Integrated Study of the Effects of Probiotics, Plant Extracts, and Laboratory Analyses on Plant Health, Food Product Quality, and Clinical Applications Using Innovative Biotechnological Techniques

Editors

Amna Mohammed Khudhr
Al-Farabi University College of Science Department of Biology

Dalal Hussein Jasim Mohammed
College of Science University of Baghdad Department of Biotechnology

Sarah Abbas Ali Kadhim Alghanmi
Agriculture College University of kerbala Department of plant protection

Doaa Adil Rabee Abdali
Agriculture College University of Kerbala Department of food science

Riyam Dakhil Mohsin Hargoosee
University of Karbala College of Applied Medical Sciences Department of Pathological Analysis

Bright Sky Publications ™
New Delhi

Published By: *Bright Sky Publications*

Bright Sky Publication
Office No. 3, 1st Floor,
Pocket - H34, SEC-3,
Rohini, Delhi, 110085, India

Editors: *Amna Mohammed Khudhr, Dalal Hussein Jasim Mohammed, Sarah Abbas Ali Kadhim Alghanmi, Doaa Adil Rabee Abdali and Riyam Dakhil Mohsin Hargoosee*

Edition: *1st*

Publication Year: *2025*

Pages: *110*

Paperback ISBN: *978-93-6233-393-3*

E-Book ISBN: *978-93-6233-588-3*

DOI: *https://doi.org/10.62906/bs.book.474*

Price: *₹545/-*

Abstract

The core objectives, scope, and underlying hypotheses of an integrated study evaluating probiotics and plant extracts in agriculture, food, and clinical applications are synthesized. The primary aim is to elucidate interactive mechanisms, with supporting objectives focused on enhancing product quality, establishing safety profiles, and enabling clinical translational, all underpinned by diverse biotechnological techniques. Laboratory-based analyses provide the foundation for interactions among probiotic, phytochemical, and pathogenic systems, with omics approaches furthering understanding of probiotic activity and guiding translational application. Evidence for the action and interaction of probiotics and phytochemicals in promoting plant health and subsequent food stability and safety, along with the nurturing of gut microbiota and immunomodulatory support, lies at the study's heart. Measurable indicators spanning agricultural, food, clinical, and laboratory domains act as benchmarks, with feasibility constrained by installation and operational costs.

Modern biotechnology relies on a suite of established technologies, especially fermentation (mainly lactic acid bacterial fermentation), extraction (of the soluble liquid or complex), chromatography, spectroscopy, and the so-called omics technologies, which include genomics, proteomics, transcriptomics, and metabolomics. Biotechnology is essentially an intertwining and intersection of disciplines in biology, chemistry, chemical and biological engineering, and data science. Modern biotechnology constitutes a union of long-established disciplines (especially microbiology), with the discovery of novel microbial and archaeal species and the

assembly of comprehensive biochemical and genetic databases providing the springboard for recent advances in its diverse fields of application and specialization.

Contents

Chapter - 1

Introduction to Integrated Biotechnological Approaches

As with science and technology, the boundaries between disciplines often impose restrictions on research and innovation. With these boundaries in mind, the proposed work involves research on probiotics and plant extracts. Going beyond laboratory analyses, the experimental results are then integrated by virtually (bioinformatics approaches) melding the results into a single comprehensive discussion during the interpretation phase. Three major fields are represented—agriculture, food science, and medicine. With their mechanisms of action characterized, the probiotics and/or plant extracts are then supplied to other fields for evaluation, thereby extending the analytical needs beyond the traditional laboratory scope. The work synthesizes the basic science underlying these areas and provides details of supporting experimental data, the overarching goal being to explore the possibility of enhancing both the food system and human clinical conditions by making use of related technologies.

Probiotics and plant extracts are of prime importance for agriculture (promoting plant health and also aiding soil health), food science (extending shelf-life, serving as natural preservatives, enhancing flavor and aroma, imparting texture, and serving as nutrient boosters), and clinical research (providing antioxidant and anti-inflammatory actions, modulating gut health, and preventing or controlling a range of diseases). Despite their importance in these three domains of modern applied/industrial biotechnology, the interfaces and interactions among the disciplines have not been widely explored. A detailed analysis of the involved mechanisms and techniques shows that the three major branches have critically overlapping areas of interest [1, 2, 3].

Overview of Modern Biotechnology

Modern biotechnology emerges from interdisciplinary linkages between biology, chemistry, engineering, and computer science. Laboratory-dependent technological advances in fermentation, extraction, chromatography, spectroscopy, and omics enable the systematic evaluation of probiotics and plant extracts, their interactions, and their impact on plant health, food quality, and human well-being.

The application of modern biotechnology has undergone significant evolution. The earliest applications involved the use of living microorganisms and enzyme preparations for fermentation and the discovery of antibiotics for therapeutic purposes. Research in laboratory-based Biotechnology has subsequently advanced to the systematic application of all biological systems and products, driven by the commercialization of integrated biological models through the mechanisms of Biotechnology and Genetic Management. Recent advances in molecular biology and the development of removal and identification technologies for bioactive components have led to the emergence of the new branches of omics and their application in the experimental evaluation of bioactive and bioactivate systems. These technologies provide the means of establishing detailed integration among Life Sciences, Health Sciences and Engineering, Environmental Sciences, and Food Sciences [4, 5, 6].

Interdisciplinary Relevance of Probiotics and Plant Extracts

The three domains of application are closely linked at the biochemical level by their association with living organisms and their metabolites. Probiotic microorganisms can enhance soil microbial quality through introduction, and phytochemical-based products can support soil microorganisms. The two approaches thus act in synergy to improve soil health, plant health, and food quality and safety. Standard microbiological methods can characterize these interactions in detail.

Interactions among bioactive components should enhance therapeutic efficiency in clinical practice as well. For example, combined treatment with probiotics and phytochemical-rich plant extracts is anticipated to yield greater health benefits than either treatment alone. Integrated use of the two bioactive groups should reduce the dosage burden associated with clinical application. Safety and potential toxicity concerns linked to high doses of chemical drugs may also be alleviated [7, 8, 9].

Importance of Laboratory-Based Analytical Techniques

Probiotics, phytochemicals, and their interaction can be quantified, identified, and functionally assessed by various laboratory-based analytical techniques. Chromatography, including separation techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC), plays a central role in quantifying and identifying bioactive phytochemicals from plant extracts. Spectroscopic techniques such as ultraviolet-visible (UV-Vis) spectrophotometry, nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and mass spectrometry (MS) are essential for the characterization and profiling of natural compounds. Microbiological and molecular biology assays are used to determine the growth-inhibitory effects of extracts against pathogenic and spoilage microorganisms and to assess important probiotic attributes of potential probiotic microorganisms. Other microbiological assays (e.g., for antioxidant activity) and molecular biology assays (e.g., for measuring expression of defense-related genes) are employed in the assessment of the activity of bioactive combinations in plant models. Protocols for method validation, calibration, and quality assurance, such as those of Good Laboratory Practice (GLP), are applied.

The high degree of development in applied biotechnology demands rigorous analytical techniques at the laboratory level for the quantification and identification of bioactive compounds and the evaluation of their action and activity. Probiotics and phytochemicals from terrestrial and aquatic plants are receiving considerable attention as a safe and effective way to promote plant growth and protect against disease, while also potentially enhancing food quality and supplementing or improving treatment of various diseases in humans and animals [10, 11, 3].

Rationale and Objectives of the Integrated Study

The primary aims of this integrated study are to obtain a detailed understanding of the interaction between probiotics and phytochemicals, ascertain the impact of individual and combined action on plant growth and resilience, and identify factors that govern interaction efficacy. Correlating the multitiered evidence will reveal mechanisms of action, potential for food quality and safety enhancement, and therapeutic efficiency against infections and inflammatory diseases. The underlying hypothesis posits that probiotics serve as a matrix for the application of phytochemicals and facilitate features beyond the individual action of either bioactive agent.

The probiotics employed belong to a community of multiple species and genera, all investigated under identical conditions, and produced from one origin and environment.

Correspondingly, the phytochemicals include extracts from diverse medicinal plants classed into groups of similar components and functions, targeting the collective probiotic community. Measurable indicators encompass analytical factories of concentration, quantity, and composition of bioactive components; quality and security parameters of food; and empirical evaluations of resilience against biotic and abiotic stresses, pungency, rot, redness, and inflammation. Potential limitations arise from heterogeneity among chemical groups, possible antagonism with fungicides, and the limited understanding of combinatorial and environmental rules [12, 13, 14].

Chapter - 2

Fundamentals of Probiotics and Their Bioactive Roles

Probiotic microorganisms are pivotal to sustaining ecosystem health. They play key roles in human and animal health, food production and preservation, and agricultural practices. These functions derive from three organizing principles: probiotic microorganisms comprise a defined group of microorganisms with specific characteristics; they confer health benefits to the host in which they reside or to other organisms via chemical-mediated multi-sensory mechanisms and by affecting the activity of other microorganisms; and they perform their roles in multiple biological systems, ranging from hosting the human gut microbiome to contributing to soil fertility.

In industry, probiotic microorganisms are predominantly sourced from fermented milk products and the human intestine, with Lactobacillus and Bifidobacterium species being the most widely used. Fermentation and incomplete scaling-up are the chief impediments to practical application, but innovative strategies can mitigate these challenges. Probiotics have proved beneficial in recent studies of agricultural applications related to cholera, biocontrol, and flavor enhancement, as well as in food safety, health promotion, and ameliorating chronic diseases [15, 16, 17].

Classification and Characteristics of Probiotic Microorganisms

Probiotic microorganisms are living microorganisms that exert beneficial effects on the host when consumed in adequate amounts. They are usually derived from the gut microflora of healthy humans and animals. The most widely used probiotic constituents belong to Lactobacillus and Bifidobacterium, with medicinal properties attributed to other bacterial taxa resistant to bile acids and acid, such as Saccharomyces, Enterococcus, Streptococcus, Lactococcus, and Bacillus. A diverse spectrum of probiotic strains is also extracted from non-conventional sources.

The classification of probiotic microorganisms is based on the principles of the International Code of Nomenclature for Bacteria and is especially consistent with the criteria of Schneider *et al*. Goldman *et al*., and Bostan *et al*. on probiotic species. An analysis of these studies concentrates on different probiotic genera. Compounds grouped within microbial derivatives, also known as postbiotics, offer health benefits with therapeutic potential. Examples of microbial derivatives include peptidoglycan, polysaccharides, arginine, glutamic acid, lactic acid, acetylcholine, and heat-killed or inactivated microorganisms. Microbial fermentation products found in food and feeds, such as sake, wine, and vinegar, offer health benefits and support the term probiotic [18, 19, 20].

Mechanisms of Action in Biological Systems

In recent years, probiotics, phytochemicals, and combinations thereof have gained popularity as bioactive agents capable of improving plant health, enhancing food quality and security, supporting animal and human health, and modulating the microbiome. Mechanistic investigations in all application areas are now essential for maximizing their bioactivity and determining pathway control variables. Probiotics are defined as live microorganisms that confer health benefits on the host upon adequate intake. They can act in a range of environments and exert direct and indirect benefits on plants. Phytochemicals are natural secondary metabolites produced by plants for self-defense against biotic and abiotic stresses. Phytochemicals from diverse plant families are used in agriculture.

Synergism between phytochemicals and probiotics at the level of the host, food products, and pathogenic organisms can enhance therapeutic efficiency. Such synergistic action has been supported by limited clinical and *in vivo* study evidence. Validating and expanding this knowledge base may lead to novel formulations with more pronounced health benefits for humans and animals. Integration within an experimental framework that covers agricultural, food science, and clinical applications can provide a richer understanding of these interactions and their regulating factors, with potential contributions to data-sharing platforms and interdisciplinary collaboration structures [21, 22, 23].

Industrial Sources and Cultivation Techniques

A variety of probiotic microorganisms can be obtained from diverse sources. Probiotics that are added to food or feed must fulfill certain requirements such as being free of pathogenicity, showing adequate growth in the product matrix, and withstanding the storage and passage through the gastrointestinal tract. In addition, to ensure safety with respect to their application at different dosage levels, their consumption, vegetation, and metabolic properties also need to be evaluated. Probiotic microorganisms are typically produced in separate manufacturing processes and subsequently formulated together in the final products. Such mixtures are nevertheless susceptible to nutrient competition or antagonistic effects.

Fermented dairy and non-dairy products are rich sources of water-soluble vitamins, which can potentially be absorbed through the skin. Regular consumption of probiotic fermented products or direct topical application of certain strains can possibly improve the overall health of the skin. In order to utilize the essential components secreted during fermentation by probiotics, some companies commercialize probiotic fermentation filtrates. Whole-cell or cell-free filtrates of probiotics can also be used as ingredients for food and feed formulations. The production of any ingredients, including probiotic fermented filtrates, must be scaled up to fit the demand in industry [24, 25, 26].

Applications in Agriculture, Food Science, and Medicine

Probiotic microorganisms and plant extracts interact through shared biological mechanisms, and their combined application can augment biological processes in agriculture, food science, and medicine. Probiotic microorganisms profit plants primarily through plant growth-promoting properties, while secondary metabolites of plants possess antimicrobial properties, ameliorating food spoilage and pathogen accumulation during storage. Moreover, evidence points toward synergistic health benefits of probiotic and phytochemical combinations. For example, these combined agents possess superior antioxidant and anti-inflammatory activities compared with either group alone, and such a composition could serve as a nutraceutical formulation, modulating gut health by balancing dysbiosis and enhancing immunity.

Plant Growth-Promoting Rhizobacteria (PGPR) are Plant Growth-Promoting Rhizobacteria (PGPR) are microorganisms that benefit plants by augmenting root development, improving soil-plant nutrient transfer, and affecting signaling pathways. Various mechanisms underlie these effects. PGPR secrete plant hormones such as auxin, gibberellins, and cytokinins that promote vegetation and radial growth. Decomposition of soil organic matter by PGPR releases substrates that encourage fungal, actinomycete, and free-living nitrogen-fixing bacterial growth, ultimately enhancing plant nutrition. In addition, PGPR can optimize carbon, nitrogen, and phosphorus cycling in soil as co-occurring species, affecting nutrient availability in specific croplands.

PGPR microorganisms are also considered biocontrol agents based on their ability to induce systemic resistance in plants against specific pathogens. The overexpression of pathogenesis-related (PR) genes, such as β-1,3-glucanase, chitinase, PR-2, PR-3, PR-4, and PR-5 further demonstrates this function. These bacteria prevent pathogen establishment by secreting antimicrobial compounds, degrading cell walls, or inducing ectopic or localized hypersensitive responses in host plants. Overall, these defense mechanisms lower pathogen-induced yield loss and promote agricultural sustainability [27, 28, 29].

Chapter - 3

Phytochemicals and Plant Extracts: Composition and Functional Properties

A broad array of phytochemicals can be recognised in the plant kingdom, including flavonoids, phenolic acids, tannins, terpenoids, alkaloids, and glucosinolates, with each group constituting a large family of compounds with varying structures and functions. A few well-studied representatives are provided in Table 1.9. Collections of phytochemicals synthesized within a single plant or in a particular plant part may be grouped into corresponding extracts and water, alcohol, methanol, or acetone used for extraction. Sugar, pH, anthocyanin concentration, and other parameters influence the stability of extracts. Quality control constitutes an important consideration in applying extracts and includes the determination of total polyphenol or flavonoid content and the quantification of implicated antimicrobial, antioxidant, and anti-inflammatory constituents. Primary bioactivity studies typically involve the examination of antioxidant capacity using DPPH, ABTS, ORAC, or FRAP protocols as well as antimicrobial testing against bacteria, fungi, or viruses, with additional assays employed according to the intended applications of the extracts. Phytochemicals exert bactericidal, cytotoxic, and genotoxic effects on preclinical pathogens, with the potential to modulate the gut microbiota. Extracts from sources long used in traditional medicine have been shown to elicit a spectrum of therapeutic actions.

Plant extracts with multiple properties offer possibilities for preservation in food. For example, the efficacy of rosemary extract for controlling antimicrobial pathogens in foods has been demonstrated, with potential enhancement of shelf life. Plant extracts may also be used to control food spoilage organisms. Active components not only contribute to the preservation of food quality but may also enhance sensory attributes and nutritional value. The presence of these compounds can also minimize changes in colour, texture, and flavour during storage, providing a possible strategy for increasing the shelf life of perishable bonito fish. In addition, leaf extracts from a diverse range of plants have been successfully tested for enhancing beneficial functions in food like non-meat sources and have exhibited enhancement of sensory attributes [30, 31, 32].

Major Classes of Bioactive Phytochemicals

Plant-derived chemicals with important biological activities are called phytochemicals. Although phytochemicals are not nutrients, they support biological functions and are involved in plant defense. The most widely distributed and consumed phytochemicals in the human diet are secondary metabolites, the most important of which include flavonoids (e.g., flavones, flavonols, flavanones, isoflavones, flavan-3-ols, anthocyanins), terpenoids (e.g., monoterpenes, sesquiterpenes, sterols, carotenoids), alkaloids, glucosinolates, glycosides, saponins, tannins, and phenolic acids. However, alkaloids are generally toxic or psychoactive in high concentrations and are not typically consumed as health-promoting components. Major classes of phytochemicals, along with representative compounds, are listed. Identifying their chemical nature is crucial for understanding biological action.

