

Laboratory VI

Code: 100975 ECTS Credits: 3

2024/2025

Degree	Туре	Year
2500502 Microbiology	ОВ	3

Contact

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Teachers

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Teaching groups languages

You can view this information at the <u>end</u> of this document.

Prerequisites

Students are advised to review the scientific-theoretical contents on which this subject is based.

It is also appropriate that this subject is taken simultaneously or subsequently to other subjects scheduled for the second semester of the third year of the Bachelor's Degree in Microbiology.

To take this course, students must have passed the safety and biosafety tests available in the corresponding educational space of the Campus Virtual or Moodle.

The information to pass these tests is in the communication area of the Microbiology degree. Furthermore, it is essential for students to follow the work rules as directed by teachers and to accept and follow the rules of operation of the teaching laboratories of the Faculty of Biosciences.

For security reasons, if any of the tests have not been passed or the student does not bring a lab coat and safety glasses, they will not be allowed to enter the laboratory.

Objectives and Contextualisation

This is a compulsory third year corecourse of the Bachelor's Degree in Microbiology, which introduces students to the use of a set of basic microbiology techniques for experimentation in an industrial Microbiology and Environmental Microbiology laboratory.

The knowledge gained in this course will enable students to acquire skills in other theoretical or practical subjects that make up the degree in Microbiology and are scheduled to be taken simultaneously or subsequently.

The specific objectives to be achieved by the students are defined in the following points:

- 1.- The students will become familiar with the isolation and selection techniques of microorganisms of industrial interest.
- 2.- The students will manipulate a microorganism to convert it into a producer of an industrial activity of interest using molecular methods.
- 3.- The students will carry out a process to obtain a product of industrial interest.
- 4.- The students will analyze the microbiological quality of the air and the surfaces of the practice facilities as a model.
- 5.- The students will determine the microbiological quality of water.
- 6.- The students will evaluate the disinfectant effect of chemical products.
- 7.-The students will become familiar with the methodology for converting industrial wastes into high protein content products.
- 8.-The students will select and evaluate the activity of microorganisms involved in soil bioremediation.
- 9.-The students will demonstrate the action of bacteria on the inhibition of the growth of phytopathogenic fungi.
- 10.-The students will collect, analyze and interpret the data obtained during the practical work.
- 11.-The students will expose in a concise way the results and conclusions derived from the practices performed in group.
- 12.-The students will become familiar with the use of a laboratory notebook to elaborate reports in which all the activity performed canbe traced.
- 13.- The students will describe the procedures of good laboratory practices and biosafety with microorganisms used in industrial and environmental microbiology.

The specific learning outcomes (RAs) for this subject are:

- 1.- To know how to isolate and select microorganisms of industrial interest from a collection of microorganisms (associated to RA: SM26).
- 2.- Manipulate a microorganism to convert it into a producer of an industrial activity of interest using molecular methods (associated to RA: SM27).
- 3.- Develop a process to obtain a product of industrial interest from transformed microorganisms (associated to RA: SM28).
- 4.- Determine the microbiological quality of air and surfaces of industrial facilities (associated to RA: SM25).
- 5.- Determine the microbiological quality of water independently of its origin (associated to RA: SM25).
- 6.- Describe the disinfecting effect of chemical products (associated to RA: KM25).
- 7.- Convert industrial wastes into high protein content products using the "single-cell protein" concept (associated to RA: SM28).
- 8.- Select and evaluate the activity of microorganisms involved in soil bioremediation (associated to RA: SM27).

- 9.-Demonstrate the action of bacteria on the inhibition of the growth of phytopathogenic fungi (associated to RA: SM27).
- 10.-Analyze experimental data of industrial and environmental microbiology with computer resources (associated to RA: SM25).
- 11.- Expose in a concise way the results and conclusions derived from the practices carried out in group (associated to RA: CM17).
- 12.-Elaborate laboratory reports in which all the activity carried out individually or in group can be traced (associated to RAs: CM18).
- 13.-Describe the methodology used in the laboratory to manipulate microorganisms in the field of industrial and environmental microbiology considering the aspects of good laboratory practices and biosafety (associated to RA: KM26).

