UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

COURSE SYLLABUS

Probiotic Microbes: Biology and Industrial Applications

2627-1-F0803Q085

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.

Making judgements
Students will acquire the ability to critically evaluate the characteristics of commercial probiotic products, considering their microbiological composition, the consistency between formulation and scientific rationale, the plausibility of the proposed claims, and aspects related to product quality and stability. They will also develop independent judgement regarding the potential risks associated with the intake of live or inactivated microbial biomasses and will be able to distinguish the contexts in which the concept of live biotherapeutics should replace that of probiotics.

Critical thinking and judgement skills will be developed through interactive teaching activities (DI), including the guided analysis of real industrial case studies, controversial scientific publications and regulatory dossiers. During

these activities, students will be invited to discuss the experimental reliability of the available evidence, the robustness of the biological rationale, the methodological consistency of the studies, and the possible applicative, regulatory and socio-economic implications.

Structured guided discussion/debate activities may also be organised on controversial issues related to probiotic microorganisms, such as the relationship between clinical efficacy and marketing claims, the differences between the European and US regulatory approaches, or the distinction between probiotics, postbiotics and live biotherapeutics. These activities will have formative and self-assessment purposes and will not directly contribute to the determination of the final grade.

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

- 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, Escherichia coli*, and *Heyndrickxia 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

Teaching methods

The course includes a total of 45 hours, all delivered in person.

Teaching activities include 35 hours of expository teaching (DE), dedicated to the systematic presentation of the scientific, technical, industrial and regulatory contents of the course. Within the expository teaching activities, 2–4 hours of seminars delivered by experts from companies in the sector may be included, aimed at exploring applied aspects related to industrial production, quality control, formulation, stability and regulatory issues concerning products containing probiotic microorganisms or other microbial biomasses of interest for human health.

The course also includes 10 hours of interactive teaching (DI), dedicated to the application of the acquired knowledge to concrete problems. Interactive activities will include quizzes, problem-solving activities, guided discussion of case studies, critical analysis of scientific publications and regulatory dossiers, as well as structured guided discussion/debate activities on controversial issues related to probiotic microorganisms. These activities will have formative and self-assessment purposes and will not directly contribute to the determination of the final grade.

The course is also connected to the “Workplace Experience Activity”, which includes a one-day immersion in a real industrial setting, with technical-scientific seminars, a visit to production facilities, and direct interaction with professionals working in the field of microbial biomass production for probiotic products or other biotechnological applications.

No remote teaching activities are planned, except in the case of extraordinary circumstances.

The course is delivered in Italian. Teaching materials and supplementary resources are predominantly provided in English.

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.
Lecture recordings, for the part delivered in expository teaching mode, will be made available after each class.

Semester

Second semester.

Assessment method

The assessment consists of an individual written examination, lasting approximately 60–90 minutes, aimed at verifying the achievement of the expected learning outcomes.

The written examination includes 6–10 open-ended questions covering the topics addressed during the course. Questions may require the description of theoretical concepts, the critical interpretation of scientific publications, the application of principles related to the selection, characterisation, production and quality control of probiotic microorganisms to concrete cases, the analysis of issues related to the safety of microbial biomasses, and the discussion of regulatory, industrial and applicative aspects related to probiotic products, live biotherapeutics and other “biotic” products.

Each question is assigned a score ranging indicatively from 2 to 6 points, depending on its complexity. The sum of the scores determines the final grade, expressed on a 30-point scale. The examination is passed with a minimum grade of 18/30.

The assessment is based on the following criteria:

- accuracy, completeness and up-to-date knowledge of the contents;
- correct use of specialist terminology;
- ability to apply theoretical knowledge to concrete problems related to the selection, characterisation, production and quality control of probiotic microorganisms;
- ability to critically interpret scientific publications, regulatory dossiers and industrial case studies;
- ability to evaluate the safety, functional plausibility and scientific rationale of products containing live or inactivated microorganisms;
- ability to connect the different topics of the course, including microbiological, biotechnological, industrial, regulatory and applicative aspects;
- relevance, methodological rigour and logical coherence of the argumentation;
- clarity of exposition and appropriate use of technical language.

The written examination therefore assesses knowledge and understanding of the course contents, the ability to apply such knowledge to specific cases, independent judgement in the interpretation of scientific, industrial and regulatory issues, and communication skills through the formulation of structured and technically accurate written answers.

An optional oral examination may be held at the request of the student or the lecturer. The oral examination consists of a discussion of the course topics and/or of the written examination and allows the overall assessment to be completed, with particular reference to the ability to argue critically, connect the different topics of the course and use appropriate technical language. The oral examination may modify the grade obtained in the written examination, either upwards or downwards, based on the outcome of the discussion.

No graded mid-term examinations are planned. Examples of questions or mock examination materials may be made available during lectures and/or on the course e-learning page.

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