Natural extracts play a vital role in several fields of life, including medicine, agriculture, food preservation, the cosmetic industry, and in nutrition. They are used as health care products in various forms including dietary supplements, medications, teas, cosmetics, and functional foods in commerce all over the world. They have been used either as a complementary treatment for specific diseases or as nutraceuticals for potential health benefits. Health-promoting effects of natural extracts, including antimicrobial, antioxidant, anticancer, anti-inflammatory, antidiabetic, anticardiovascular, and neuroprotective activities, are well documented. Extracts from ginger, curcuma, onion, green tea, red grapes, citrus peels, medicinal mushrooms, and other sources exert protective effects against sodium azide-induced toxicity. Extracts also exhibit anticancer potential against various cancers, including colorectal, breast, lung, skin, and prostate. More recently, extracts from piper longum, momordica charantia, solanum nigrum, asparagopsis taxiformis, and feijoa sellowiana were reported to ameliorate DNA damage [33, 34, 35].

Extraction Methods and Standardization of Plant Extracts

Numerous extraction techniques are available for the recovery of bioactive phytochemicals from plant tissues suited to specific metabolites, including steam distillation, cold expression, maceration, reflux extraction, microwave-assisted extraction, solvent extraction, enzyme-assisted extraction, supercritical fluid extraction, and ultrasound-assisted extraction. Proximal estimation helps to categorize the extract quality by different aspects. Substantial quantities of moisture, ash, and soluble carbohydrates may indicate a low concentration of bioactive compounds, while high lipid content means limited thermal stability, low oleoresin consistency, and poor functional properties. Water-extractable polysaccharides (WEP) exert major roles in health benefits, while non-reducing sugars protect sensitive biological systems from injury and facilitate cryoconcentration and cryopreservation.

The standardization and quality control of phytochemicals demand analysis of identity, purity, composition, potency, strength, and stability. Proper quality maintenance is necessary to prevent contamination, maintain activity, and conserve therapeutic potential. Vitamin C, phenolic content, antioxidant capacity, and antimicrobial properties of various plant extracts and essential oils are well established. Standardization of extracts with bioactive compounds is essential for better production and commercialization, achieved by comparing sample concentration with standards [36, 37, 38].

Antimicrobial, Antioxidant, and Anti-Inflammatory Properties

Probiotics and phytochemicals possess intrinsic antimicrobial, antioxidant, and anti-inflammatory properties. Probiotics combat infections via multiple mechanisms, while phytochemicals deploy diverse strategies against pathogens. Notably, combinations of probiotics and phytochemicals may synergistically enhance these effects. Demonstrating such interactions can open novel avenues for clinical applications and integrated strategies for food safety, quality, sustainability, and plant health.

Characterizing the antimicrobial, antioxidant, and anti-inflammatory potential of probiotics and herbal extracts is crucial for assessing their suitability in combined therapeutic applications. Synergistic benefits are indicated by emerging evidence and remain to be systematically explored. Interactions between Lactobacillus reuteri and Berberis vulgaris or silver nanomaterials represent promising leads. Combined bioactive applications offer exciting prospects in various sectors, with an expanding knowledge base in microbiology, molecular biology, and omics technologies fuelling development [39, 40, 41].

Applications in Agriculture, Food Preservation, and Therapy

Bioactive plant molecules, although developed mainly for their therapeutic properties, are increasingly recognized for their modulatory action on the terrestrial and aquatic microbiomes and their ability to interact with probiotic microorganisms. The combined use of probiotic genera and bioactive phytochemicals for the improvement of plant health and growth, the preservation and stability of food products, and the modulation of chronic diseases has attracted considerable research interest. The joint application of probiotics and plant extracts not only generates enhanced protective and restorative effects on biological systems, but also fosters health-promoting properties that are greater than the sum of the individual components. However, the efficacy of such cross-functional responses is not only governed by intrinsic characteristics of the probiotic strains and phytochemicals involved, but can also be modulated by extrinsic factors such as the synthesis conditions of the plant extract and its administration route.

The application of probiotics in agriculture, food science, and therapy is underpinned by a variety of mechanisms and system responses. In agriculture, probiotics play a direct role in plant growth promotion and developmental disease resistance or act indirectly by enhancing soil microbial diversity and fertility. For food science, probiotics improve fermentation of food products and beverages, extend shelf life by reducing spoilage organisms, and modulate food flavour and quality. In the clinical context, probiotics support human health and prevent disease through various pathways that are also accessible to non-viable cells or cell-free supernatants. The complementary and synergistic action between probiotic strains and plant-derived phytochemicals further underscores the relevance of probiotics as supporting agents in the application of bioactive interventions in agriculture, food science, and clinical settings [42, 43, 11].

Chapter - 4

Interaction of Probiotics and Plant Extracts in Enhancing Biological Systems

Contributions to plant health, food quality, and clinical outcomes share a common foundation in the interaction of probiotics and plant extracts. In environmental contexts, probiotic microorganisms improve soil fertility, while phytochemicals incorporated into the soil enhance microbial activity, nutrient availability, and plant growth. In product contexts, probiotic starter cultures drive desirable fermentations and inhibit spoilage and pathogenic organisms, while plant extracts extend shelf life and improve sensory qualities. Acceptance of both probiotics and phytochemicals into nutraceutical formulations stems from supporting evidence of health benefits, and advantages of combining both classes are substantiated by physiological and biochemical analyses.

These interactions should be exploited and thoroughly evaluated for optimal gain. In plant systems, enhanced probiotic activity could be fostered with carefully selected phytochemical amendments. In food systems, an extra dimension of safety could be provided with phytochemical-rich packaging materials. In clinical applications, the therapeutic potential of probiotics could be further augmented with phytochemical supplements. For all applications, the interaction could be fine-tuned by proper selection of the specific probiotics and phytochemicals under consideration [44, 45, 46].

Synergistic Effects on Plant Health

Mutually beneficial interactions between probiotics and phytochemicals enhance plant health, improving growth, resilience, and disease resistance. Probiotics promote growth as plant growth-promoting rhizobacteria (PGPR), whereas phytochemicals stimulate plant defenses by acting as natural elicitors. Consequently, the combination of probiotics with phytochemicals from plant extracts offers a powerful tool for promoting plant health and tolerance to the effects of climate change. These products prevent biological and chemical diseases and enhance plant growth through induction of systemic resistance.

Plant growth-promoting rhizobacteria (PGPR) are associated with root systems and are capable of stimulating plant growth by improving nutrient availability and modulating phytohormone levels. Probiotics may enhance resistance against pathogens and pests by producing bioactive compounds with antimicrobial activity or acting as a source of natural elicitors. The production of good-quality seedlings and the addition of these probiotic products to soil or growth medium before sowing can be an efficient strategy for improving the performance of food crops [2, 1, 47].

Combined Action in Food Stability and Safety

Probiotics and plant extracts exert independent and combined antimicrobial activity on spoilage and pathogenic microorganisms in food products. Probiotic microorganisms and lactic acid bacteria (LAB) from fermented foods impart distinctive flavors and aromas and help maintain quality during storage. METAFOOD (meta-analysis of a database of experiments with edible plants and their chemical constituents) identified natural preservatives present in edible plants and fruits that can be used in food products to extend shelf life. Antimicrobial plant extracts not only preserve food products for longer periods but also increase the safety of food products by reducing spoilage and pathogenic microorganisms.

Prototype salads with leaf lettuce and regional herbs (basil, rosemary, and thyme) were developed using calcium lactate as a firming agent. LAB fermentation improved the bacterial quality and stability of salads, affecting taste and aroma. During storage, flavor stability was maintained, but fermentative activity was detected after 12 days at 5 °C. Olives and salsa with Lactobacillus plantarum, pumpkin, beetroot, and cucumber pickles with Leuconostoc mesenteroides maintained quality during storage. In cider, rhythmic oscillation of Lactobacillus helveticus prevented spoilage, and the product was stable for 12 months. Novel tomato sauce containing grape must as a sweetener, Basilicum essential oil, and low sugar content (38%) fermented by Lactobacillus pentosus showed freshness and coffee aroma after 6 months of storage at cold temperature.

Beverages with probiotic bacteria in high-carbohydrate-rich base materials (coconut milk, pumpkin syrup, sapodilla syrup) developed in carbonated forms induced health effects by modulating gastrointestinal activity. Fermentation improved sensory quality and may control the growth of spoilage microorganisms during storage. Fermented soy-and-kadalai flower milk mixture with higher antioxidant properties was shown to modulate the gut microbiota of albino rats, thereby reducing colitis symptoms. When consumed by humans, kola powder-enriched fermented milk improved the total antioxidant capacity and reduced the symptoms of atopic dermatitis [48, 49, 50].

Enhanced Therapeutic Efficiency in Clinical Settings

Combined application of probiotics and plant extracts can enhance the therapeutic efficiency of bioactive agents in clinical settings, adding to the indications for probiotic-phytochemical products. Evidence accumulated to date suggests that probiotics modulate gut microbiota composition and activity, stimulate local mucosal immunity, and secrete bioactive substances with beneficial effects for the host. Active compounds from plant extracts exert analogous effects, ameliorating intestinal and systemic diseases by acting directly against microbial pathogens, restoring homeostasis, relieving inflammation, and diminishing oxidative stress. Therapeutic outcomes may be further improved when probiotics and plant extracts are administered together, their combined action and physiological role appearing to be more effective than that of either group alone.

Such synergistic effects have been also observed in animal experiments. Indications for the development of probiotic-phytochemical medicines can also be derived from synergistic processes, but further toxicity assessments are necessary to establish dose safety and set the acceptable range for human consumption. Therapeutic applications depend on the results of double-blind placebo-controlled studies and meta-analyses evaluating the efficacy of probiotics in different human diseases [51, 23, 52].

Factors Affecting Interaction Efficiency

The aforementioned examinations have elucidated that the co-application of probiotics and plant phytochemicals can produce enhanced benefits in diverse systems compared to individual application. However, certain factors should be taken into consideration to harness the synergistic effects of probiotics and plant extracts in biological systems.

Microbiome composition appears to play a pivotal role, as a specific coryneform bacterium and Lactobacillus plantarum were crucial for the probiotic-synergized protective effects conferred by a plant extract on apple fruit. The biological properties of both probiotics and plant extracts also govern their interaction efficiency; foliar application of L. plantarum combined with an Artemisia vulgaris extract improved the quality and defense capacity of black mulberry fruit, while co-treatment of kiwi seedlings with a Bifidobacterium and a Rosa rugosa extract conferred osmotic-stress tolerance. External conditions such as raw-material ratios and fermentation periods affect the overall efficacy when probiotics and plant extracts are employed in combination [53, 54, 55, 53, 54, 55].

Chapter - 5

Advanced Laboratory Methods for Analyzing Bioactive Compounds

Advanced laboratory methods for quantifying, identifying, and functionally testing bioactive compounds of probiotics and plant extracts utilize well-established microscale assays in microbiology, molecular biology, chemistry, bioinformatics, and data science. Quality control relies on calibration against suitable reference standards and use of positive and negative controls. Methodological validation, standard operating procedures (SOPs) for Good Laboratory Practice (GLP), and quality assurance (QA) processes ensure precision, accuracy, reproducibility, and compliance with applicable regulatory guidelines.

Profiling phytochemicals and other low-molecular-weight bioactive compounds often employs gas chromatography (GC) or high-performance liquid chromatography (HPLC), combined with mass spectrometry (MS) or diode-array detection (DAD). Infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy also support structural elucidation. Selection is based on the biological question, the chemical nature of the original extract or the analyte of interest, sensitivity requirements, and availability of equipment. GC provides the highest sensitivity and resolution, but requires volatile and thermally stable samples, with detection limited to mass- or fragmentation-type characteristics. HPLC is widely applicable but less sensitive; additional detection methods are often required to enhance selectivity. HPLC-DAD and HPLC-NMR offer almost universal applicability, but without the high sensitivity of other LC techniques. Quality control of chromatographic method development relies on calibration with suitable, preferably certified, external reference standards. The choice of positive and negative controls supports both testing for biological activity and for safety/risk assessment.

Molecular biology assays in the field of bioanalysis include qualitative detection of target nucleic acids by polymerase chain reaction (PCR) and calmodulin-binding protein (CaM) genes at low copy number using sensitive forms of real-time PCR. Specific protocols are available for a wide range of pathogenic microorganisms to determine their presence or absence in environmental samples. For several of the main spoilage and pathogenic microorganisms in foods, the development of multiplex assays allows the detection of multiple organisms in a single reaction mixture, enabling significant reductions in analysis time and cost. Quantitative systems, such as TaqMan assays, allow the dynamic determination of the level of such microorganisms, further improving the level of information obtained from the analysis.

Chromatography and Spectroscopy Techniques

Chromatography and spectroscopy techniques are commonly employed for the qualitative and quantitative analysis of bioactive compounds in natural products, helping to elucidate their chemical profiles and nutrient compositions. Chromatographic methods, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC), are considered versatile separation techniques and have been widely used for the identification and quantification of phytochemical constituents. UV-visible, fluorescence, and the Fourier-Transform Infrared (FTIR) spectroscopies are used in conjunction with chromatographic techniques for the identification and characterization of compounds, provided significant fingerprinting in food analysis and organic materials.

Though chromatographic techniques coupled with mass spectrometry (MS) are effective in separation and identification of a large range of chemical compounds, with low detection limits, the cost of equipment and complexity of sample preparation make these approaches less accessible for many laboratories. However, recent years have brought several innovations and improvements in general MS analysis systems. These include ionization methods such as desorption atmospheric pressure chemical ionization (DAPCI), laser ablation electrospray ionization (LAESI) for direct mass analysis of solid tissues, and laser ablation whisker nanospray. Despite the substantial contributions that these methods can provide to chemical analysis, they should always be carefully used in conjunction with additional techniques that allow for the detection of other properties [56, 57, 58].

Microbiological and Molecular Biology Assays

Microbiological and ML Assays for Assessing Antimicrobial Activity and Safety of Bioactive Agents Microbiological techniques are critical for determining the antimicrobial properties of test agents against pathogenic microorganisms of plant, food, and clinical relevance. Antimicrobial activity is commonly evaluated through disk-diffusion, well-diffusion, or minimum inhibitory/ minimum bactericide concentration assays. Detection of specific pathogens in test matrices ensures food product safety and contributes to disease diagnosis in clinical settings. Quantification of total aerobic and/or coliform bacteria populations along with yeasts and molds indicates general hygiene quality during food storage. The presence of fecal contamination in foods is assessed by detecting Escherichia coli or E. coli O157:H7 using selective media, confirmatory tests, or molecular assays. The safety of probiotic microorganisms is evaluated by detecting virulence factors involved in intestinal and extra-intestinal infections.

Molecular biological techniques enable the detection of bioactive components and their corresponding genes. Identification traces phylogenetic relationships among different organisms. qPCR quantifies probiotic microorganisms in fermented products, evaluates the abundance of specific bacterial groups in complex samples, and detects pathogens or their virulence genes. Detection of antibiotic resistance genes associated with soil and probiotic bacteria is essential for evaluating the safety of health-associated preparations. The presence of high-grade bacterial or plasmid DNA in probiotic products promotes market acceptability. Molecular detection of mycotoxin-producing fungi in various matrices enhances safety, and polymerase chain reaction-enzyme linked immunosorbent assay detection contributes to rapid screening for plant pathogens. Integrating the above-mentioned techniques supports the comprehensive analysis of biological products and processes [59, 60, 61].

Innovations in Bioactive Component Detection

Detecting and quantifying bioactive components is crucial for assessing functional properties. Chromatography and spectroscopy techniques are widely used to profile phytochemicals, while microbiological assays model antimicrobial activity. Molecular biology techniques screen for pathogenicity factors, determine microorganism identity, and characterize genetic diversity.

Recent innovations in bioactive detection enable quantifying health-promoting compounds with high throughput.

Phytochemical content is commonly analyzed through HPLC and GC-MS profiling, using method-dependent calibration with genuine standards for quantification upon detection. These sophisticated methods, capable of profiling a wide variety of compounds, remain expensive and inaccessible to many labs. As a simple alternative for routinely detecting seven major flavonoids, an HPTLC-based method was developed, validated, and offered calibration equations for each compound. The bacterial culture-based inhibition assay remains the most widely adopted microbial antagonist testing method. Novel composite-agar approaches allow testing multiple phytochemicals against a panel of human pathogens simultaneously for screening extracts with multifaceted bioactivity. Molecular assays enable rapid screening of hundreds of pathogens for one or more target genes with carefully selected primers, providing confidence in pathogen detection.

Laboratory data contribute information-rich biosystem signatures when combined in a cohesive tree structure. Patterns emerge among homologous samples, defined through inter-relationship linking. The integration process guarantees accuracy, precision, and reliability, thus supporting dependable analysis and interpretation of complex data sets [62, 63, 64].

Interpretation of Laboratory Data and Quality Control

Laboratory data generated through chromatographic, spectroscopic, microbiological, and molecular methods undergo interpretation contingent upon the nature of information being scrutinized. To strengthen the integrity of the data generated through testing, quality control measures, including calibration and validation, are urgently applied. Chromatographic methods, as typified by HPLC/PDA analysis, are verified through the inclusion of standard curves, whereas calibration of contrary chromatography is necessitated. Environmental fluctuation may significantly impede accurate reporting of laboratory data; thus, rigorous quality assurance measures must govern each stage of sampling, detection assessment, and valiant validation of methodological parameters for all kinds of biosystems underlined for scrutinization.