Learning Outcomes

- 1. CM17 (Competence) Critically evaluate experimental results in the field of microbiology for their presentation clearly and concisely.
- 2. CM18 (Competence) Integrate knowledge and skills for the design of experiments in the field of microbiology and the interpretation of their results working individually and in teams.
- 3. KM25 (Knowledge) Describe the theoretical foundations and instrumentation used in basic and advanced experimental techniques in microbiology and other related sciences, including sterilization and microbial load reduction procedures in industrial, clinical and experimental environments.
- 4. KM26 (Knowledge) Identify the principles and standards of good laboratory and biosafety practices.
- 5. SM25 (Skill) Manage computer resources for the treatment of experimental data within the field of microbiology and other biosciences.
- 6. SM26 (Skill) Apply conventional microbiological techniques that allow differentiating and characterizing different microbial groups and manipulate materials and samples under aseptic conditions.
- 7. SM27 (Skill) Develop appropriate methodologies to sample, characterise and manipulate microbial populations and communities in natural and artificial ecosystems.
- 8. SM28 (Skill) Use different indicators and tests based on microorganisms or their components for industrial, sanitary, biotechnological purposes or to assess environmental impacts.

Content

The course is divided into the following sections:

Section 1: Industrial Microbiology

Section 2: Environmental Microbiology

Each section occupies approximately 24 hours.

Section 1: Industrial Microbiology

Content: This section consists of seven sessions focusing on the isolation and the use of microorganisms to obtain products of industrial interest. Lab work in this section will be performed under the safety and biosafety regulations indicated at the beginning of each session.

- 1. Isolation and selection of microorganisms of industrial interest-MI1
- 2. Screening for activity-MI2

- 3. Isolation of genes of industrial interest-MI3
- 4. Cloning of overexpression vector-MI4
- 5. Development of-production method MI5
- 6. Assessment-MI6

Section 2: Environmental Microbiology

Content: This section consists of eight sessions focusing on detection of microbial environmental contamination, the use of microorganisms in bioremediation and recovery of waste, as well as in plague control and plant diseases.

- 1. Waste management
- Bioremediation in contaminated soil-MA1
- Production of single-cell protein starting from waste-MA2
- 2. Control of environmental pollution
- Air and surface quality in industrial facilities-MA3
- Microbiological quality of water-MA4
- Disinfection of surfaces-MA5
- 3. Biological control
- Isolation of bacteria from the phylosphere.
- Determination of their potential as natural enemies of plant pathogens -MA6

The contents per session that will be taught in each of the modules are as follows:

Section 1: Industrial Microbiology

Content	Session	Activities
MI1	1	- Enrichment and isolation of Actinomycetes from soil samples
	2	 Enrichment and isolation of <i>Bacillus</i> from soil samples Identification, counting and isolation of <i>Actinomycetes</i> clones
	3	- Identification, counting and isolation of Bacillus clones
MI2	3	 Detection of the antibiotic activity of Actinomycetes-plating Detection of the enzymatic activity of Actinomycetes-plating
	4	- Detection of the antibiotic activity of <i>Bacillus</i> -plating

		- Detection of the enzymatic activity of Bacillus-plating
	5	 Evaluation of the enzymatic activity of Actinomycetes Detection of the enzymatic activity of Actinomycetes-double layered plating
	6	- Detection of the enzymatic activity of Bacillus-double layered plating
	7	- Evaluation of the antibiotic activity of <i>Bacillus</i> - Evaluation of the enzymatic activity of <i>Bacillus</i>
		- Evaluation of the antibiotic activity of <i>Actinomycetes</i>
		- Evaluation and discussion of results
MI3	1	- Purification of genomic DNA of <i>Bacillus</i>
		- PCR to amplify genes of industrial interest
	2	- Agarose gel to determine the DNA amplification of the gene of industrial interest
MI4	2	- Restriction enzyme digestion of the <i>E. coli</i> expression vector
		- Restriction enzyme digestion of DNA fragments containing the gene of industrial interest
		- Dephosphorylation and purification of the linearised <i>E. coli</i> expression vector
	3	- Ligation of the gene of industrial interest
		- Transformation of the ligation reaction
	4	- Detection of productive transformants
MI5	5	- Inoculation of microorganisms producing enzymatic activities of industrial interest
	6	- Batch experiment: reinoculation of the culture and growth curve monitoring of microorganisms producing enzymatic activities of industrial interest
MI6	6	- Evaluation of the production and activity of enzymes of industrial interest produced by microorganisms

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- Evaluation and discussion of results

