gene manipulation. Prentice Hall, Inc., Englewood Cliffs, New Jersey (available at Microbiology Lab).

5. Assessment:

1. To have the frequency:

The students could not miss more than 5 sessions in the semester and to have his Laboratory notebook up-date.

2. Examination

The students have two options:

2.1. Continuous examination

On the basis of : a) three written questionnaires concerning the content of the 3 didactic units (50%); b) one seminar (25%) and c) the personal laboratory notebook (25%).

2.2. Final examination

On the basis of a written exam concerning theoretical and practical courses.

6. Estimated Workload:	168	Hours
7. Last Update:	19/7/2010	