Validation, when mentioned, encompasses quality determination for an analytical method—be it method development or sensory assessment of olives, substitutes of saturated fatty acids, or edible coatings from red prickly pear stem. Validation establishes the reliability, precision, efficacy, reproducibility, linearity, sensitivity to minor stress factors, and authentication of generation of tested compounds (antioxidant and antibacterial). For molecular biology assays, the adopted standards, though not yet implied, may be shown, availed through other proclamations. Quality assurance of standardization of probiotics as inputs and of bacterial culture production follows the guidelines of Good Manufacturing Practices (GMP) [65, 66, 67].

Chapter - 6

Soil Microbiome Improvement through Bioactive Interventions

Soil microorganisms play essential roles in natural ecosystems, contributing to organic matter decomposition, nutrient recycling, and mediation of plant disease. The presence of diverse and abundant microbial communities in agricultural soils is a prerequisite for sustainable agriculture that seeks to maximize yield without irreversible degradation of soil health. However, soil microbial diversity continues to decline through the constant application of chemical fertilizers and pesticides. Recently, there has been considerable interest in improving soil conditions and nutrient availability through microbial and phytochemical interventions.

Probiotic microorganisms improve soil fertility by mobilizing soil nutrients, enhancing soil structure, and supplementing organic matter. Phytochemical-based soil amendments recruit beneficial microorganisms, thereby stimulating ecological niche specialization, enhancing organic matter decomposition, and accelerating nutrient cycling. Moreover, biochemical constituents that naturally occur in crop residues and plant-extract-based manures can offer a protective effect in soils. Integrated application of these bioactive compounds in sustainable agriculture has received relatively less attention and deserves further consideration. Bioactivity-based nutrient and soil management can be practiced in conjunction with other biological strategies to increase soil fertility in the long run [68, 44, 69].

Soil Microbial Ecology and Nutrient Cycling

Soil microbes play important roles in nutrient cycling with a broad phylogenetic diversity. Probiotic microorganisms are a group of beneficial bacteria that help improve the fertility of the soil ecosystem when applied as an agro-input. Plant phytochemicals are the main components of natural soil amendments. Their application has positive effects on soil microbial populations by increasing microbial biomass, activity, and diversity. Treatments of soil with phytochemicals or phytochemical-rich plant extracts also enhance the transformation and bioavailability of different soil nutrients.

Soil microbes are broadly classified into two groups according to their ecological functions in the nutrient cycling processes: (a) heterotrophic bacteria (including fungi and actinobacteria) that decompose organic matter, and (b) autotrophic bacteria capable of deriving their energy from inorganic sources, principally nitrifying bacteria (Nitrosomonas, Nitrobacter) that oxidize ammonium to nitrite and nitrite to nitrate. All the biochemical reactions carried out by soil microbes are important, because they increase the concentration of nutrients in the available forms usable by plants. The application of probiotics has been reported to enhance the activities of microbial populations such as soil bacteria, fungi, and actinomycetes, and the levels of N, P, and K in soil. Spore-forming probiotic microorganisms produce several beneficial enzymes, hormones, and bioproducts and can also enhance the nitrogen status of soil.

Soil health is characterized by a stable and functional soil microbial community, which is affected by various climatic and management factors. Changes in soil microbial community composition can have implications for soil functioning. Phytochemicals from plant resources also serve as important soil amendments to improve soil health and fertility. Phytochemical-based soil treatments stimulate feedback processes that enhance the productivity and sustainability of various agroecosystems, which could be a promising approach for sustainable soil management [70, 71, 72].

Role of Probiotic Microorganisms in Soil Fertility

Study of soil microbiome improvement through bioactive treatment, both from microbial and chemical sources, is an important part of sustainable farming. The soil microbial community mediates critical processes, including organic matter decomposition, nutrient availability, plant disease resistance, soil salinity and infertility management, and soil structure and aeration improvement. In this context, probiotic microorganisms such as plant growth-promoting rhizobacteria (PGPR), mycorrhizae, and nitrogen fixers help build up soil fertility, targeting different soil attributes, through various mechanisms. Other bioactive amendments, especially natural phytochemicals, can also modulate key soil microorganisms, enhancing the fertility of unhealthy soil. Amending soil with these microorganisms or phytochemicals is expected to increase crop yields while reducing inorganic fertilizer and chemical pesticide requirements. Integration of these bioactive methods within sustainable farming systems appears to be a viable solution to improving soil fertility, crop productivity, and overall ecosystem health.

The soil microbiome is the hub of plant growth and development. It plays an important role in soil fertility, nutrient cycling, carbon sequestration, plant health, and ecological balance. Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizae are the two most important bioinoculants for augmenting soil nutrients, while other probiotics such as nitrogen-fixing and phosphate-solubilizing microbes, and actinobacteria, also play significant roles. PGPR influence soil physical, chemical, and biological properties, affecting important soil attributes such as pH, organic carbon, electrical conductivity (EC), nitrogen, phosphorus, potassium, and micronutrient concentrations, enzymatic activity, respiration, and microbial biomass [73, 74, 75].

Phytochemical-Based Soil Amendments

Healthy soil nurtures healthy plants. A balanced microbial ecosystem sustains the soil's ability to recycle nutrients, but factors like abuse, pollution, and climate change lead to a loss of microbial diversity. Several authors have reported that the addition of organic matter in the form of compost or other organic amendments can restore soil microbiota. Probiotics can reinvigorate soil during the composting process, while phytochemical-rich herbal extracts facilitate microbial growth and nutrient cycling. Phytochemical-based soil amendments for crop production offer various advantages, including increased soil fertility, supplemented plant nutrient needs, and improved crop yield and quality.

Soil is the natural habitat of different groups and diverse microorganisms that are involved in nutrient cycling and the conversion of organic matter. The activities of these microorganisms help to maintain soil fertility. Probiotic microorganisms are a natural component of the soil microbial ecosystem and can be used as biofertilizers. The use of specific phytochemical-rich herbal extracts can serve as soil amendments compatible with probiotics and help increase the activity of soil microorganisms during the composting process. These amendments can boost the levels of fungi, actinomycetes, bacteria, and nematodes in the final compost product. The incorporation of these amendments into the soil can provide a viable source of organic matter during early-stage crop establishment while fulfilling the nutrient demands of the growing crop and potentially increasing both quantity and quality of produce [68, 76, 77].

Integration of Bioactive Methods in Sustainable Farming

At the intersection of modern agriculture and biotechnology, the microbial and phytochemical commerce in soils is crucial for plant nutrition. Soil microorganisms form an active part of nutrient cycling, and altered populations may lead to reduced soil fertility. Probiotics, such as plant growth-promoting rhizobacteria (PGPR), can facilitate fertilisation processes and promote sustainable farming. Further, low-cost and eco-friendly amendments based on phytochemicals can improve soil fertility and stimulate biogeochemical processes. Using both bioactive methods, therefore, can enhance soil microbial diversity and functionality, reinforce plant health, and support sustainable agriculture. In the wake of climate change, such integrated approaches are essential for developing sustainable agricultural systems.

Soil microbial communities, comprising diverse bacteria, fungi, and protozoa, shape soil health and quality; significantly, only a small fraction of soil microorganisms can be cultured and identified. The main functions attributed to soil microorganisms relate to organic matter decomposition, nutrient recycling, plant disease suppression, and soil structure. Among these, the decomposition of organic matter releases nutrients for plants and other organisms. The bioavailability of these nutrients is a function of microbial metabolism. Different groups of microorganisms are involved in the decomposition of different organic substances at different rates. The addition of organic amendments to soil alters the community composition and microbial biomass, thus stimulating the activity of soil microbes [78, 79, 80].

Chapter - 7

Enhancement of Plant Growth and Disease Resistance

Probiotic metabolites and plant-derived compounds share important antimicrobially active components, enabling synergistic effects against harmful microorganisms. The probiotic ability to secrete various hydrolytic enzymes enhances plant disease resistance, while antimicrobial compounds in phytochemical extracts can be deployed for the biocontrol of economically important plant diseases. The significant role of probiotic microorganisms in promoting plant growth and natural resistance against different pathogens in various biological systems is clearly demonstrated by several studies.

The concept of plant growth-promoting rhizobacteria (PGPR) refers to the beneficial effects of probiotic microorganisms in the soil and immediate surroundings of plants. A large number of PGPR have been identified, expanding the range of plant species in which these beneficial effects are expressed in the presence of the microorganisms. These beneficial effects occur either directly, for example, through nitrogen fixation, stimulation of phytohormone synthesis, or solubilization of important nutrients such as phosphate, or indirectly, through increased resistance to pathogens or environmental stresses. The latter is mediated either by the action of antimicrobial compounds in the root exudates or by induction of physical or chemical mechanisms in the plant [42, 81, 82, 42, 81, 82, 42, 81, 82].

Probiotic-Based Plant Growth-Promoting Rhizobacteria (PGPR)

Probiotics, including bacteria, fungi, and viruses, are routinely administered to humans and animals, often through food. Probiotic-based PGPR can promote seed germination and seedling growth, enhance plant tolerance, reduce soil-borne diseases, and encourage soil fertility. Anaerobic LAB are quick-growing, Grampositive bacteria that play key roles in dairy, meat, and vegetable fermentation, with production of lactic acid and other organic acids lowering pH and creating an inhospitable environment for spoilage and foodborne pathogenic bacteria. LAB improve the flavour and texture of fermented products, increase shelf life and safety, and support a healthy gut environment.

LAB are used in chemical-free vegetable production and postharvest preservation by preventing fungal spoilage, while others possess antagonistic activity against postharvest pathogens and spoilage organisms. Research on Leuconostoc mesenteroides, Lactobacillus sakei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus chiayiensis, and Pediococcus pentosaceus has shown these LAB alter postharvest disease development in vegetables. Whether grown on dairy or vegetable media, these LAB generate probiotics that enhance shelf-life and gut health in humans, and further exploration of these probiotics for use in fermentation and preservation is warranted.

Antimicrobial and Defense-Inducing Properties of Plant Extracts

Phytochemicals exhibit direct antimicrobial effects, serving as natural alternatives to synthetic chemicals. Additionally, plants respond to pathogenic infection by synthesizing diverse chemical compounds to induce systemic resistance. Thus, the application of phytochemical-rich extracts with broad-spectrum antimicrobial activity can protect against various diseases, and exposure to specific classes of phytochemicals (e.g., β-glucans) can induce defense mechanisms. These characteristics can be exploited for developing crop protection with minimal ecological impact.

Reduction of Chemical Fungicides by Natural Substitutes

The overuse of chemical fungicides to manage plant diseases has led to toxicological risks, secondary infections, and the appearance of resistant pathotypes. Against this backdrop, natural phytochemicals and microorganisms have been identified as potential alternatives that can protect agricultural and horticultural crops. Plant extracts are excellent sources of antifungal compounds and their application can reduce the amount of synthetic fungicides used to manage plant diseases..

Plant extracts are complex mixtures of secondary metabolites with the potential for antifungal action. They can be used alone or in combination with very low concentrations of synthetic fungicide. The development of Agri-Nano Polymers (ANPs) containing plant extract-based active ingredients for seed and foliar application can enhance crop yield and control important phytopathogens, extending the technology's application beyond the laboratory stage. In this approach, ANPs carrying combinations of Azadirachta indica, Chenopodium album, Mentha piperita, and Zingiber officinale appear highly effective for controlling principal diseases of Phaseolus vulgaris and Z. mays.

Biocontrol of Plant Pathogens

Probiotics and plant extracts can serve as alternative or supplementary strategies for disease control in many crops. Probiotic microorganisms protect plants from disease by producing antibiotics and other antimicrobial compounds and by inducing systemic resistance against invading pathogens. Antimicrobial phytochemicals from plant extracts also directly inhibit fungal, bacterial, and viral pathogens or induce plant defense responses leading to improved resistance. Combinations of probiotics and phytochemicals may therefore enhance disease suppression effects. Several studies illustrate the roles of bioactive agents in controlling plant diseases and disorders.

Probiotics are beneficial microorganisms that promote plant growth and health. They establish colonization in the rhizosphere and collectively function as plant growth-promoting rhizobacteria (PGPR). Mechanisms include nitrogen fixation, phosphate solubilization, and production of phytohormones and secondary metabolites. PGPR also enhance plant resistance to biotic and abiotic stresses via the actions of various metabolites. Bioactive secondary metabolites produced by medicinal and aromatic plants can inhibit many important phytopathogens such as fungi, bacteria, and viruses [42, 83, 84].

Case Studies on Crop Improvement

Successful applications of probiotics and/or phytochemicals in crop cultivation demonstrate the effectiveness of bioactive interventions in agriculture. Poultry probiotic microflora was administered to tomato plants, which exhibited improved growth traits along with increased antioxidant properties. Algal probiotics were used on Vigna radiata, resulting in plant response to biotic stress and enhanced growth. Probiotics isolated from a saline-alkaline habitat exerted plant growth-promoting ability by producing IAA, solubilizing phosphorus, and enriching potassium. Four strains were evaluated on sesame plants, leading to improved germination rates and reduced disease severity (Myrothecium roridum). Application of Bacillus cereus on soybean seeds with simultaneous foliar spray augmented seed yield and oil content—the synergism attributed to greater nodulation and nitrogen accumulation. A combination of plant extracts providing different modes of action improved metabolic energy, performance, and immune responses in poultry.

The toxicity of Alternaria alternata on the leaf of Passiflora edulis was minimized through mixed culture of Bacillus cereus, Pseudomonas fluorescens, and Enterobacter cloaceae, resulting in a healthy and functional ecosystem. Antimicrobial activity of Trichoderma harzianum against Cylindrocladium floridanum was evaluated on Coffea canephora along with the pathogenic host, and T. harzianum application conferred significant resistance against wilt disease. The interaction of probiotics with plant-growth-promoting ability avoided carbon, flower, and fruit losses and improved fruit quality of diosgenin-rich Dioscorea deltoidea. Natural anti-estrogens, alone or in combination with Trichoderma viride, managed Fusarium wilt incidence of pearl millet and minimized deoxynivalenol levels in grains. Two probiotics effectively reduced the severity of Fusarium wilt in chickpea and promoted growth, yield, and merchantable quality. The efficacy of Bacillus and Pseudomonas for suppressing damping-off and associated growth-promoting effects in radish was also demonstrated [85, 86, 87].

Chapter - 8

Omics Technologies in Plant-Microbe Interactions

Advances in omics technologies open new horizons for understanding complex biological systems. The range of responses of both host and associated organisms to exogenic treatments is documented through principles of genomics, transcriptomics, proteomics, metabolomics, and metagenomics. Genomics provides the blueprint for molecular studies, pinpointing important pathways active in the interaction of interest, while transcriptomics identifies molecular signals involved in different stages of the interaction and exposes which pathways are switched on or off in a specific environment. As genes are ultimately translated into proteins, proteomics captures the last step preceding the multiple biological functions played by metabolites. Integrated analysis of gene transcripts, proteins, and metabolites in tandem with metagenomics of microbiota turnover reveals not only functional responses of the host partner but also elucidates changes in the community structure of associated organisms.

Genomics, the study of whole genes, relies on accurate genome assembly and annotation. Such resources can be used to facilitate expression studies through quantitative PCR or parallel analysis of the complete transcriptome profile during an interaction. Proteomics examines the presence or absence of certain proteins at particular times during the interaction, as well as their relative abundance. Analyses of the information content of a proteome open an unprecedented window into metabolic processes, enabling the functional analysis of the whole set of proteins encoded by an organism's genome. Metabolomics investigates the entire set of metabolites accumulated in an organism at a defined lipid, protein, or nucleic acid composition. Metagenomics enables the characterization of complex communities of microorganisms that are clustered in specific environmental niches [88, 89, 90].

Genomics: Decoding Genetic Responses

Genomics enables researchers to decode the organismal response to different stimuli by analyzing changes in the DNA sequence. Numerous studies have employed genomic techniques to delineate host responses to probiotic treatment. Microorganisms such as Lactobacillus, Bifidobacterium, and Saccharomyces cerevisiae have been reported to enhance the expression levels of genes associated with gut health, transport processes, metabolism, the immune system, and inflammation in mice and human intestinal cells. Moreover, probiotics have been shown to suppress the expression of inflammation-related genes, reduce shigella-induced apoptosis, and enhance intestinal epithelial defense responses in mice. Owing to these merits, combined probiotic use, especially that of bacterial-fungal consortia, is being explored for applications in clinical health management. A metagenomic analysis on the effect of the probiotic Synlogic SL1344 on gut and liver tissues demonstrated successful conditioning of the gut and liver microbiomes toward an anti-inflammatory state. Metagenomic pathways related to copper homeostasis, fatty acid metabolism, and histidine and tryptophan biosynthesis were found to be enriched.

Genomics, especially sequencing technologies, have been employed to identify genes involved in the interaction of beneficial microbes with plant hosts. Metagenomic techniques have been successfully applied to characterize the structure and functional potentials of the root-associated microbial community under PGPR treatment. These studies revealed the active involvement of PGPR in driving the nitrogen cycle and a complex functional interaction among the root system, associated microbiome, and soil environment in the nutrient-poor tropical forest ecosystem. Moreover, genes of plant defense, disease resistance, cell rescue, and oxidative stress response showed significant positive correlations with the relative abundance of Sphingomonas and Burkholderia-Caballeronia-Paraburkholderia. A metatranscriptome-guided assembly of an annotated Humibacter sp. genome was also reported, contributing to the understanding of the assembly and function of the humic matter microbiome in the reed rhizosphere [91, 92, 93].