Section 2: Environmental Microbiology

Content	Session	Activities
MA1	4	- Enrichment and isolation of hydrocarbon-degrading bacteria from contaminated soil samples
		- Determination of the microbial activity of contaminated soil samples
	8	- Reading total heterotrophs
		- Detection of total hydrocarbon degrading microorganisms
		- Detection and enumeration of microorganisms degrading polycyclic aromatic hydrocarbons
		- Detection of n-hexadecane degrading microorganisms
	9	- Enumeration of total hydrocarbon degrading microorganisms
		- Enumeration of n-hexadecane degrading microorganisms
		- Evaluation and discussion of results
MA2	1	- Inoculation of bioreactors with microorganisms in media with different concentrations of sugars
		- Initial sampling of the culture: viable cell plating, sugars, OD
		- Analysis of samples: viable cell plating, OD
	2	- Sampling of the culture: viable cell plating, sugars, OD
		- Analysis of samples: viable cell counting, growth rate of the culture, doubling time, OD, determination of sugar consumption
	3	Final sampling of the culture:
		- Analysis of the samples:
		- Cell counting and graphs
		- Determination of sugar consumption
		- Determination of the biomass of the culture

		- Calculations
		- Evaluation and discussion of results
MA3	5	- Detection ofmicroorganisms in air samples
		- Detection of microorganisms in surface samples
	7	- Observation and counting of microorganisms of air and surface samples
		- Evaluation and discussion of results
MA4	5	- Plating of viable microorganisms from water samples
		- Detection and counts of coliform bacteria and Escherichia coli
		- Intestinal Enterococcal detection and counting
		- Detection and counts of Clostridium perfringens
	6	- Evaluation and test to confirm the presence of Escherichia coli
		- Evaluation and test to confirm the presence of Clostridium perfringens
	7	- Evaluation of the presence of <i>Escherichia coli</i> in water samples
		- Evaluation and test to confirm the presence of faecal enterococci
	8	- Count of viable microorganisms from water samples
		- Evaluation and discussion of results
MA5	6	- Detection of microorganisms in disinfected surface samples
	8	- Observation and counting of microorganisms from disinfected surfaces
		- Evaluation and discussion of results
MA6	5	- Isolation of epiphytic bacteria from leaves of different plant species
	6	- Preparation of axenic cultures of leaf epiphytic bacteria
	7	- Conducting the biological control test of fungi by leaf epiphytic bacteria

- Evaluation of the results obtained from the antagonistic potential of leaf epiphytic bacteria on a phytopathogenic fungus
- Evaluation and discussion of results

Activities and Methodology

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Practical Laboratory Classes	45	1.8	CM17, CM18, KM25, KM26, SM25, SM26, SM27, SM28, CM17
Type: Supervised			
Tutoring	1	0.04	CM17, CM18, KM25, KM26, SM25, SM26, SM27, SM28, CM17
Type: Autonomous			
Comprehensive reading of the practical sessions laboratory handbook	5	0.2	KM25, KM26, SM25, SM26, SM27, SM28, KM25
Drafting of laboratory notebook	6	0.24	CM17, CM18, SM25, CM17
Preparation of oral presentations	3	0.12	CM17, CM18, SM25, CM17
Study	12	0.48	KM25, KM26, SM25, SM26, SM27, SM28, KM25

This subject will be taught in three small groups of students (maximum 24 students per session). Attendance of the classes is obligatory to be able to acquire the competences of the subject. If a student, for justified and unforeseeable reasons (such as a health problem, death of a relative up to second degree, accident, enjoying the status of elite athlete and having a competition or sports activity that they are obliged to attend, etc.), has not attended a session/practice session, they will have to speak with the teacher in charge and present the corresponding proof as soon as possible (official medical certificate stating explicitly the inability to attend the session/practice session, police report, justification from the competent sports organisation, etc.). In the case of a strike, if a student decides to exercise their right to strike, they must notify the subject coordinator within a maximum period of 48 hours after the day of the strike. In no event shall the absence exceed 20% of the programmed activities. The maximum absence in each of the sections (industrial or environmental microbiology) is set at a maximum of 10% in order to be assessed. If this value is exceeded, the subject will be classified as Non-assessable.

