



UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

COURSE SYLLABUS

Probiotic Microbes: Biology and Industrial Applications

2425-1-F0802Q085

Aims

Knowledge and understanding

Students will acquire knowledge concerning:

- The meaning of probiotics, starter cultures, live biotherapeutics, and other "biotic" products;
- The methodologies adopted in research and industry for the identification, typing, and functional characterization of probiotic microorganisms;
- The main microorganisms used as probiotics;
- The principles of industrial production of microbial biomass;
- The main aspects related to the safety of using microorganisms, in accordance with current regulations;
- The potential applications of microorganisms in supporting human health beyond the concept of probiotics.

Ability to apply knowledge and understanding

Students will develop the necessary competence to interpret scientific publications involving the use of microorganisms to support human health and will know how to choose the most suitable scientific methodologies for the selection and characterization of a probiotic microorganism. Moreover, knowledge of the methodologies used in scientific research, combined with the knowledge of industrial practices for microbial biomass production, will allow them to apply what they have learned to the needs of the industry in the sector.

Autonomy of judgment

Students will acquire the ability to critically examine the characteristics of commercial probiotic products. They will gain autonomy of judgment regarding the potential risks associated with the consumption of microbial biomass. Additionally, they will be able to distinguish contexts in which the concept of live biotherapeutics should replace that of probiotics.

Communication skills

Students will be able to communicate the essential characteristics of a good probiotic product with appropriate language, both from a microbiological perspective and in terms of product formulation and packaging. Furthermore, they will be able to clearly describe the potential risks associated with the consumption of probiotics and translate

the results of recent scientific research into useful knowledge for solving industrial issues related to the production and commercialization of probiotics.

Learning Ability

At the end of the course, students will be able to continue learning through consulting the most recent literature and regulations in the field of probiotics and live biotherapeutics. Knowledge of the main industrial aspects of production and commercialization of probiotics will guide students towards integrating knowledge on the topic in a broad and multidisciplinary way.

Contents

- The Origins of the Probiotic Concept
 - Microbial starters
 - Taxonomy and genetic typing of probiotics
 - Functional characterization of probiotics
 - Safety of probiotic use
 - Next-generation probiotics, live biotherapeutics, and other “biotics”
 - Main industrial aspects of probiotic products
 - Probiotics and human trials
 - Probiotics and legislation
 - The use of microorganisms for human health benefits beyond the concept of probiotics
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- A visit will be organized to a company that produces microbial biomass used as food starters and probiotics, during which one of the production plants will be toured.
 - Seminars by experts from the industry are planned.

Detailed program

- The origins of the probiotic concept

Interactions between microorganisms and the human organism (overview of the concepts of holobiont, microbiota, and microbiome). Definitions of probiotics (FAO/WHO and Italian Ministry of Health). FAO/WHO guidelines: description of the procedure required for evaluating a probiotic according to FAO/WHO. Microorganisms present in foods as potential probiotics: yogurt and traditional fermented products. The ISAPP consensus statement.

- Microbial starters

Various definitions of microbial starter. Differences between microbial starters and probiotic microorganisms. Classification of starter cultures. Microbial starters and safety issues. Industrial production processes of starter cultures. Methods of preservation and distribution of microbial starters. Microbial starters and traditional and artisanal productions (indigenous starters).

- Taxonomy and genetic typing of probiotics

Taxonomic identification of genus and species. Microorganisms used as probiotics: lactic acid bacteria, bifidobacteria, and other microorganisms used as probiotics (*Saccharomyces*, *E. coli*, and *Weizmannia coagulans*). Strain-level characterization: international collections of microbial strains; molecular fingerprinting techniques for strain characterization. The most commonly used probiotic strains in the industry. Strain-specificity according to the ISAPP consensus statement.

- Functional characterization of probiotics

Assessment of resistance to gastrointestinal transit. Strategies to increase survival during gastrointestinal transit:

microencapsulation. Bacterial adhesion to the intestinal epithelium and methods for studying bacterial adhesion. Measurement of trans-epithelial electrical resistance (TEER). Immunomodulation assays (e.g., reporter system for studying NF-kappaB activation in Caco-2 cell line). Antagonism and antimicrobial activity of probiotics against pathogens (assessment of antagonistic activity, bacteriocins, reuterin).