Proteomics and Metabolomics in Functional Analysis

Genomic investigations reveal the roles of different genes in functional responses of biological systems, while proteomics, an analysis of the entire protein landscape at a given time, automate the checking of changes over the proteome for various treatments. Post-transcriptional and post-translational modifications are also evaluated by proteome analysis. Similarly, metabolomics, which looks at the entire range of metabolites, provides actual functional profiles and predicts responses in a biological system and its interactions.

Metagenomics, with its focus on community DNA, provides profiles of community members involved in a particular treatment. A combination of analyses is used to exam the response of any biological system or analyze the interaction of two different components. Omics technologies increase the understanding of interacting components and provide data for system-level integration and interpretation. Understanding genes, proteins, and primary metabolites is essential for analysis and interpretation of secondary metabolite levels in any biological system [94, 95, 96].

Metagenomics in Microbial Community Profiling

Due to the rapid decline of traditional culture-dependent methods in microbial identification by means of process complexity and phylogenetic variability of the target microbial group, studies focused on microbial communities have largely benefited from the application of metagenomics. Metagenomics comprises a set of next-generation sequencing techniques that permit the study of whole complex communities without prior isolation of individual constituents.

In metagenomic studies, the use of universal primers facilitates amplification of the rRNA genes of both eubacteria and archaea domains, thereby enabling the examination of the composition and structure of total soil microbial communities, and the effects of selected treatments on the dominant groups. The assembled OTU sequences of distinct sample units can be clustered into the same taxonomic groups, confirming the reliability and applicability of metagenomic techniques for analysing total microbial communities of soil samples subjected to PGP treatments [97, 98, 99].

Data Integration for System-Level Understanding

Omics technologies provide complementary information about biological systems at the genetic, protein, or metabolite level. Genomics decodes the genetic response of a system to a specific treatment, enabling identification of genes, transcription factors, and transcriptional pathways related to the phenomenon under study. Integrating transcriptomes with proteomic data allows for the assessment of actual gene product levels and functional status, while correlation with metabolomic profiles yields insights into the metabolic state of the system. In addition, metagenomics enables direct analysis of the genetic material contained in an environmental sample, revealing the presence and relative abundance of taxonomic groups and potential functional traits.

In the interaction of probiotics with plants or food products, genomics contributes to understanding the effect of the probiotic on plant growth, defense response, or food spoilage reduction through the identification of genes responding to the probiotic. Corresponding to these genomic changes, proteomic and metabolomic analyses perform detailed functional validation by providing information on the actual protein levels in the sample and the metabolite composition under different treatments. Metagenomic analyses of probiotic-plant or probiotic-food interaction offer further insight into community dynamics of the microbiome involved in the interaction. Integration of these different levels of biological information enables a more complete system-level understanding of probiotic actions [100, 42, 101].

Chapter - 9

Biotechnological Approaches to Enhancing Food Quality and Safety

Probiotics, known for their beneficial impacts on gut health, find additional applications in food production. Fermented dairy products enriched with Lactobacillus are increasingly popular, serving as dietary vehicles for live probiotics. These microorganisms have been characterized in various food products, such as yogurt, cheeses, and fermented pickles, conferring additional health benefits beyond basic nutrition. Furthermore, they play a crucial role in extending the shelf life of fermented beverages, minimizing spoilage. Recent studies highlight the efficacy of probiotics in mitigating spoilage and pathogenic organisms in various foods, including fresh produce, dairy, meat, and fish.

Plant extracts are valuable in food manufacturing and preservation. Experiments demonstrate the shelf-life-extending capacity of extracts from rosemary, basil, and tarragon in different food products. Experimental probiotics such as Lactobacillus pentosus and Lactobacillus plantarum have emerged in food production for their antagonistic properties against food spoilage and pathogenic species. In addition to augmenting shelf life, practical combinations of these microorganisms with plant extracts are being developed to enhance both sensory and nutritional quality. The resulting innovations are expected to meet rising consumer demand for healthy and safe food products [102, 103, 104].

Role of Probiotics in Food Fermentation and Preservation

Various fermented foods like yogurt, cheese, kefir, vinegar, fermented sausage, and fermented fish are produced mainly with probiotics. Fermentation is a very useful method to preserve food for a longer period of time without any synthetic preservatives. Naturally fermented foods have been prepared since ancient times and have their origin in regional food cultures around the world, but now laboratory preparations are becoming more common. Lactic acid bacteria (LAB), yeasts, and vinegar-producing strains of acetic acid bacteria (AAB) are mostly used in food fermentation. Modern biotechnology may use isolated strains or mixed cultures of these probiotics. Microorganisms used in fermentation play an important role in preservation and may also improve the organoleptic and nutritional qualities of food. During fermentation, microorganisms outcompete spoilage organisms, and acid and other metabolites produced inhibit the growth of potential spoilage and pathogenic organisms. Additionally, some probiotic microorganisms produce bacteriocins and hydrogen peroxide, which can enhance the antimicrobial properties of fermented food.

As most fermented foods are produced using LAB and yeast as starter cultures, these fermented foods and their by-products have proved to be quite safe for human consumption. Additionally, several fermented foods have generally recognized as safe (GRAS) status from the U.S. Food and Drug Administration. Nevertheless, occasional infections and side effects such as gut bloating have been reported, although they are rare. Thus, systematic practice and quality control during manufacture are required. Furthermore, the possibility that perishable food may be fermented (most often spontaneously) by natural microflora during storage complicates safety considerations; uncontrolled fermentation may lead to spoilage or formation of a potentially unsafe product [105, 106, 107].

Application of Plant Extracts in Extending Shelf Life

Probiotics are microorganisms considered beneficial to health when administered in adequate amounts. They are usually lactic acid bacteria (Lactobacillus, Bifidobacterium, and Streptococcus), yeasts (Saccharomyces, Kluyveromyces), and some nonpathogenic strains of Escherichia coli. During fermentation, they produce various compounds (short-chain fatty acids, bacteriocins, and hydrogen peroxide) that help inhibit spoilage pathogens and spoilage organisms in fermented foods, as well as extend the shelf life of unfermented foods. At an industrial scale, Lactobacillus, Streptococcus, Bifidobacterium, and other bacteria are used for the preparation of yogurts, low-fat dairy products, fermented milks, and acidophilus milks.

Plant extracts contain high concentrations of phytochemicals, including phenolics, flavonoids, essential oils, alkaloids, and terpenoids. Different extraction and isolation processes are employed to obtain a variety of extracts with proven antimicrobial and antioxidant activities. The quality and concentration of bioactive compounds in different extracts are standardized before being incorporated into food products to enhance their antimicrobial effectiveness and prolong shelf life.

Analyses of literature indicate that probiotics have been studied in fermentation and preservation processes, while plant extracts are mostly added for extended shelf life and reduced microbial activity during storage. Such interactions are necessary to avoid food spoilage during storage. Their combined incorporation to target both fermentation and shelf life is crucial for the stability and safety of foods. Moreover, the concentration of both probiotics and extracts must be synergistically evaluated for cost-effective applications without compromising sensory quality [108, 109, 110, 108, 109, 110].

Reduction of Pathogens and Spoilage Organisms

Probiotics and plant extracts can act in concert to suppress the growth of pathogens and other microorganisms responsible for spoilage. Probiotics contribute to this process by competing for space and resources, tuning the microbial milieu, and secreting antimicrobial metabolites. Plant extracts exert their antimicrobial effects by directly inhibiting pathogen growth and inducing defence responses in the host. Collectively, these properties play a key role in extending the shelf life of various food products.

Fermented dairy products are a prominent global category of functional foods tailored for the inclusion of probiotics, mainly because of their compatibility with the human gut microbiota and the considerable advantages associated with their consumption. The fermentation process itself has a positive effect on the preservation of food and also promotes sensory quality. Lactic acid bacteria (LAB) and yeasts that produce volatile compounds contribute to the typical aroma, and the low pH favours the hydrolysis of proteins and lipids, resulting in a creamy texture and a rich aromatic diversity. Yogurts, however, are more susceptible to spoilage than other fermented products: high humidity levels favour the growth of spoilage bacteria. Probiotic LAB strains are recognised for their ability to compete with spoilage bacteria for the available space, but microbiological analyses have indicated that combinations of probiotic strains are more effective in controlling cold spoilage than either strain alone. This observation underscores the importance of a well-balanced composition when including probiotics to enhance the shelf life of fermented dairy products.

Sensory and Nutritional Quality Improvement

Probiotics play a key role in fermentation and food preservation. They can improve the sensory composition of products, enhance their nutritional value through the degradation of anti-nutritional compounds or the biosynthesis of vitamins, and increase health-promoting effects. Furthermore, foods containing probiotics are less susceptible to contamination by spoilage or pathogenic microorganisms and show a longer shelf life. Phytochemicals from both essential oils and extracts can also be used to extend the shelf life of food products and improve their sensory quality, safe with sensory evaluation techniques, and exploit the establishment of a sensory profile through panel testing. The sensory quality may be enhanced by reducing anti-nutritional factors and off-flavor compounds, while the consumption of foods enriched with phytochemicals and/or their fermentation products may lead to both odor pleasantness and acceptability.

Synergistic effects between probiotics and phytochemicals can strongly contribute to sensory and nutritional quality improvement. Evidence has highlighted that the combination of these bioactive ingredients can enhance antioxidant and anti-inflammatory properties, ameliorate dysbiosis in different pathological conditions, and improve the organoleptic properties of food products. Such combined action deserves deeper investigation to explore new opportunities for product development. Specifically, the development of probiotic-enriched foods that are further integrated with phytochemical-rich extracts is proposed, together with an evaluation of the product stability and shelf life in relation to packaging conditions and consumer acceptance trends [23, 10, 3].

Chapter - 10

Development of Functional Foods Enriched With Bioactive Components

Probiotic-enriched food formulations can deliver live probiotics or their metabolites and contribute to flavor and texture. Phytochemical-rich extracts can also be integrated to yield healthier foods with antioxidant, antimicrobial, and preservative properties. Product stability, shelf life, packaging materials, consumer acceptance, and market demand are critical considerations.

Food fermentation is an ancient practice that predates scientific understanding of microorganisms, yet it remains a widespread method of preparation and preservation. Fermented products often contain naturally occurring probiotics generated during fermentation, usually with beneficial effects on human health. Other foods infused with probiotics are also becoming popular, especially among health-conscious consumers. Potable yoghurts fortified with probiotics are the most common of these, but other formulations are gaining traction despite formulation challenges, including long-term preservation and consumer acceptance. Commercial fermented milks without preservatives typically require cold chains, face distribution challenges, and have short shelf lives.

Nutraceuticals are defined as food ingredients with nutritional, medicinal, and pharmaceutical effects. Recent research has indicated that probiotics and phytochemicals exert complementary effects on human health when combined, leading to the concept of probiotic-phytochemical nutraceuticals. These products may contain a source of probiotics, such as dairy products or fruit juices, augmented with phytochemicals for added health benefits. Products enriched with elements such as ginger-honey or saffron-garlic can also be formulated. Special consideration should be given to product stability, shelf life, packaging materials, consumer acceptance, and current market demand in the development of probiotic-phytochemical nutraceuticals [23, 10, 3].

Probiotic-Enriched Food Formulation

Commercial products with probiotic activity have increasingly entered the market in recent years. Fulfilling consumer preferences requires consideration of probiotic stability and usability aspects throughout production, transport, and storage, as well as impact on organoleptic, sensory, and nutritional properties. Exploring the use of cheese- and yogurt-type formulations, along with beverages such as kefir, complementary rice- and sago-based foods, protein- or lipid-rich light dairy products, and curry powder, are various avenues for probiotic food development. Integration of probiotic- and phytochemical-rich ingredients represents an additional direction within functional food research. Elements that determine product shelf life—especially packaging materials and conditions—and the influence of dressing types on the sensory acceptance of probiotic salad dressing products have been assessed in this context. Specifically, the stability of probiotic strains in enriched functional bread has been investigated, along with the suitable combination of rice, corn, and lascor at different fermentation times, supported by sensory evaluation for consumer acceptance.

Various types of probiotic food products with enriched nutrition are proposed, with some supporting the concept of functional food intake for particular diseases or health conditions.

Antibiotic-free, gluten-free, and fortified functional crackers, along with probiotic-rich cookies, are included. Food preparation using standardized probiotic and phytochemical-rich extracts holds substantial promise, with studies confirming the feasibility of functional pancake and dosa formulations. However, the stability and shelf life of probiotic cookies and cereal-based products—including a cornbased subbite with 21% exo-polysaccharide production by Lactobacillus rhamnosus GG and whole sorghum-based pitha enriched with skim milk powder, D-mannitol, or wheat flour in combination with ginger root—remain key challenges for these and other products [111, 9, 112].

Incorporation of Phytochemical-Rich Extracts

Major Class of Phytochemicals and Different Phytocompound-rich Plant Extracts

Phytochemicals, bioactive compounds produced by plants, fall into two main classes—primary metabolites, responsible for normal cellular function involved in growth and development, and secondary metabolites, produced for interaction with the environment. The latter class consists of a wide range of compounds synthesized during the life cycle of the plant, including phenolics, alkaloids, terpenes, flavonoids, saccharides, glycosides, and tetracyclics. Phytochemicals play key roles in the protection of plants against biotic and abiotic stresses. These include phenolic compounds that provide mechanical and biological resistance against fungal infections, flavonoids which exhibit antioxidant activity in plants, and a range of other components that confer resistance to nematodes and bacteria.

On account of their biological properties, several phytochemical-rich plant extracts—including ginger, turmeric root, cinnamon, onion, garlic, red and black pepper, coriander leaves, basil, fennel, oreganum, rosemary, thyme, and neem—can be used separately or in combination as antimicrobial agents in agriculture and food preservation. The antimicrobial properties of the extracts of coriander leaves, ginger, garlic, local onion, red pepper, and turmeric against Colletotrichum species, Rhizopus species, Penicillium species, candida, Escherichia coli, and Staphylococcus aureus also support their potential applications as preservatives. Laboratory-based analytical techniques such as chromatography, spectroscopy, microbiological assays, and molecular biology assays are crucial for the quantification of bioactive phytocompounds, the validation of extracts' antibacterial potential, and the evaluation of different factors influencing extraction.The major class of phytochemicals and the different phytocompound-rich plant extracts are summarized in Table 1 [113, 114, 115].

Stability, Shelf Life, and Packaging Considerations

Curiosity about food safety and quality often leads to questions about what causes food spoilage. While microorganisms are frequently blamed for food spoilage and contamination, they are not always the culprits. Numerous factors contribute to food spoilage, such as microbiological, biochemical, chemical, and physical factors, and food preservation methods are classified and defined according to these spoilage factors. A food product can be preserved only in the particular environmental conditions that are adverse for the spoiling organisms or agents. Sensory quality, including flavor, aroma, taste, and texture, plays a vital role in consumer preferences for foods. Moreover, the flavor, nutritive value, and acceptability of many fermented foods are largely attributed to complex biochemical undercurrents caused by microorganisms during fermentation. Shelf life is defined as the duration of time that a product may be stored without spoiling. The use of probiotics and plant extracts to improve food shelf life and quality is becoming increasingly popular.

The right combination of sensory and nutritive qualities of food is the key to its marketability. For instance, product characteristics might include making the product appealing to eyes and nose, improving flavors, mouth-feel, and quality, and prolonging shelf life and storage stability. Sensory evaluation methods confirm whether a product is suitable for consumers. Probiotics help to maintain and enhance the sensory qualities of food, broaden consumer acceptance, and minimize health risks by inhibiting spoilage bacteria. The inclusion of phytochemical-rich extracts in various food items not only improves healthfulness but also enhances the sensory attributes of the products. Research has shown that these extracts help in the management of foodborne pathogenic microorganisms. The quality parameters of food can be enriched with these components, thus catering to consumer desire for nutraceuticals with clear health benefits [65, 116, 117].

Consumer Acceptance and Market Trends

Widely in food processing and development, probiotic-phytochemical combinations have received only limited attention. Research evidence suggests that the application of both types of bioactive agents together could lead to synergistic benefits. Strengthening of the vertebrate immune system (especially in children and older adults) and inhibition of inflammatory disorders of various origins have been observed. Probiotic-phytochemical combinations might provide broad health-benefit spectra, yet it remains unclear whether regulatory or commercial factors will finally steer the market toward this direction, as sufficient scientific evidence has yet to be presented.

Consumer demands for enhanced product quality have rendered probiotics a major attribute of a wide range of foods. These fermentation starters not only convert a substrate into palatable food but also provide health benefits for many consumers by augmenting or modulating gut microbiota ecology. The maintenance or enhancement of the organoleptic properties of food is equally important, since any unpleasant off-flavor or rapid spoilage will either detract from its marketability or, in some cases, pose serious health risks. To this end, the incorporation of nontoxic spoilage-inhibiting or sensory-enhancing agents into the matrix, coupled with appropriate packaging to minimize contamination during storage, can extend shelf life and enhance the overall quality of the product [118, 119, 120].

Chapter - 11

Nutraceutical and Therapeutic Potential of Combined Bioactive Agents

Combined application of probiotics and phytochemical-rich extracts is supported by preliminary evidence indicating synergistic health benefits. Their combined use is further substantiated by antioxidant and anti-inflammatory applications. Interactions with gut microbiota and effects on the immune system are particularly important for supporting human health. Commercialized probiotics often lack broad-spectrum preventive action, which can be addressed through co-administration with phytochemicals. Owing to their therapeutic and synergistic potential, composite probiotic-phytochemical extracts should be developed and evaluated for nutraceutical and medicinal applications across various disease conditions.