Students will have a Handbook of the subject before the beginning of the practical sessions. At each practical session it is compulsory for the student to wear their own <u>lab coat</u>, laboratory <u>glasses</u>, and have a permanent <u>marker</u>, <u>calculator</u> and the <u>Handbook</u> of the subject that will be available on the Campus Virtual, Moodle platform, or where indicated by the teachers. It is also necessary to have a <u>notebook</u> (Miquelrius, Abacus or Oxford) with stiched pages, where each student will note the laboratory observations. To undertake the practical sessions the students will work in pairs and under the supervision of the teacher. At the beginning of and/or during each session the teacher will make brief theoretical explanations of the content of the practicals and procedures to be carried out by the students, as well as the specific safety measures and the treatment of the different chemical and biological waste generated.

In order to achieve good performance and to acquire the competences corresponding to this subject, it is essential that students undertake a comprehensive reading of the Handbook for the subject, becoming familiar with the practices that will be carried out in each session, as well as with the methodology that they will have to apply in each case.

Annotation: Within the schedule set by the centre or degree programme, 15 minutes of one class will be reserved for students to evaluate their lecturers and their courses or modules through questionnaires.

Assessment

Continous Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Delivery of laboratory notebook	20%	0	0	CM17, CM18, SM25
Evaluation of Section 1 Industrial Microbiology: multiple-choice test	20%	0.5	0.02	KM25, KM26, SM26, SM27, SM28
Evaluation of Section 1 Industrial Microbiology: oral presentation and class participation	20%	1	0.04	CM17, CM18, SM25
Evaluation of Section 2 Environmental Microbiology: delivery of a report	3,3%	0	0	CM17, CM18, SM25
Evaluation of Section 2 Environmental Microbiology: multiple-choice test	30%	0.5	0.02	KM25, KM26, SM25, SM26
Evaluation of Section 2 Environmental Microbiology: oral presentation and class participation	6,7%	1	0.04	KM25, KM26, SM26, SM27, SM28

The assessment of the subject will be conducted by section and will be continuous. The weight of the assessment on the final grade of the subject of each section is: Section 1, 50%, Section 2, 50%. It will be necessary to obtain a grade equal to or higher than 5 in each of the practical sections separately to be able to pass the subject.

The assessment of each section will be carried out as follows:

Section 1. Industrial Microbiology

Continuous assessment of group work

Oral presentation, discussion of the results of the practical sessions and participation in the practical sessions (20%)

Individual assessment of the content

Daily follow-up of the notebook and work in the laboratory (10%)

Individual questionnaire with multiple-choice questions (20%)*

* The individual questionnaire can be set at any time during the practice sessions and there may be more than one.

Section 2: Environmental Microbiology

Continuous assessment of group work

Delivery of a final report Bioreactors (3.3%)

Oral presentation discussion of the results of the practical sessions and participation in the practical sessions (6.7%)

Individual evaluation of the contents

Daily follow-up of the laboratory book (10%). Individual multiple-choice test (30%)

In each section, the student's attitude in the laboratory, punctuality, use of laboratory equipment (lab coat and goggles), compliance with safety and biosecurity regulations, and understanding and follow-up of the Handbook of the subject will be assessed. This assessment does not entail an increase in the grade, but it can mean a reduction of up to 20% of the final grade obtained in each module.

Since attendance of the activities scheduled in this subject is mandatory, absence from any of the sessions must be justified and may not exceed 20%. In any case, this 20% can not be accumulated in a single section, the maximum of each being 10% absence. If this value is exceeded, the student will be gradedas Non-assessable (*No avaluable*).

Students who do not pass the assessments for the different sections of the subject will be able to retake them on the date scheduled at the end of the semester (retakes), answering a multiple-choice test associated with the section not previously passed (20% and 30%, respectively for the Individual multiple-choice test). Retake exams will not be scheduled for the other activities.

Students who do not obtain the minimum grade required to pass each of the sections of the integrated laboratory, will not be able to pass the subject. In this case, the final maximum grade of the subject will be 4.

Because this subject is split into modules, from the second registration, students will only have to be assessed in the specific sections that have not been passed.

Single assessment

The students who choose the single assessment must do the laboratory practices in face-to-face sessions since they are mandatory teaching activities.

The single assessment consists of a final exam that may contain multiple choice questions, short questions and problems to develop on theoretical and practical contents of the subject. The grade obtained in this synthesis test represents 50 % of the final grade of the subject. This single assessment test will be held coinciding with the same date for the last continuous assessment test. The same criterion will be applied to pass the subject as for the continuous assessment.

The continuous assessment of the individual and teamwork, the daily follow-up of the laboratory notebook, the oral presentations and discussions of the results and the written report of the second part, will be the remaining 50 % of the final grade and will be done on the same dates set for the continuous assessment.