- *Safety of probiotic use*

Tests for evaluating probiotic safety: bile salt deconjugation; D-lactic acid production by probiotics and D-lactic acidosis; bacterial-induced hemolysis; determination of probiotic infectivity in animal models. Evaluation of probiotic safety: antibiotic resistance in probiotics (intrinsic vs acquired resistance; resistance mechanisms; EFSA breakpoints). Health risks associated with probiotic consumption: discussion of clinical cases. Use of probiotics for managing adverse effects associated with antibiotic consumption: discussion of recent scientific literature.

- *Next-generation probiotics, live biotherapeutics, and other "biotics"*

Definitions and regulations (FDA and Pharmacopeia). Use of genetically modified microorganisms for human health: potential (drug delivery system) and industrial applications. Cartagena Protocol on Biosafety. The case of *Akkermansia muciniphila*. Products containing inactivated probiotic microorganisms (paraprobiotics or postbiotics?). Definitions of synbiotics, bifidogenic, and metabiotics.

- *Main industrial aspects of probiotic products*

Industrial production of microbial biomass. Importance of packaging for probiotic products. Labeling of probiotic products. Overlooked microbial elements in commercial probiotic formulations. Viability/death assessment of bacterial cells using propidium iodide/SYTO staining and flow cytometry. Main types of microbiological issues encountered in probiotic products.

- *Probiotics and human trials*

Markers type a, b, and c. Example of type a marker study: recovery studies. Difficulties associated with studying probiotic efficacy. Health benefits attributed to probiotics (evaluation of available meta-analyses). Brief description of possible mechanisms of action of probiotics.

- Probiotics and legislation
European legislation on probiotics (Regulation 258/97/EC on novel foods, the concept of Qualified Presumption of Safety; QPS, Regulation 1924/2006 on health claims, consequences of Regulation EC 1924/2006). Probiotics in animal husbandry: Regulation (EC) No. 1831/2003. Guidelines from the Italian Ministry of Health. Legislation on probiotics outside of Europe. Nagoya Protocol.
- Use of microorganisms for human health benefits beyond the concept of probiotics
 - (i) Use beyond the gastrointestinal tract: commercial products for the oral cavity, vaginal mucosa, and skin;
 - (ii) Phage therapy;
 - (iii) Fecal microbiota transplantation.
- A visit will be organized to a company that produces microbial biomass used as food starters and probiotics, during which one of the production plants will be toured.
- Seminars by experts from the industry are planned..

Prerequisites

Knowledge acquired in the courses of Microbiology and Biochemistry is required.

Basic knowledge of Immunology and Genetics is necessary.

Basic knowledge related to molecular biology technologies (PCR, gene expression analysis, electrophoresis, cloning) is required.

Teaching form

LECTURES. 19 lectures of 2 hours each, consisting of:

- a section of delivered didactics (Didattica erogativa, DE) focused on the presentation of scientific and technical contents by the lecturer. One lecture will be given by an expert from the industry.
- a section of interactive teaching (Didattica Interattiva, DI) including supplementary didactic interventions, brief presentations by the students, and additional demonstrations of practical applications of the content covered in the lecture-based part. Specifically, at the end of each major topic of the course (e.g., industrial production, safety of use, legislation, etc.), the use of Audience Engagement platforms will allow students to answer questions designed to apply the acquired knowledge to real-world issues (industrial, commercial, legal, or health-related) in the context of probiotics. The collected responses will serve as a basis for a debate, which the instructor will moderate and enrich by showing scientific publications, popular articles, industrial case studies, and media news.

EDUCATIONAL VISIT. A visit to the production site of a company in the sector is planned (estimated duration: 4 hours).

All activities are conducted in person.

The course is conducted in Italian.

Textbook and teaching resource

The teaching material (slides) and supplementary material (scientific publications, videos, and popular science texts) are available on the e-learning page of the course.

The video recording of each lecture (*didattica erogativa*) will be made available at the end of it.

Semester

Second semester.

Assessment method

Written test organized in open-ended questions (from 8 to 10); duration of 90 minutes. Questions may cover any topic presented during the lectures. Each student may also request to take an oral exam (in the form of a discussion on the topics covered in class) after the written test.

Evaluation criteria: demonstration of successful acquisition of knowledge; ability to organize knowledge discursively. Correct use of specialized vocabulary will be given importance. Through specific questions, the candidate's autonomy of judgment will also be considered and evaluated.

No interim assessments are scheduled.

Office hours

On appointment (in person or via video chat), upon prior request by email (simone.guglielmetti@unimib.it) or in classroom.

Sustainable Development Goals

GOOD HEALTH AND WELL-BEING | QUALITY EDUCATION | INDUSTRY, INNOVATION AND INFRASTRUCTURE