Clinical studies confirm the therapeutic potential of probiotics and phytochemical-rich extracts in chronic and infectious diseases such as cardiovascular disorders, neurodegenerative diseases, and SARS-CoV-2 infection. Recent data suggest antidepressant activity in a viral-infected model; however, the modulatory effect and functioning of gut microbiota along with underlying mechanisms remain controvertible. Clinical safety concerns regarding probiotics and herbal remedies during pregnancy are debated; appropriate dosage recommendations are required. There is an urgent need to assess their combined therapeutic use for COVID-19 and other severe infections, particularly in critically ill patients [7, 9, 121].

Synergistic Health Benefits

Growing empirical evidence supports the health-promoting synergism of probiotics and plant-derived phytochemicals at both cellular and holistic levels. A probiotic-plant-extract combination shows potential for enhancing antioxidant and anti-inflammatory efficiency as well as preventive efficacy against enteric infections and pyogenic infections. Several studies suggest the potential integration of select probiotics and phytochemical compounds in a single nutraceutical formulation targeting oxidative stress and inflammatory processesdriving lifestyle diseases associated with chronic low-grade inflammation. Moreover, supporting evidence points to the possibility of a more effective therapeutic outcome through the coadministration of probiotics and phytochemical-rich extracts, particularly involving nutraceuticals from extracts with demonstrated antimicrobial or antimycotic action.

Despite the recognized benefits of such a combined approach, commercial availability remains extremely limited. Challenges include the need for strict quality control over viable probiotics in nutraceutical formulations, potential interactions (positive or negative) between probiotics and plant extracts, proper delivery system development, and a thorough analysis of sensory properties. Nevertheless, these challenges must be balanced against the possible takeaway benefits such as reduced dosages of medicinal drugs, health-related food versus health-supporting recipes, and stimulation of consumption in predisposed subpopulations [3, 1, 122].

Antioxidant and Anti-Inflammatory Applications

Accumulating evidence points to the potential for synergistic action. For example, antioxidants derived from probiotic microorganisms and plant extracts may jointly alleviate oxidative stress and inflammation. Several studies have indicated that probiotics and plant extracts can confer antioxidant protection *in vitro* by quenching free radicals and enhancing the activities of antioxidant enzymes. Combined use has also been shown to upregulate expression of the nuclear factor erythroid-related factor 2 (Nrf2) gene. In mice with acute liver injury, co-administration greatly reduced malondialdehyde and TNF-alpha levels and elevated superoxide dismutase activity, providing stronger hepatoprotection than either agent alone.

Stimulation of Toll-like receptors (TLR) on innate immune cells is one of the primary mechanisms through which probiotics exert anti-inflammatory effects, especially in intestinal tissues. Probiotics can also produce bioactive peptides that inhibit TLR signaling. Plant extracts can suppress TLR expression or modulate signaling cascades downstream of TLR activation. Given their potential for complementary action, combined administration of probiotics and phytochemical-rich extracts may provide enhanced protection against inflammation in both the gastrointestinal tract and other organs [7, 9, 8].

Gut Microbiota Modulation and Immune Support

Bacterial dysbiosis is associated with the development of various diseases. Evidence suggests that combination of probiotics with anti-inflammatory and antioxidant compounds present in medicinal plants may enhance the regulation of gut microbiota and support immune function. Probiotics are well-known for their pivotal role in the maintenance of gut health and modulation of the gut microbiota, engaging the immune system to efficiently overcome health threats. However, even though probiotics have long been used in the form of fermented dairy products, their approaches remain limited and are gradually becoming stagnant, leading further exploration and identification in the area of functional foods. Phytochemical-rich plant extracts, because of their broad-spectrum anti-inflammatory and antioxidant activities, can be another interesting component selection for functional foods. The positive interaction of both probiotics and plant extracts could enhance their respective therapeutic effects. There are many available studies that together provide a glimpse of the pro-health properties of medicinal plants, possibly increase the market of functional food and probiotic products. Their mode of synergistic action clarifies that probiotics may have a role in the effective modulation of the gut microbiome and in the enhancement of immune responses [22, 123, 122].

Commercialization of Probiotic-Phytochemical Products

Commercialization pathways for products that combine plant bioactives and probiotics have yet to be fully explored. Several factors favor such development. Many recent clinical trials indicate that probiotics—particularly multi-strain formulations—offer health benefits. These agents can form the basis of functional foods for which consumers demonstrate a strong preference. On the other hand, plant extracts rich in antioxidants also rank high on consumer-wish lists and serve as natural bio-preservatives in food products. Consequently, products that contain high concentrations of bioactive plant components and probiotics can address several important market segments simultaneously.

Several commercial probiotic-phytochemical formulations exist, and many more are under development. Smoothies and herbal teas enriched with both probiotics and antifungal extracts have been successfully developed and analyzed for compositional and sensory attributes. Research addresses the probiotic characteristics of lactic acid bacteria and bifidobacteria isolated from herbal teas, as well as their ability to survive in herbal beverages. Products are also being developed that combine probiotic bacteria with formulations containing garlic extract to enhance sensory characteristics, flavor, and shelf life. Pre-biotic powders fortified with probiotics and plant extracts are emerging as suitable ingredients for probiotic-delivering foodstuffs in powder form [124, 125, 126].

Chapter - 12

Clinical Applications and Evaluation of Bioactive Treatments

Recent scientific literature supports the beneficial effects of both probiotics and plant extracts in human health. These studies demonstrate the potential for reducing inflammation, controlling metabolic disease in diabetes mellitus and obesity, ameliorating the gut microbiota, and supporting immune responses against infectious diseases. Unfortunately, numerous patients experience recurrences after treatment with plant extracts or probiotics, prompting further investigation into the effectiveness of combinations. Recent studies indicate that some probiotic-phytochemical combinations can enhance health effects. However, few studies have specifically addressed these combinations in clinical practice, focusing mainly on mechanism exploration or animal experiments.

Despite promising synergy, the clinical use of probiotics and phytochemical combinations remains nascent. Clinical protocols must be refined and tested, including evaluation of interactions, potential synergistic effects, and proper doses. Safety, toxicity, and regulatory compliance must also be considered, especially for long-term administration or in vulnerable populations such as pregnant women, children, and immunocompromised patients. Progress in these areas could facilitate the clinical application of probiotics and phytochemicals in metabolic, inflammatory, respiratory, and infectious diseases [7, 22, 127].

Clinical Studies on Probiotics and Plant Extracts

Clinical studies support the beneficial effects of probiotics, plant extracts, and their combinations. Probiotic products are generally considered safe when consumed as directed, but side effects may arise in rare cases. Among patients with COVID-19 and chronic inflammatory conditions, administration of Aloe vera extract has been shown to alleviate symptoms. The daily intake of various plant extracts exhibits favourable effects in healthy individuals and patients. Nevertheless, the synergistic effects of probiotics combined with plant extracts in improving health, preventing disease, and supporting recovery after illness remain less explored.

While physiological factors, allergic reactions, and other disease conditions may influence treatment effects, toxicity and overdose must also be considered. For example, high doses of vitamin C may cause gastrointestinal problems. Furthermore, probiotics should not be administered intravenously in critically ill patients without proper indication. Hence, specific caution is warranted when providing concentrated natural extracts to clinical populations, and possible adverse events must be exhaustively investigated [128, 129, 130].

Safety, Dosage, and Toxicity Considerations

Consideration of safety, dosage, and toxicity is crucial for the effective application of probiotics and bioactive products in humans, animals, and plants. The potential adverse effects of probiotics have been examined in patients with severely weakened immune systems, particularly during the use of immunosuppressive drugs, in patients with underlying heart conditions, and in individuals with implanted central venous catheters. Nevertheless, multiple reviews conclude that clinically proven probiotics are safe for human use. Concerns regarding epidemiological studies suggesting a hazard for patients with severe pancreatitis are contradicted by a recent large clinical trial. For healthy populations, the determination of minimal, optimal, and maximal doses is more relevant than the outright safety of probiotics. In general, no adverse effects were substantiated for dosages <10^9 CFU/day; center recom-mendations for healthy infants and children for oral rehydration and other indications are 10^10 CFU/day; and for adults, 10^11 CFU/day is a common level in European foods. For many specific applications, dosages markedly higher than 10^11 CFU/day are used. Dosages ≥10^9 CFU/day are generally used in animals, mainly with young or stressed animals, or for reduction of certain pathogens.

When using plant extracts for medicinal purposes, safety and toxicity issues occupy a primary place on the agenda. Although many plants can be considered safe, deeply investigated studies focusing on extract safety and toxicity are necessary. The available data concerning the toxicity of the extracts may not be sufficient, especially when used in long-term visions or in treated patients suffering from important medical ailments. Traditional uses of plants together with adequate study for attention to toxicity, dosage, synergism, and bioavailability can guarantee the efficacy and commercial acceptance of herbal products in curing or alleviating various human ailments. Multidrug-resistant pathogenic bacteria are presently a burning concern in the treatment of infectious diseases. Efforts made to study the antibacterial activity of plant extracts against these pathogens should take care to test their toxicity together with the underlying causes of action [131, 132, 133].

Therapeutic Use in Chronic and Infectious Diseases

The safety and efficacy of probiotic and phytochemical formulations have been evaluated in clinical studies, and a variety of combinations of both agents represent promising candidates for the treatment of chronic and infectious diseases. Probiotics, herbal extracts, and a combination of both have been evaluated in patients with chronic conditions such as atopic dermatitis, recurrent urinary tract infection, and irritable bowel syndrome, as well as in patients with infections. However, not all clinical trials indicate beneficial effects associated with probiotic treatment. Additional research is needed to support the prophylactic or therapeutic use of specific probiotics and phytochemical combinations, especially for important diseases like infections. Limitations of the use of probiotics in clinical settings include doubts about their safety for immune-compromised patients, possible transient bacteremia, especially with Enterococcus species, and the lack of data supporting the efficacy of combining multiple probiotics. Standard clinical guidelines for dosage or treatment duration are also still lacking. Phytochemical, probiotic, and prebiotic antagonistic effects on bacterial pathogens have yet to be comprehensively demonstrated in human infection models, and remaining safety or toxicity issues still need to be addressed. Nevertheless, the available data indicate that combinations of probiotics and phytochemicals can exert additive or synergistic effects, and further investigations in this direction should be prioritized.

Probiotics are often considered safe for healthy individuals, but they still have the potential to cause adverse events, especially in special populations such as the immunocompromised. Safety and toxicity study in humans with chronic and multicenter disease are strongly recommended, and until now, limited clinical trial sensory acceptability has confirmed the analyzed combinations [134, 135, 136].

Challenges in Clinical Implementation

Low probiotic incorporation levels in dietary habits and cultural resistance to fermented foods in some countries hinder achieving therapeutic benefits in conditions such as ulcerative colitis, Crohn's disease, and antibiotic-associated diarrhoea (AAD). High doses of probiotics (more than 10^9 CFU/g) are commonly administered in clinical trials to support their efficacy. However, critical therapeutic concentrations for individual probiotic isolates are often not evaluated in AAD studies. Further clinical validation is necessary to confirm the efficacy of combined mycobiota-modulating therapies that also consider balance shifts in Candida-Altermonadaceae-Moraxella and Lachnoclostridium-Phascolarctobacterium-Faecalibacterium coronaviruses.

Probiotics rarely trigger adverse reactions when consumed in appropriate doses, although isolated cases of infections or toxic reactions have been reported. This low incidence may be linked to antimicrobial substances like bacteriocin and reuterin produced during the fermentation process. Nevertheless, such effects have not been rigorously evaluated in clinical practice. Another concern is the susceptibility of probiotic bacteria to commonly prescribed antibiotics. Strains used in clinical trials are usually screened for such susceptibility, but isolates from different geographical regions require screening too. These aspects have important safety implications, as patients using antibiotics are vulnerable to gut infections or imbalances, which antioxidant activity alone cannot correct [137, 138, 139].

Chapter - 13

Experimental Models for Bioactive Research

Experimental models serve as indispensable tools for investigating the efficacy of bioactive compounds and facilitating their incorporation into food, agriculture, and medicine. For quality assessment, diverse *in vitro* models are employed to test antioxidant and antimicrobial properties. Furthermore, *in vivo* animal models are widely used for experimental validation of these assessments. Plant models, both *in vivo* and *in vitro*, are applied to determine the potential of bioactive agents to enhance growth, resistance, and tolerance against various environmental stresses. These model systems offer the feasibility to comprehensively evaluate the bioactive potential, either in isolation or in combination, for desired beneficial outcomes in the respective areas. Nevertheless, each system usually has its own advantages and limitations, and careful model selection is therefore vital.

Here, we focus on the most popular experimental models for investigating the action of bioactive compounds and their integrated applications for improved biological effects. The consideration of main model systems used for testing the bioactivity of phytochemicals, probiotics, and their interactions is outlined. Additionally, the importance of model choice in obtaining credible results for both laboratory-scale validation and large-scale implementation is discussed [140, 61, 141].

In vitro Methods for Antioxidant and Antimicrobial Testing

Probiotic microorganisms and plant extracts have been found to have a number of synergetic effects that may work in both food and therapeutic contexts. When these compounds are combined, they can offer higher protection from fungal infections and provide greater direct clinical benefit than either agent alone.

Testing these anti-oxidant and anti-microbial effects can be performed using a variety of in-vitro methods, each assessing different aspects or effects with various materials. Common tests include the DPPH test and the Agar Disc Diffusion test. The DPPH test measures free radical scavenging activity within a sample by providing an indicator (DPPH), which changes colour as it is neutralized by free radical scavenging action, thus indicating the total electro-donating capacity of the extract. The Agar Disc Diffusion test is a well-established method for checking the antimicrobial activity of different materials. A microbial culture is grown on an agar plate, and soaked filters containing the test substance are placed on the surface. After proper incubation, the characteristic parameters of the moulding activity are evaluated.

Beyond these common methods, several sophisticated techniques have been proposed to overcome the limitations of traditional strategies. Mitochondrial membrane permeability assays, for example, probe the permeability of the inner mitochondrial membrane, which is critical to mitochondrial function. Also notable is the Redox Sensor of Gene Expression, which provides a fluorescent *in vivo* assay suitable for studying the role of intracellular redox status in bacterial physiology, pathogenesis, and host interactions [142, 143, 144].

In vivo Animal Models for Efficacy Assessment

Suitable *in vivo* animal models are crucial for evaluating the therapeutic action of bioactive treatments on biological systems and diseases. Endogenous animal models reflect complex interactions among organs, physiological functions, cell types, and communities of microorganisms, making them irreplaceable for determining therapeutic efficiency and safety. *In vivo* models share species, metabolic processes, specific organs, or physiological functions with humans to portray human disease and therapeutic responses. Popular models for checking antioxidant, antimicrobial, and anticancer effects include the Wistar, Sprague Dawley, and Balb C mouse/syrian hamster/rabbit models.

Selection of a suitable model depends on the action being explored. Anticancer activity is tested in rodents with chemically induced solid tumors, while the MMTV-neu mouse model is often employed to evaluate the anticancer efficacy of natural products against human breast cancer. The ApcMin mouse model is used to identify substances that may inhibit intestinal cancer, whereas azoxymethane-induced colonic preneoplastic lesion models help assess the potential of probiotics. The carrageenan and histamine-induced mouse paw edema models are widely used to evaluate anti-inflammatory activity in mice and/or rats. A variety of other inflammatory models have also been utilized to examine the anti-inflammatory activity of several probiotics, for example, the allergic rhinitis model in mice, the ovalbumin-induced eosinophil infiltration model in a rat videoendoscopic and histological evaluation, and the dextran-sulfate-sodium-salt-induced colitis model [145, 146, 147].

Plant Bioassays for Growth and Defense Evaluation

In evaluating plant responses to probiotic and phytochemical treatments, bioassays can provide valuable insights. Germination and seedling growth assays assess direct phytotoxic effects, while foliar-applied treatments may be screened using cut leaf segments. Whole-plant assays within controlled environments can also serve as preliminary tests. To evaluate the effects of putative biocontrol agents or defense-inducing treatments against soilborne plant diseases, pathogen challenge assays are essential. Commonly employed pathosystems for such screening include those involving Pythium spp. on cucumber and tomato, Fusarium spp. on cucumber, and Macrophomina spp. on tomato.

Bioassays may be highly controlled in growth cabinets or simpler setups involving plastic pots placed outdoors. Depending on the nature and mode of application of the treatment, additional controls without treatments, without inoculum, and/or without both treatments and inoculum are included. Combinations of several assays in one experiment can provide a more comprehensive understanding of the biological effects of a particular treatment. Factors such as soil nutrient status, moisture content, and root temperature are kept optimal for the plants and the soilborne pathogen. Assays in which treatments are administered at different stages of the host-pathogen interaction allow evaluation of defense induction and its persistence before challenge [148, 149, 150].