The same retake system as for the continuous assessment will be applied. The revision of the final qualification follows the same procedure as for the continuous assessment.

Bibliography

Bibliography and web links are indicated in the corresponding sections of the laboratory manual.

Class schedules of the subject can be obtained in the information section of the Microbiology Degree website.

Industrial Microbiology

- -R.S. Burlage, R. Atlas, D. Stahl, G. Geesey and G. Sayler, (1998). Techniques in Microbial Ecology. New York, NY. Oxford University Press.
- -L-M. Prescott (2002). Microbiology. Capter 42: Industrial Microbiology and biotechnology, 5th Edition, New York, NY. The McGraw-Hill Companies.
- -M. Rabbani, H.M. Sadeghi, F. Moazen, M. Rahimi and G. Salehi. (2011). Cloning and Expression of Randomly Mutated Bacillus subtilis α -Amylase Genes in HB101. Biotechnology Research International 2011:305956. doi:10.4061/2011/305956
- -R.C. Cadwell and G.F. Joyce. (1994). Mutagenic PCR. Genome Res. 3: S136-S140.
- -M.J. Waites, N.L. Morgan, J.S. Rockey and G. Higton (2001) Industrial Microbiology: an introduction. London, UK. Blackwell Science Ltd.
- -M.R. Ladisch, N.S., Mosier, (2009) Modern Biotechnology. John Wiley & Sons, Inc. https://doi.org/10.1002/9780470473412
- -Alpha amylase activity protocol: http://www.worthington-biochem.com/aa/assay.html
- -Preparation of phosphate buffers: http://openwetware.org/wiki/Phosphate_buffer

Environmental Microbiology

- 1.-Waste treatment
- -Martin Alexander (1999) Biodegradation and Bioremediation. 2nd Edition. Cornell University, Ithaca, New York, U.S.A. AcademicPress.
- -Ajay Singh, Ramesh C. Kuhad, Owen P. Ward. (2009) Advances in applied bioremediation. Berlin, Heidelberg. Springer-Verlag. https://dx.doi.org/10.1007/978-3-540-89621-0
- -Ralph, M. and Ji-Dong, G. (eds) (2010) Environmental Microbiology. 2nd Edition. Wiley-Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9780470495117
- -Yates, M. V. et al. (eds) (2016) Manual of Environmental Microbiology. 4th Edition. ASM Press. doi: 10.1128/9781555818821.
- 2.-Control of the environmental contamination
- -Wen-TsoLiu, Janet K. Caister (2010) Microbiology. Norfolk, UK. Academic Press.
- -Harley-Prescott. (2002) Laboratory exercises in microbiology (5th Edition). Boston, Mass. The McGraw-Hill companies.
- -Delort, A.-M. and Amato, P. (eds) (2017) Microbiology of aerosols. Wiley Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9781119132318
- -Mohee, R. and Mudhoo, A. (eds) (2012) Bioremediation and Sustainability: Research and Applications. Wiley-Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9781118371220
- 3.-Biological control
- H.J. Benson, (2001) Microbiological applications. Laboratory manual in general microbiology (8th edition). Boston, Mass. The McGraw-Hill companies.

- B. Prapagdee, C. Kuekulvong and S. Mongkolsuk (2008). Antifungal Potential of Extracellular Metabolites Produced by Streptomyces hygroscopicus against Phytopathogenic Fungi. International Journal of Biological Sciences 4:330-337.
- -Ghannoum, M. et al. (eds) (2015) Microbial Biofilms. Second Edi. ASM Press. doi: 10.1128/9781555817466.

General Microbiology

-Martín A., Béjar V., Gutierrez J.C., Llagostera M. y Quesada E. 2019. Microbiología Esencial. 1ª edición. Editorial Médica Panamericana. https://www.medicapanamericana.com/VisorEbookV2/Ebook/9788491102427

Software

No specific software is foreseen

Language list

Name	Group	Language	Semester	Turn
(PAUL) Classroom practices	731	Catalan/Spanish	second semester	morning-mixed
(PLAB) Practical laboratories	731	Catalan/Spanish	second semester	morning-mixed
(PLAB) Practical laboratories	732	Catalan/Spanish	second semester	morning-mixed
(PLAB) Practical laboratories	733	Catalan/Spanish	second semester	morning-mixed