

Miroslava Kačániová, Natália Čmiková

Microbiological laboratory methods

Nitra 2025



Title: Microbiological laboratory methods

Authors:prof. Ing. Miroslava Kačániová, PhD. (1.71 AQ)Slovak University of Agriculture in NitraFaculty of Horticulture and Landscape EngineeringInstitute of Horticulture

Ing. Natália Čmiková (1.71 AQ) Slovak University of Agriculture in Nitra Faculty of Horticulture and Landscape Engineering Institute of Horticulture

Reviewers: prof. RNDr. Leona Buňková, Ph.D. Tomas Bata University in Zlín, Faculty of Technology, Institute of Environmental Engineering

> doc. Ing. Simona Kunová, PhD. Slovak University of Agriculture in Nitra Faculty of Biotechnology and Food Science Institute of Food Science

The manual was published with the financial support of the KEGA project 023SPU-4/2024.

Approved by the Rector of the Slovak University of Agriculture in Nitra on 20. 2. 2025 as textbook for students SUA in Nitra.

ISBN 978-80-552-2841-9

Content

List of figures
Introduction
1. Safety Guidelines for the Microbiology Laboratory7
2. Preparation of Culture Media for Microorganism Cultivation16
3. Sample Preparation for Microbiological Quality Testing
4. Preparation of Dilutions for Microbiological Analysis
5. Inoculation of Culture Media with Microorganisms
6. Preparation of Microorganisms for Antimicrobial Activity Measurement and Optical Density of Microbial Inoculum Measurement
7. Disk Diffusion Method for Antimicrobial Activity Testing
8. Minimum Inhibitory Concentration (MIC) Testing
9. Sample Preparation for <i>In Situ</i> Antimicrobial Activity
10. Preparation of Colonies for MALDI-TOF Analysis
11. MALDI-TOF Analysis for Microorganism Identification
Conclusion
Final Thoughts
References
Sources of images



List of figures

Figure 1. Showing some of the principles of working in a microbiology laboratory (URL 1).
Figure 2. Procedure for the preparation of the culture medium (URL 2)
Figure 3. Preparation of samples for microbiological analysis (URL 3)
Figure 4. 10-fold dilutions for microbiological analysis (URL 4)
Figure 5. Inoculation of microorganisms (URL 5)
Figure 6. Petri dishes showing different levels of optical density of micro-organisms
(URL 6)
Figure 7. Disk diffusion method (URL 7)
Figure 8. Minimum Inhibitory Concentration (URL 8)
Figure 9. Vapor phase in situ analysis
Figure 10. Preparation of samples for identification by mass spectrometry (URL 9, edited). 75
Figure 11. Identification of microorganisms by MALDI-TOF MS Biotyper (URL 10) 83

Introduction

Microbiological laboratory techniques are essential for understanding processes and analyses related to microorganisms, which play a crucial role in various industries. This methodological manual serves as a comprehensive resource for students, providing a structured approach to mastering both basic and advanced laboratory methods. Each section includes step-by-step instructions, outlines different scenarios that may arise during microbiological analyses, and helps students develop both manual and technical skills. Additionally, accompanying videos enhance comprehension through visual demonstrations.

Why is this methodological manual important?

Microorganisms influence various aspects of life, from supporting specific ecosystems and processes to affecting human health-both positively and negatively. Understanding their behavior, interactions, and characteristics is essential for addressing global challenges such as antimicrobial resistance, foodborne diseases, and the development of biotechnological solutions, as well as the coexistence of microorganisms with plants or the contamination of various commodities.

This manual highlights not only the technical skills required for working with microorganisms but also the importance of critical thinking and precision in each step of laboratory work. Mastering the selected methodological procedures presented in this manual ensures the accuracy and reliability of students' experiments. The experience gained will provide a deeper understanding of microbiological analytical methods, which can be applied in laboratory exercises for other subjects. Moreover, it prepares students for work in similar laboratories, which they may encounter in their future careers.

This methodological manual is divided into 11 sections, each focusing on a specific aspect of microbiological analysis and its procedures. These are essential for laboratory work during the courses **Bacterial Diseases of Plants** and **Microbial Biotechnology in Horticulture**. Together, these methods provide a comprehensive workflow, covering everything from laboratory safety and microorganism handling to the use of advanced techniques necessary for microorganism identification.



Through this manual, students will not only acquire manual and technical skills but also gain a broader understanding of microbiological methods, data analysis, and result evaluation. During practical exercises, they will develop precision, critical thinking, and problem-solving skills, which are essential for handling microorganisms and other materials. These competencies are necessary for contributing to scientific progress and solving real-world challenges related to microbiological techniques and data interpretation.

Whether students aim to gain a foundational grasp of laboratory techniques to advance their careers in research, diagnostics—such as identifying plant pathogens—or within horticultural industries, this methodological manual serves as an invaluable resource for their academic and professional journey. It may also help them assess microbial risks in everyday life.



Authors: prof. Ing. Miroslava Kačániová, PhD. Ing. Natália Čmiková

Titlle: Microbiological Laboratory Methods

Published by: Slovak University of Agriculture in Nitra Edition: first Copies: 50 Year of Publication: 2025 Number of pages: 90 AQ - PQ: 3.42 - 3.58Not edited in Publishing centre of the SUA in Nitra.

ISBN 978-80-552-2841-9

The publication was printed on ecological papers.