Model Selection, Validation, and Limitations

Experimental models for in-depth research on interactions between probiotics and phytochemicals are essential. Their sensitivity permits evaluation of antioxidant, antimicrobial, and plant-growing-promoting efficacy through *in vitro* bioassays, confirming the functional potential of probiotics and phytochemicals. *In vivo* trial systems using model or test species representative of real-production ecosystems further demonstrate the usefulness of bioactive agents as soil amendments, growth-promoting substances, bio-control agents, or disease-suppressive formulations. Selection of a particular testing system depends on the functional capability being probed. Validation and limitation statements accompany each experimental model to ensure reliable interpretation of test results and their translation into field performance.

In vitro systems are suitable for preliminary screening. Although sensitive methods can quantify the antioxidant and antimicrobial effects of probiotics or plant extracts, the combined role of these active substances in suppressing pathogens or extending shelf life remains ill-defined. *In vivo* studies with animal species, including simians, often require special setup, expense, and ethical approval, and results cannot be directly correlated with human trials. These limitations underscore the importance of conducting comparative experiments in relevant mammalian disease models. Similarly, while plant bioassays are reliable for screening the promoting or inhibiting activity of bioactive components on growth, defense, and productivity, field trials are essential for real-performance evaluation [151, 152, 153, 154].

Chapter - 14

Quality Control and Standardization in Bioactive Product Development

Development of Quality-Control and Safety-Determining Standards for Probiotics and Plant Phytochemicals Enriched With Knowledge-Based Quantification, Validation, And GMP Guidelines

Products formulated with bioactive substances must be composed, controlled, and managed with the utmost responsibility. Thus, differentiation of biosafety standards for probiotics, and safety-determining analysis of plant phytochemicals, is essential for the complete biotechnological development involved in applied sciences. Such development must include the production, scaling, storage, and use of these products in various areas of human health and the environment for the benefit of humanity.

Oral administration of probiotics for human pharmaceutical/nutraceutical applications must be statistically confirmed in bacterial dispensing products. In plant extracts, the detection, identification, and quantification of phytochemicals must be unity-based to guarantee organism safety. Phytochemicals for product development must be biologically profile-validated in different *in vitro* and animal systems for quality control during preparation and in the market when consumed. For established commercialization levels, Good Manufacturing Practices (GMP) guarantee correct product formulation, uniformity, and standardized consumer safety [155, 156, 157].

Analytical Standards for Probiotics

Analytical standards in the quality assessment of probiotics/synbiotics are requisite for characterizations and applications. The World Health Organization (WHO) set standards for probiotics that include cell identification, quantification, assay of intrinsic properties, characterization of health benefits, and proof of survival during processing and shelf life. Probiotic strains intended for therapeutic use are additionally subjected to qualitative safety screening. Quantification of viable cells is accomplished by cultivation methods and molecular methods that include quantitative polymerase chain reaction (qPCR). For standard quality assurance, oral therapeutic doses of 109-1012 colony-forming units are applied in clinical trials.

Efficacy of probiotics in synbiotics formulations with phytochemicals or food products necessitates quantification of phytochemicals in a wide variety of raw materials, semi-finished and finished products. Analytical techniques for quantification of maximum classes of phytochemicals at different concentration levels were described. An integrated approach by liquid chromatography-mass spectrometry (LC-MS) and qPCR enables simultaneous quantification of six flavonoids and one phenolic acid present in herbal extracts. For control purposes, internal standards and validation by validation parameters are essential. The use of Good Manufacturing Practices (GMP) is fundamental for industrial production [158, 159, 160].

Quantification and Validation of Phytochemicals

Various standards are needed for the accurate quantification of phytochemicals, which must then be identified and validated in biological systems for well-founded applications. The progress of these criteria is described below.

Analytical standards—pure crystalline preparations of natural bioactive compounds that meet the criteria of a certified reference material—are essential for reliable quantification and validation of phytochemicals. External standards, either primary or secondary, should be used for GC-MS and LC-MS applications, respectively. Concentrations of the bioactive compounds in plant extracts should be expressed in terms of established standard quantifiers (e.g. catechin equivalents, gallic acid equivalents, or ellagic acid equivalents). Calibration curves should demonstrate a linear correlation within the determined ranges; suitability for quantification should be confirmed by performing back-calculation studies. The limits of detection and quantification must be established. Quantifying the main classes of phytochemicals in plant extracts, including total phenolics, total flavonoids, and tannins, provides valuable information with an internal standard that need not be validated.

Methods for quantifying major groups of phytochemicals and the main classes of secondary metabolites involved in determining the activity of plant extracts—viz. phenolics, flavonoids, and alkaloids—should be validated accordingly. Validation studies should also assess other aspects of practical quality assurance recognized in pharmacopoeial guidance, such as precision, robustness, and recovery for the specific use of each procedure, as well as possible application to other groups of bioactives. Good Manufacturing Practice (GMP) guidelines should govern production of bioactive substances, the manufacture of marketed products should comply with applicable guidelines, and certification according to well-recognized regulatory requirements should enable worldwide acceptance [161, 162, 163].

Good Manufacturing Practices (GMP)

And adherence to other analytical standards, are vital considerations for the commercial development of probiotic formulations and phytochemical-rich products. Confirming the identity and efficacy of probiotic strains requires enumeration and genotyping. Quantification of major bioactive phytochemicals is incorporated into production protocols for rhizome extracts from ginger (Zingiber officinale), turmeric (Curcuma longa), and galangal (Alpinia galanga) to support the safe application of these extracts.

To support regulatory compliance, external certification bodies provide guidance on production, testing, and packaging procedures for products intended for human consumption. Such Guidelines provide a framework for a numerically and routinely defined degree of quality control applied during the manufacture of such products. The Guidelines are complementary to food safety authorities (e.g., Codex Alimentarius) which provide protection against foodborne and waterborne illness and unsatisfactory consumption of food. While such testing protocols cover the rules for probiotics and other products, the more recent guidelines focus on phytopharmaceuticals for therapeutic purposes [164, 165, 166].

Regulatory Compliance and Certification

To meet consumer demands and ensure clinical efficacy, probiotics and herbal drugs must pass safety evaluations along with clinical, pre-clinical, and toxicological studies as per government regulatory requirements. Before a biopesticide is approved for market release, data should support its target specificity, safety to humans, animals, non-target organisms, and the environment. Regulatory agencies worldwide exhort the assessment of safety and toxicology of such products before their commercialization in the market. Guidelines for investigating biopesticides for submission to regulatory authorities must be established.

Good Manufacturing Practices (GMP) is a quality assurance system covering all aspects of development and production including raw materials, formulation design, production, quality control, storage, distribution, and analysis to ensure that the criteria are met for clinical use. A GMP-certified facility must be established for the production and quality control of probiotics and phytochemical-based products such as medicines, dietary supplements, and food products incorporated with herbal extracts that fulfill the needs of the consumers. Additionally, probiotics and phytochemicals supplements must comply with national and international regulatory guidelines such as the FDA, EFSA, FSSAI, and DSIR in India [65, 167, 168].

Chapter - 15

Regulatory, Ethical, and Environmental Issues in Biotechnology

The deployment of defined or unmarked bioactive phytochemicals in agriculture, food science, and medicine is generally considered low risk. Nevertheless, the potential hazards posed by large-scale production and use should be examined, as should the potential environmental advantages of minimising chemical use. Moreover, ethical concerns regarding human testing of probiotic-phytochemical formulations must be taken into account, and the implementation of such combinations for disease treatment should undergo thorough evaluation.

The microbiological and toxicological aspects of probiotics are reviewed in detail in Section 6.4. Concerns about the safety of probiotics prompted the establishment of the International Scientific Association for Probiotics and Prebiotics (ISAPP), which publishes guidance on the characterization, safety evaluation, and health claims of probiotic preparations. Preclinical investigations serve to delineate these aspects before the formulations progress to clinical trials aimed at evaluating their efficacy and determining appropriate dosage regimens. Such studies assess additional aspects of safety, such as the risk of transmission of antibiotic-resistant genes, and investigate potential negative interactions with medications. As probiotics differ in species, strain, and origin, such evaluations must be conducted for each marketing preparation.

The large-scale application of bioactive chemicals in modern agricultural practices raises environmental concerns. For example, excessive use of nitrogen-based fertilizers results in the accumulation of reactive nitrogen in the ecosystem, affecting air quality and terrestrial and aquatic ecosystems. Consequently, there is growing interest in boosting soil-fertility mechanisms through the addition of bioactive-molecule-based nutritional compounds. Fertility amendments that modulate the composition, structure, and function of the soil microbiome can enhance nutrient cycling, improve soil aggregates and structure, and decrease the abundance of pathogens, potential human pathogens, multiresistant bacteria, and plant pathogens responsible for soilborne diseases. Unsurprisingly, microbes have been proposed to function as natural bioindicators of soil quality [169, 170, 171].

Safety and Ethical Concerns of Bioactive Applications

The extensive market penetration of probiotics and plant extracts has injected significant investment into biomedical research aimed at elucidating their therapeutic potential. Investigators are thereby empowered to reproduce the plethora of health benefits claimed by the manufacturers of bioactive products. Nevertheless, despite the corroboration of several clinical benefits through multiple investigations, safety and efficacy concerns remain. Those questions often stem from insufficient characterization of the bioactive agent or a lack of understanding of the mechanism underlying the health benefit. Safety and ethical issues related to consumption of plant extracts or probiotics should therefore be thoroughly investigated before large-scale production becomes a reality.

Probiotics are heralded as generally recognized as safe (GRAS) by the United States Food and Drug Administration (USFDA), and toxicological evaluation is not mandatory. Toxicity assessment of probiotic microorganisms is nevertheless critical before their introduction into clinical practice, particularly for strains which deviate from the original species profile or which have an extensive history of exposure in food systems. High doses can trigger gastrointestinal disturbances, and caution is also advisable during use in infected or immunocompromised patients. Such caveats also hold for phytochemical-rich plant extracts. Although extensive human consumption may obviate considerable toxicological assessment, extraction method, solvent type, presence of undesirable coextracted compounds, and dosage nevertheless affect the safety profile of these preparations. Nevertheless, meta-analytical studies highlight the antioxidant and anti-inflammatory potential of plant extract-probiotic combinations, particularly in conditions that predispose to oxidative stress. In such cases, provided that both the probiotic and the extract are safe, the synergistic interaction augurs well for their combined therapeutic use without adverse effects [172, 173, 174].

Environmental Impacts of Large-Scale Biotechnological Use

Large-scale application of probiotics and phytochemicals in agriculture, food systems, and medicine could provoke harmful environmental consequences, including development of resistance in spoilage and infectious organisms. To mitigate this potential threat, intensity and dosage should minimized, altered, or avoided and combined application with other agents considered.

Probiotics showcase substantial versatility in biotechnology. Used in food agroindustry, they spur resurgence of traditional food products; they are paramount for complimentary feeding of nursing babies and pregnant, internal and external immunity, therapeutic and gut-microbiome advantages. As ecological fertilizers, they bolster soil and plant development. Probiotics rapidly proliferate and displace epiphytic spoilage microorganisms through competitive antagonism during food fermentation. Bioactive components produced with probiotic yeasts impart distinct flavors, modify organoleptic features, and provide health advantages. Yet large-scale use poses an impending threat: the potential for spoilage organisms to develop resistance against the myriad of bioactive compounds produced by different yeast and bacterial strains.

Substantial reduction of food spoilage and disease-causing organisms could offer an intelligent approach to therapeutic and preventative use of probiotics in pain-relief foods. Application of phytochemicals as natural preservatives constitutes another attractive solution: they very often delay food spoilage without health hazards. Nevertheless—much as for probiotics—their large-scale use can provoke development of resistance to spoilage and alimentary-intestinal-disease-causing organisms. Successful management of these potential dangers would require careful planning of application protocols [175, 176, 177].

International Regulatory Frameworks

The international regulatory framework for the use of probiotics and plant extracts has been established to safeguard practical applications and ensure public safety. The incorporation of active microbiota or plant-derived ingredients in food or as drugs can lead to ecological or health-related problems. The products obtained through modern biotechnology are routinely recognized as safe. Tuberculosis, cholera, and typhoid vaccines have been successfully developed using probiotics. Some countries have accepted the mass production of biologically active substances from microorganisms. However, most applications still require preliminary research and long-term evaluation of potential adverse effects. Probiotics or pharmaceuticals containing probiotics, as well as functional foods, are classified as biological or pharmaceutical products.

The regulatory approval of probiotics and their use in food and feed systems requires efficacy and safety studies. Safety assessment of probiotics is usually evaluated through toxicity studies, metabolic effect studies, and duration of administration of products containing probiotics. The use of probiotics in food systems for longer periods requires a grasp of their safety, efficacy, and adverse effects in infants, children, pregnant women, and the elderly. Further, both countries and the European Food Safety Authority have emphasized the need for controlled human studies to establish criteria for the application of probiotics and offer guidance on dosage, duration, and type of clinical disease to be treated. The United States Food and Drug Administration has classified probiotics as generally recognized as safe and has provided guidance for development [178, 179].

Future Policy Directions

Concentration of probiotic microorganisms, phytochemicals derived from medicinal plants, and natural plant extracts in industrial applications should be a primary area of focus. Biosafety and ethical concerns surrounding the use of probiotics, phytochemicals, and plant-based crude extracts are minimal compared with genetically modified organisms. Consequently, the promotion of bioactive compounds with respect to environmental safety and ecology is advisable. An interdisciplinary, integrated, and regulatory-compliant program contributes significantly to subsequent product development and commercialization. A sustainable research and development program in biotech-based bioactive products ensures environmental restoration, maintenance of soil health, the nutrition value in food, and improved therapeutic effects in diseases.

Integrated probiotics and medicinal plant-based microbe-plant interaction products for agricultural applications, the quality and the safety of food production and preservation, and probiotic therapy with plant-based functional food or nutraceutical products provide full-fledged solutions for plant disease management, increase biological food production, ensure food safety, and aid in treating emerging disorders and diseases, such as gut-related infections and metabolic disorders associated with type 2 diabetes or associated inflammatory responses. Data-sharing updates on research may ultimately lead to translational or product development, because microbiomes are universal, and the half-life of metabolites/bioactive compounds is also short for both fermentative and medicinal plant-based phytochemicals [180, 11, 181].

Chapter - 16

Future Perspectives and Innovations in Applied Biotechnology

The analysis and application of probiotics and plant extracts have improved significantly with the introduction of "omics" technologies. Advances in genomics, proteomics, metabolomics, and metagenomics and their data integration have provided an in-depth understanding of the genetic, regulatory, metabolic, and community functions involved in different processes in biosystems. With the decreasing costs of these technologies and increasing accessibility of open-source databases and bioinformatics tools, the expectation of similar user-friendly interfaces for analytical biotechnological methods including chromatography, spectroscopy, and microbiological assays is on the rise. Automation of laboratory processes is another focus area that promises to avoid human-induced errors, ensure reproducibility, reduce analysis time, and increase practical outputs.

The pair of probiotics and natural phytochemicals has demonstrated significant health benefits in humans through sample studies. Advancement is now anticipated in the combined use of probiotics and phytochemicals among food and health product manufacturers. Attention is also directed to the environment, its microbiome, and ecosystem conservation. Recent investigations underscore wise activity of the probiotics' community and their bioprospecting in the soil microbiome for ecosystem health. The meticulous yet sustainability-hungry approach of biotechnology points at sustainable agricultural practices carrying a full bioactive folio for the inoculation of all possible support agents in the soil-plant-ecosystem-environment razor on the globe [10, 127, 112].

Emerging Technologies in Bioactive Research

Developments in applied biotechnology aim to improve the health of humans, plants, and the environment. Future innovations will emerge not only from biotechnology itself but also from data science, artificial intelligence (AI), and other disciplines. Recent breakthroughs in the following areas are expected to yield significant advances: (1) cultivation of genetically modified organisms, (2) automated monitoring of environmental parameters, such as temperature and pH, (3) high-throughput screening of active compounds in bioactive-rich extracts, (4) novel probiotics based on metagenomic studies, (5) machine-learning-assisted exploration of omics data, and (6) laboratory methods that conform with Good Manufacturing Practice (GMP). When implemented consistently, these strategies should enable interdisciplinary studies that address a wide range of research questions related to plant health, food safety, and industrial development. Translating these advances into sustainable solutions requires cleantech systems that prevent pollution from the onset.

In conclusion, the research described in this work serves as a foundation for future innovations through the applied biotechnological study of probiotics and plant extracts. Biotechnology represents a crucial area for the integrated development of scientific, technical, and infrastructure aspects. For instance, crop improvement through functionally rich quality ingredients that enhance sensory, nutritional, and health properties could open immense market opportunities. Internationally, the demand for products enriched with bioactive components is on the rise, as is consumer knowledge of their effects. Both academic and industrial sectors can play key roles in developing such product categories.

Sustainable Biotechnological Solutions

Sustainable solutions for diverse applications require multidisciplinary research inputs capable of generating widespread benefits. Synergistic advances among the three disciplines currently represented by agriculture, food science, and medical sciences would help achieve applied targets while supporting greater progress across each dedicatory area of emphasis. Areas with tangible potential for mutual contribution include the integrated analysis of bioactive compounds produced by probiotic microorganisms and plant extracts with concentrations of phytochemicals, particularly in terms of better understanding the mechanisms underpinning the observed interactive effects, delineating their usage in production processes, support of preservation, and improvement of consumer health.

The imminent integration of artificial intelligence is set to transform biotechnological solutions via automation of analysis and data interpretation. Automaton-based synthesis of experimental conditions will impact cutting-edge methodologies such as screening of microbial communities driving specific biogeochemical cycles and metabolic crackling elucidation, opening numerous new paths and enabling novel adaptive efforts in unexplored domains. Through extrapolation and cross-validation, discoveries arising from focused technical variations in specific areas will progressively culminate in comprehensive progress in all these supporting disciplines, generating solutions capable of overcoming obstacles of practical articulation hitherto deemed intractable [182, 183, 184].

Integration of AI and Automation in Laboratory Analyses

Biotechnology has made use of different advances that have contributed to supply, improve, and expand products and processes in all areas of knowledge. The great development of biotechnology depends not only on the applied knowledge in biology, chemistry, and engineering, but also on new technologies, especially those related to data analysis. The development of new algorithms associated with different Artificial Intelligence techniques, as well as speech and image recognition, have had a growing impact on the applied research carried out around the world. Data analyses in many research areas that traditionally required prolonged experimental periods can now be executed in a fraction of the time, with results that are comparable to those generated by the best and most experienced researchers in the area. Because of the capacity of these technics to access an almost unlimited number of references and experimental data, it is also possible to apply them in areas that require innovation and creativity, helping in the development of new proposals.

Nevertheless, the laboratory work has also changed with the advent of laboratory automation. With the miniaturization of systems and the increase in precision and sensitivity of detection systems, many experimental procedures can now be carried out in small-volume experiments, which enables the use of robotic systems. Robotic laboratories can execute a great number of experiments in parallel, producing tens of thousands or even more results per week. Because of its high speed and low cost, laboratory automation may increase the number of tests and eliminate the data-limited problem that traditional approaches usually face. Automated laboratory analysis is generally designed to be carried out with little technician involvement during the execution stage. Repetition of the same procedure in successive experiments can lead to a decrease in the requirements of supervision, as well as the possible optimization of methods and protocols. The procedures and results have to be carefully validated, and the laboratory performance is also controlled using samples prepared by qualified personnel, with known composition and properties, which are subjected to analysis along with the tested materials [185, 186, 187].

Global Opportunities for Interdisciplinary Innovations

Numerous opportunities exist to implement and innovate bioactive applications by integrating different areas of biotechnology. For example, combining probiotics with short- and long-chain fatty acids can develop cosmeceutical products for skin diseases. In another area, the biosurfactant-producing capacity of probiotics could be utilized in the food and cosmetics industry. Integrating molecular techniques with a knowledge-based system in the poultry sector would lead to precision feeding that is cost-effective, eco-friendly, and disease-free and produces eggs with a longer shelf life and high nutritional value. In the clothing industry, the crucial role of soil fungi and the loamy nature of soil maintains maximum stability and less absorptivity compared to treatments with various kinds of azo dyes. In recent years, mushroom walls have been used as wastewater treatment; evidence shows that wastewater treated with the requisite number of mushrooms has no impact on chemical oxygen demand (COD) levels and pH. In addition, the expansion of mushroom cultivation in the state will support ruminant and poultry production, leading to economically sustainable development.

Automation and artificial intelligence can play key roles in quantitative and qualitative expression information. For instance, the analysis of images using artificial intelligence is now revolutionizing the identification of fresh and processed fruits, vegetables, and fish using trained computer models. Artificial intelligence is being applied to different areas of metagenomics. Future presentations of metagenomic data will have an integrated holistic structure—visualization, supervised or unsupervised annotation, feature selection, model generation, and interpretation of important features in known class distinction—thus minimizing redundant stages in the development pipeline. Artificial intelligence tools can also intensively and efficiently map ontogenetic states and identify sensitive traits and models that can be used as a source of tolerance for the future. In another area, new nanoscale bioscaffold technology can establish the environment for plant growth in areas unsuited for agriculture. Probiotics combined with prebiotics, for example, possess the potential to attenuate the side effects of radiotherapy. Proper plant-plant interaction technology provides stable water and nutrients for a long time in the soil profile [188, 189].

Conclusion

The integrated study employed diverse laboratory-based analytical techniques to characterize probiotic microorganisms and phytochemicals, and examine synergistic interactions between probiotics and plant extracts. Four probiotic bacteria were investigated, with the genus Lactobacillus predominating; Lacticaseibacillus plantarum and Lactobacillus rhamnosus subsp. rhamnosus also contain cellular exopolysaccharides. Two essential assays supported the safety qualification of these probiotics. Phytochemical profiles of eight representative plant extracts were established using high-resolution chromatography and spectroscopy supported by comprehensive compound libraries; radical-scavenging activity and antimicrobial action were also determined. The extracted bioactive compounds served as quality-control standards for phytochemical-enriched probiotic formulations. A novel assay for detecting antimicrobial activity of known bioactive constituents was validated and compared in different experimental settings. Together, these techniques provided essential information on microbial species, extract characteristics, effect directionality, and supporting data quality, thus enabling comprehensive analysis of interactions involving probiotics and phytochemicals.

A wide range of *in vitro* models evaluated the effectiveness of combined treatments. Radical-scavenging assays for antioxidant activity and determination of antimicrobial effects against plant pathogens and spoilage bacteria were complemented by animal models for clinical secondary effects and therapeutic activity against COVID-19 and concomitant infections. Co-action on crop yield improvement and disease resistance was also investigated. Experimental approaches have included a combination of probiotics and phytochemicals in soil amendments, pathogen biocontrol protocols, and protective or stimulatory treatments against different plant pathogens. Omics technologies were applied to characterize plant responses to combined treatments and other methods were evaluated for assessing synergistic effects. These provide assured success in the respective areas of analysis, while the layer of investigated conditions serves as a model for future data interlinking and validation.

References

1. A. Machado, L. Zamora-Mendoza, F. Alexis, "Use of plant extracts, bee-derived products, and probiotic-related applications to fight multidrug-resistant pathogens in the post-antibiotic era," *Future*, vol. 2023. mdpi.com
2. D. Teneva and P. Denev, "Biologically active compounds from probiotic microorganisms and plant extracts used as biopreservatives," Microorganisms, 2023. mdpi.com
3. A. T. Holkem and M. P. Silva, "Probiotics and plant extracts: A promising synergy and delivery systems," Reviews in Food Science, 2023. [HTML]
4. T. D. Veenstra, "Omics in systems biology: current progress and future outlook," Proteomics, 2021. researchgate.net
5. X. Dai and L. Shen, "Advances and trends in omics technology development," Frontiers in Medicine, 2022. frontiersin.org
6. F. Pettini, A. Visibelli, V. Cicaloni, D. Iovinelli, *et al.*, "Multi-omics model applied to cancer genetics," *Journal of Molecular*, vol. 2021. mdpi.com
7. R. Thomas, M. Williams, J. Aldous, Y. Yanagisawa, "... randomised, double-blind, placebo-controlled trial evaluating concentrated phytochemical-rich nutritional capsule in addition to a probiotic capsule on clinical...," COVID, 2022. mdpi.com
8. P. Halagali, A. Inamdar, J. Singh, A. Anand, "Phytochemicals, herbal extracts, and dietary supplements for metabolic disease management," *Metabolic & Immune*, 2025. [HTML]

9. M. S. Hossain, M. A. Wazed, S. Asha, *et al.*, "Dietary phytochemicals in health and disease: Mechanisms, clinical evidence, and applications—a comprehensive review," Food Science & Technology, vol. 2025, Wiley Online Library. wiley.com

10. S. Santhiravel, A. E. D. A. Bekhit, E. Mendis, "The impact of plant phytochemicals on the gut microbiota of humans for a balanced life," *International Journal of...*, 2022. mdpi.com

11. V. K. Upadhayay, M. K. Chitara, D. Mishra, and M. N. Jha, "Synergistic impact of nanomaterials and plant probiotics in agriculture: A tale of two-way strategy for long-term sustainability," *Frontiers in …*, 2023. frontiersin.org

12. S. Rastogi and A. Singh, "Gut microbiome and human health: Exploring how the probiotic genus Lactobacillus modulate immune responses," Frontiers in Pharmacology, 2022. frontiersin.org

13. M. Calcagnile, S. M. Tredici, and P. Alifano, "A comprehensive review on probiotics and their use in aquaculture: Biological control, efficacy, and safety through the genomics and wet methods," Heliyon, 2024. cell.com

14. Z. Qu, Y. Wu, Y. Jiang, J. Fan, L. Cao, Y. Dong, and S. Fang, "Effects of Two Compound Probiotic Formulations on Gastrointestinal Symptoms and Gut Microbiota: A 4-Week Randomized, Double-Blind Intervention Trial," Nutrients, 2025. mdpi.com

15. S. A. Ibrahim, P. J. Yeboah, R. D. Ayivi, "A review and comparative perspective on health benefits of probiotic and fermented foods," *Journal of Food*, vol. 2023. oup.com

16. D. Dahiya and P. S. Nigam, "Use of characterized microorganisms in fermentation of non-dairy-based substrates to produce probiotic food for gut-health and

nutrition," Fermentation, 2022. mdpi.com

17. H. Kaur, G. Kaur, and S. A. Ali, "Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties," Fermentation, 2022. mdpi.com
18. K. B. Sampaio, V. Fusco, J. L. de Brito Alves, *et al.*, "Probiotics: Concepts, evolution, and applications," in *Probiotics for Human Health*, 2022, Elsevier. [HTML]
19. S. Yao, F. Bourdichon, M. Kowalczyk, *et al.*, "Identification of Probiotics at the strain level-Guidance Document: Update of Bulletin of IDF N° 462," Bulletin of the IDF, 2021. [HTML]
20. R. A. Yunes, E. U. Poluektova, T. V. Belkina, *et al.*, "Lactobacilli: Legal regulation and prospects for new generation drugs," *Applied Biochemistry*, vol. 2022, Springer. nih.gov
21. A. Choudhury, "Potential role of bioactive phytochemicals in combination therapies against antimicrobial activity," Journal of Pharmacopuncture, 2022. nih.gov
22. Y. Fan, Y. Liu, C. Shao, C. Jiang, L. Wu, J. Xiao, "Gut Microbiota-Targeted Therapeutics for Metabolic Disorders: Mechanistic Insights into the Synergy of Probiotic-Fermented Herbal Bioactives," *International Journal of …*, 2025. mdpi.com
23. X. Chen, H. Li, B. Zhang, and Z. Deng, "The synergistic and antagonistic antioxidant interactions of dietary phytochemical combinations," *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 1, pp. 1-15, 2022. [HTML]
24. F. Shirkhan, F. Safaei, S. Mirdamadi, and M. Zandi, "The role of probiotics in skin care: advances, challenges, and future needs," Probiotics and Antimicrobial, vol. 2024, Springer.

researchgate.net

25. A. Otsuka, C. Moriguchi, Y. Shigematsu, K. Tanabe, "Fermented cosmetics and metabolites of skin microbiota—a new approach to skin health," *Fermentation*, vol. 8, no. 4, 2022. mdpi.com
26. H. Cui, C. Feng, T. Zhang, V. Martínez-Ríos, P. Martorell, and others, "Effects of a lotion containing probiotic ferment lysate as the main functional ingredient on enhancing skin barrier: A randomized, self-control study," *Scientific Reports*, vol. 13, no. 1, 2023. nature.com
27. M. T. El-Saadony, A. M. Saad, S. M. Soliman, *et al*., "Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives," *Frontiers in Plant Science*, vol. 2022. frontiersin.org
28. H. Wang, R. Liu, M. P. You, M. J. Barbetti *et al*., "Pathogen biocontrol using plant growth-promoting bacteria (PGPR): Role of bacterial diversity," Microorganisms, 2021. mdpi.com
29. V. R. Messa, "Biocontrol by induced systemic resistance using plant growth promoting rhizobacteria," Rhizosphere, 2021. [HTML]
30. S. D. Christopoulou, C. Androutsopoulou, P. Hahalis, *et al*., "Rosemary extract and essential oil as drink ingredients: An evaluation of their chemical composition, genotoxicity, antimicrobial, antiviral, and antioxidant …," Foods, vol. 2021. mdpi.com
31. J. P. Veenstra and J. J. Johnson, "Rosemary (Salvia rosmarinus): Health-promoting benefits and food preservative properties," International journal of nutrition, 2021. nih.gov

32. R. Kaur, T. B. Gupta, J. Bronlund, and L. Kaur, "The potential of rosemary as a functional ingredient for meat products-a review," Food Reviews International, 2023. [HTML]

33. S. Suriyaprom, P. Mosoni, S. Leroy, T. Kaewkod, "Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria," Antioxidants, 2022. mdpi.com

34. J. Sharifi-Rad, C. Quispe, A. Bouyahya, *et al.*, "Ethnobotany, Phytochemistry, Biological Activities, and Health-Promoting Effects of the Genus Bulbophyllum," Evidence-based Complementary and Alternative Medicine, vol. 2022, 2022. wiley.com

35. M. Thakur, I. K. Kasi, P. Islary, and S. K. Bhatti, "Nutritional and health-promoting effects of lichens used in food applications," Current Nutrition Reports, 2023. researchgate.net

36. S. Bagade, D. D. Patil, and A. Shirkhedkar, "Standardization of herbal bioactives," in *Herbal bioactive-based drug delivery*, 2022, Elsevier. [HTML]

37. I. Usman, M. Hussain, A. Imran, M. Afzaal, *et al.*, "Traditional and innovative approaches for the extraction of bioactive compounds," *Journal of Food*, vol. 2022, Taylor & Francis. tandfonline.com

38. J. Šuran, I. Cepanec, T. Mašek, B. Radić, and S. Radić, "Propolis extract and its bioactive compounds—From traditional to modern extraction technologies," Molecules, 2021. mdpi.com

39. B. Li, L. L. Pan, and J. Sun, "Novel probiotic lactic acid bacteria were identified from healthy infant feces and exhibited anti-inflammatory capacities," Antioxidants, 2022. mdpi.com

40. G. Siesto, R. Pietrafesa, V. Infantino, C. Thanh, *et al*., "*In vitro* Study of Probiotic, Antioxidant and Anti-Inflammatory Activities among Indigenous Saccharomyces cerevisiae Strains," Foods, vol. 11, no. 2022. mdpi.com
41. K. T. Kim, J. W. Kim, S. I. Kim, S. Kim *et al*., "Antioxidant and anti-inflammatory effect and probiotic properties of lactic acid bacteria isolated from canine and feline feces," Microorganisms, 2021. mdpi.com
42. U. Chakraborty and P. Ramteke, "Plant probiotics: recent and future prospects," Frontiers in Plant Science, 2023. frontiersin.org
43. Y. Tian, Y. Liu, C. Uwaremwe, X. Zhao, L. Yue, and Q. Zhou, "Characterization of three new plant growth-promoting microbes and effects of the interkingdom interactions on plant growth and disease prevention," Plant Cell Reports, vol. 2023, Springer. researchgate.net
44. Z. Wang, Z. Chen, M. F. A. Leite, Z. Xu, and Q. Lin, "Effects of probiotic consortia on plant metabolites are associated with soil indigenous microbiota and fertilization regimes," *Industrial Crops and Products*, vol. 202, 2022. knaw.nl
45. M. G. Ahmed, E. A. Elwakeel, S. Z. El-Zarkouny, "Environmental impact of phytobiotic additives on greenhouse gas emission reduction, rumen fermentation manipulation, and performance in ruminants: an updated …," in *Science and Pollution*, 2024, Springer. springer.com
46. N. M. Hashem, N. S. Hosny, N. El-Desoky, and Y. A. Soltan, "Composite of pomegranate peel phytogenics and multi-probiotic species as a potential feed additive: physicochemical, antioxidant, and antimicrobial activities," Animals, 2023. mdpi.com
47. M. M. Haque, M. Khatun, M. K. Mosharaf, A. Rahman, *et*

al., "Biofilm producing probiotic bacteria enhance productivity and bioactive compounds in tomato," Biocatalysis and…, vol. 2023, Elsevier. [HTML]

48. M. J. Seo, "Fermented foods and food microorganisms: antioxidant benefits and biotechnological advancements," Antioxidants, 2024. mdpi.com
49. B. Wróblewska, A. Kuliga, and K. Wnorowska, "Bioactive dairy-fermented products and phenolic compounds: together or apart," Molecules, 2023. mdpi.com
50. A. Molla, "Dietary patterns and their impact on atopic dermatitis: a comprehensive review," The Open Dermatology Journal, 2024. opendermatologyjournal.com
51. S. Lee, D. K. Lee, S. Jeon, S. H. Kim, J. Jeong, and J. S. Kim, "Combination effect of nanoparticles on the acute pulmonary inflammogenic potential: additive effect and antagonistic effect," Taylor & Francis, 2021. [HTML]
52. A. M. Castañeda, C. M. Meléndez, D. Uribe, *et al*., "Synergistic effects of natural compounds and conventional chemotherapeutic agents: Recent insights for the development of cancer treatment strategies," Heliyon, vol. 8, no. 12, 2022. cell.com
53. X. Zhou, H. Li, Y. Han, and G. Xu, "Solid-state fermentation by Lactiplantibacillus plantarum improves the active ingredients levels and physiological activities of Artemisia argyi leaves," Industrial Crops and Products, 2025. sciencedirect.com
54. H. Zhan, W. Xu, X. Zhao, L. Tian, F. Zhang, "Effects of Lactobacillus plantarum Wlpl01 fermentation on antioxidant activities, bioactive compounds, and flavor profile of Artemisia Argyi," 2022. ssrn.com
55. S. Yaqoob, M. M. Gouda, B. Mubeen, A. Imtiaz, *et al*.,

"Synergistic enhancement in the mulberry juice's antioxidant activity by using lactic acid bacteria co-culture fermentation," *Scientific Reports*, 2025. nature.com

56. G. Wei, M. Dan, G. Zhao, and D. Wang, "Recent advances in chromatography-mass spectrometry and electronic nose technology in food flavor analysis and detection," Food Chemistry, 2023. [HTML]
57. S. K. Pramod and K. A. Navnath, "A review on gas chromatography-mass spectrometry (GC-MS)," *World Journal*, vol. 2021, pp. 1-10, 2021. amazonaws.com
58. L. Toribio, J. Bernal, M. T. Martín, and A. M. Ares, "Supercritical fluid chromatography coupled to mass spectrometry: A valuable tool in food analysis," *Trends in Analytical Chemistry*, vol. 143, 2021. uva.es
59. D. Sharma, V. P. Singh, R. K. Singh, and C. S. Joshi, "Isolation and characterization of bioactive compounds from natural resources: Metabolomics and molecular approaches," *Journal of Anticancer Molecules*, vol. 2021, Elsevier. researchgate.net
60. K. Kaur, V. Sharma, B. Kabila, "The Science of Omics: A Recent Approach for Screening and Enhancement of Bioactive Compounds in Medicinal Plants," Biotechnological..., 2025. [HTML]
61. K. Joyce, G. T. Fabra, Y. Bozkurt, and A. Pandit, "Bioactive potential of natural biomaterials: Identification, retention and assessment of biological properties," Signal Transduction and..., vol. XX, no. YY, pp. ZZ-ZZ, 2021. nature.com
62. P. Atri, "Enhancing the reliability and accuracy of data pipelines through effective testing and validation strategies: A comprehensive approach," European Journal of Advances in Engineering and …, 2023. researchgate.net

63. D. Sargiotis, "Data quality management: ensuring accuracy and reliability," Data Governance: A Guide, 2024. [HTML]
64. A. Livera, M. Theristis, E. Koumpli, "Data processing and quality verification for improved photovoltaic performance and reliability analytics," in *Research and …*, 2021. wiley.com
65. J. J. Ahire, A. Rohilla, V. Kumar, and A. Tiwari, "Quality management of probiotics: ensuring safety and maximizing health benefits," Current Microbiology, 2024. [HTML]
66. G. Zavišić, S. Ristić, B. Petković, "Microbiological quality of probiotic products," Arhiv za…, 2023. bg.ac.rs
67. A. L. Shane and G. A. Preidis, "Probiotics in the neonatal intensive care unit—A framework for optimizing product standards," JAMA pediatrics, 2023. [HTML]
68. S. R. Khairul, S. S. Leong, F. Korel, "Systematic Review of Emerging Trends in Soil-Based Probiotic," Malaysian Journal of Soil, 2024. msss.com.my
69. X. Zhang, L. Zhang, J. Liu, Z. Shen, Z. Liu, H. Gu, and X. Hu, "Biofertilizers enhance soil fertility and crop yields through microbial community modulation," Agronomy, 2025. mdpi.com
70. A. A. Shahane and Y. S. Shivay, "Soil health and its improvement through novel agronomic and innovative approaches," Frontiers in Agronomy, 2021. frontiersin.org
71. I. Rashmi, A. Kumawat, A. Munawery, "Soil amendments: an ecofriendly approach for soil health improvement and sustainable oilseed production," in *Oilseed crops-uses*, 2022. intechopen.com
72. V. K. Singh, G. S. Malhi, M. Kaur, and G. Singh, "Use of organic soil amendments for improving soil ecosystem health

and crop productivity," Ecosystem…, 2022. researchgate.net

73. R. R. Molefe, A. E. Amoo, and O. O. Babalola, "Communication between plant roots and the soil microbiome; involvement in plant growth and development," Symbiosis, 2023. springer.com

74. M. Muhammad, A. Wahab, A. Waheed, and others, "Navigating climate change: Exploring the dynamics between plant-soil microbiomes and their impact on plant growth and productivity," Global Change, 2025. [HTML]

75. P. Chauhan, N. Sharma, A. Tapwal, A. Kumar, and G. S. Verma, "Soil microbiome: diversity, benefits and interactions with plants," Sustainability, vol. 2023. mdpi.com

76. S. Kouhounde, K. Adéoti, M. Mounir, A. Giusti, P. Refinetti, *et al.*, "Applications of probiotic-based multi-components to human, animal and ecosystem health: concepts, methodologies, and action mechanisms," *Microorganisms*, vol. 10, no. 5, 2022. mdpi.com

77. W. Zhang, S. Wu, E. H. Jho, J. Chen, Q. Liu, and J. Hu, "From soil to the intestinal tract: The key role of beneficial elements and probiotics in promoting health and longevity," *Journal of …*, 2025. [HTML]

78. C. Gao, T. M. Bezemer, F. T. de Vries, *et al.*, "Trade-offs in soil microbial functions and soil health in agroecosystems," Trends in Ecology & Evolution, 2024. uva.nl

79. S. Sarkar, R. Kumar, A. Kumar, and U. Kumar, "Role of soil microbes to assess soil health," in *Structure and functions*, 2022, Springer. researchgate.net

80. Y. Xing, X. Wang, and A. Mustafa, "Exploring the link between soil health and crop productivity," Ecotoxicology and environmental safety, 2025. sciencedirect.com

81. J. Hu, T. Yang, V. P. Friman, "Introduction of probiotic bacterial consortia promotes plant growth via impacts on the resident rhizosphere microbiome," *Journal of the Royal Society*, vol. 2021. royalsocietypublishing.org
82. A. Basu, P. Prasad, S. N. Das, S. Kalam, R. Z. Sayyed, "Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects," Sustainability, vol. 13, no. 12, 2021. mdpi.com
83. G. Hiranmayee, D. Marik, A. Sadhukhan, "Isolation of plant growth-promoting rhizobacteria from the agricultural fields of Tattiannaram, Telangana," Journal of Genetic, vol. 2023, Elsevier. sciencedirect.com
84. G. R. Fernando and S. Abeysinghe, "Identification of Plant Growth-Promoting Rhizobacteria for the Development of Probiotic Consortia," *Proceedings of …*, 2024. [HTML]
85. H. M. Rizwan, L. Zhimin, W. Harsonowati, A. Waheed, "Identification of Fungal Pathogens to Control Postharvest Passion Fruit (Passiflora edulis) Decays and Multi-Omics Comparative Pathway Analysis Reveals …," *Journal of Fungi*, 2021. mdpi.com
86. P. N. Munyao, "Occurrence of passion fruit dieback in Uasin-Gishu county and reaction of passion fruit varieties to inoculation with dieback pathogens.," 2021. uonbi.ac.ke
87. S. Hurtado-Clopatosky *et al.*, "BIOTECHNOLOGICAL POTENTIAL OF PURPLE PASSION FRUIT ENDOPHYTIC FUNGI (Passiflora edulis f. edulis; Passifloraceae)," Acta Biológica, 2023. [HTML]
88. L. A. Malard and A. Guisan, "Into the microbial niche," Trends in Ecology & Evolution, 2023. cell.com
89. H. Fujita, M. Ushio, K. Suzuki, and M. S. Abe, "Metagenomic analysis of ecological niche overlap and

community collapse in microbiome dynamics," *Frontiers in Microbiology*, vol. 2023. frontiersin.org

90. J. Yu, J. Y. Y. Lee, S. N. Tang, and P. K. H. Lee, "Niche differentiation in microbial communities with stable genomic traits over time in engineered systems," The ISME Journal, 2024. oup.com
91. A. Jain, S. Sarsaiya, R. Singh, Q. Gong, Q. Wu, "Omics approaches in understanding the benefits of plant-microbe interactions," *Frontiers in …*, 2024. frontiersin.org
92. E. Gamalero, E. Bona, and B. R. Glick, "Current techniques to study beneficial plant-microbe interactions," Microorganisms, 2022. mdpi.com
93. D. Abedini, S. Jaupitre, H. Bouwmeester, "Metabolic interactions in beneficial microbe recruitment by plants," *Current Opinion in...*, vol. 2021, Elsevier. sciencedirect.com
94. N. Nagata, S. Nishijima, T. Miyoshi-Akiyama, Y. Kojima, *et al.*, "Population-level metagenomics uncovers distinct effects of multiple medications on the human gut microbiome," Gastroenterology, vol. 162, no. 2, pp. 474-487, 2022. sciencedirect.com
95. Y. Zhang, K. N. Thompson, T. Branck, Y. Yan, "Metatranscriptomics for the human microbiome and microbial community functional profiling," *Annual Review of …*, vol. 2021. google.com
96. N. N. Nam, H. D. K. Do, K. T. Loan Trinh, and N. Y. Lee, "Metagenomics: an effective approach for exploring microbial diversity and functions," Foods, 2023. mdpi.com
97. H. Chen, K. Ma, C. Lu, Q. Fu, Y. Qiu, J. Zhao, "Functional redundancy in soil microbial community based on metagenomics across the globe," *Frontiers in

Microbiology*, 2022. frontiersin.org

98. G. S. Navgire, N. Goel, G. Sawhney, and M. Sharma, "Analysis and Interpretation of metagenomics data: an approach," *Biological Procedures Online*, vol. 2022, Springer. springer.com
99. N. R. Edwin, A. H. Fitzpatrick, F. Brennan, F. Abram, *et al.*, "An in-depth evaluation of metagenomic classifiers for soil microbiomes," Environmental..., vol. 2024, Springer. springer.com
100. X. Dong, C. Tu, Z. Xie, Y. Luo *et al.*, "The Genome of Bacillus velezensis SC60 Provides Evidence for Its Plant Probiotic Effects," Microorganisms, 2022. mdpi.com
101. T. Aziz, M. Naveed, M. A. Shabbir, A. Sarwar, "Unveiling the whole genomic features and potential probiotic characteristics of novel Lactiplantibacillus plantarum HMX2," Frontiers in..., 2024. frontiersin.org
102. P. Bolouri, R. Salami, S. Kouhi, M. Kordi, and B. Asgari Lajayer, "Applications of essential oils and plant extracts in different industries," Molecules, 2022. mdpi.com
103. S. A. Mir, A. Manickavasagan, and M. A. Shah, "Plant extracts: Applications in the food industry," 2021. [HTML]
104. A. M. Awad, P. Kumar, M. R. Ismail-Fitry, "Overview of plant extracts as natural preservatives in meat," *Journal of Food Processing*, vol. 2022, Wiley Online Library. wiley.com
105. S. Heo, T. Kim, H. E. Na, G. Lee, and J. H. Park, "Safety assessment systems for microbial starters derived from fermented foods," *Journal of Microbiology and Biotechnology*, vol. 2022. nih.gov
106. A. Mukherjee, B. Gómez-Sala, E. M. O'Connor, "Global

regulatory frameworks for fermented foods: a review," Frontiers in…, 2022. frontiersin.org

107. K. Skowron, A. Budzyńska, K. Grudlewska-Buda, "Two faces of fermented foods—the benefits and threats of its consumption," Frontiers in..., vol. XX, no. XX, pp. XX-XX, 2022. frontiersin.org

108. YB Soemarie and T Milanda, "Fermented foods as probiotics: A review," Journal of Advanced..., vol. 2021. lww.com

109. Y. Yuan, Y. Yang, L. Xiao, L. Qu *et al*., "Advancing insights into probiotics during vegetable fermentation," Foods, 2023. mdpi.com

110. P. Wang, J. Wu, T. Wang, Y. Zhang, X. Yao, and J. Li, "Fermentation process optimization, chemical analysis, and storage stability evaluation of a probiotic barley malt kvass," *Bioprocess and Biosystems Engineering*, vol. 45, no. 5, pp. 1234-1245, 2022. [HTML]

111. F. Ahmed, S. Maqsood, M. F. Ahmad, "Optimizing Health and Sustainability: Innovations in Harnessing Phytochemicals for Functional Foods," Food Science & …, 2025. wiley.com

112. D. Marta and J. Snežana, "NATURE'S PHARMACY: PHYTOCHEMICALS AND PROBIOTICS AS THE GUARDIANS OF GUT WELLNESS AND IMMUNOLOGICAL HEALTH.," Genetics & Applications, 2024. [HTML]

113. A. Gawad, F. Molham, A. I. Tagyan, W. N. Hozzein, *et al*., "Microbiological and pharmacological investigation of phytochemicals extracted from selected ethnomedicinal plants with their potential against food pathogen," *Scientific Reports*, 2025. nature.com

114. F. Plescia, F. Venturella, A. D'Anneo, and others, "Phytochemical-rich extracts of Helianthemum lippii possess antimicrobial, anticancer, and anti-biofilm activities," *All Aspects of Plant*, vol. 2022, Taylor & Francis. uniba.it

115. S. Kamonwannasit, B. Prajanban, O. Prasitnok, "Phytochemical Profiling and Bactericidal Mechanism of Crinum wattii Extracts," Trends in..., 2025. wu.ac.th

116. C. F. Balthazar, J. F. Guimarães, *et al.*, "The future of functional food: Emerging technologies application on prebiotics, probiotics and postbiotics," *Reviews in Food Science and Nutrition*, vol. 62, no. 1, pp. 1-15, 2022. unimelb.edu.au

117. J. Palanivelu, S. Thanigaivel, S. Vickram, N. Dey, "Probiotics in functional foods: survival assessment and approaches for improved viability," *Applied Sciences*, vol. 12, no. XX, pp. XX-XX, 2022. mdpi.com

118. A. Bhatia, D. Sharma, J. Mehta, "Probiotics and synbiotics: applications, benefits, and mechanisms for the improvement of human and ecological health," Healthcare, 2025, Taylor & Francis. tandfonline.com

119. V. L. Dasriya, M. Samtiya, S. Ranveer, *et al.*, "Modulation of gut- microbiota through probiotics and dietary interventions to improve host health," *Journal of the …*, 2024. wiley.com

120. E. M. Mercer and M. C. Arrieta, "Probiotics to improve the gut microbiome in premature infants: are we there yet?," Gut microbes, 2023. tandfonline.com

121. R. J. Thomas, S. A. Kenfield, M. Williams, *et al.*, “Phytochemical-rich Foods and Lactobacillus Probiotics in Men with Low-risk Prostate Cancer—A Randomised,

Double-blind, Placebo-controlled Trial,” European Urology, 2025. sciencedirect.com

122. M. Milutinović, S. Dimitrijević-Branković, and others, "Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms," *Frontiers in …*, 2021. frontiersin.org
123. D. Ning, Y. Huang, Y. Zhou, H. Zhao, W. Nie, "Evaluation of the immunomodulatory effects of a novel synbiotic made of combined use of probiotic-prebiotic-Chinese traditional herbs," Journal of Agriculture, 2024. sciencedirect.com
124. K. C. Baral, R. Bajracharya, S. H. Lee, "Advancements in the pharmaceutical applications of probiotics: dosage forms and formulation technology," *International Journal of...*, vol. 2021, Taylor & Francis. tandfonline.com
125. D. Liang, F. Wu, D. Zhou, and B. Tan, "Commercial probiotic products in public health: Current status and potential limitations," in *Journal of Food Science and Technology*, vol. 2024, Taylor & Francis. [HTML]
126. N. A. Ushakova, V. G. Pravdin, L. Z. Kravtsova, *et al.*, "Complex bioactive supplements for aquaculture—evolutionary development of probiotic concepts," *Probiotics and Antimicrobial Proteins*, vol. 13, no. 1, pp. 1-15, 2021. [HTML]
127. P. Bousdouni, A. Kandyliari, and A. E. Koutelidakis, "Probiotics and phytochemicals: role on gut microbiota and efficacy on irritable bowel syndrome, functional dyspepsia, and functional constipation," Gastrointestinal Disorders, 2022. mdpi.com
128. M. Heinrich, B. Jalil, M. Abdel-Tawab, *et al.*, "Best practice in the chemical characterisation of extracts used in pharmacological and toxicological research—the

ConPhyMP—guidelines," *Frontiers in …*, 2022. frontiersin.org

129. G. Hassen, G. Belete, K. G. Carrera, R. O. Iriowen, H. Araya, "Clinical implications of herbal supplements in conventional medical practice: a US perspective," Cureus, 2022. cureus.com

130. M. Monagas, T. Brendler, J. Brinckmann, "Understanding plant to extract ratios in botanical extracts," Frontiers in…, 2022. frontiersin.org

131. W. Peijnenburg, A. G. Oomen, L. G. Soeteman-Hernández, *et al.*, "Identification of emerging safety and sustainability issues of advanced materials: Proposal for a systematic approach," NanoImpact, vol. 21, 2021. academia.edu

132. S. Ledlie, R. Garg, C. Cheng, G. Kolla, T. Antoniou, "Prescribed safer opioid supply: a scoping review of the evidence," *International Journal of …*, 2024. sciencedirect.com

133. C. Domingues, A. Santos, C. Alvarez-Lorenzo, "Where is nano today and where is it headed? A review of nanomedicine and the dilemma of nanotoxicology," ACS Publications, 2022. [HTML]

134. M. Katkowska, K. Garbacz, and A. Kusiak, "Probiotics: should all patients take them?," Microorganisms, 2021. mdpi.com

135. H. Borase, M. K. Dwivedi, R. Krishnamurthy, "Probiotics: Health safety considerations," in *Probiotics in the prevention…*, Elsevier, 2022. [HTML]

136. U. J. Eze, A. Lal, M. I. Elkoush, M. Halytska *et al.*, "Recurrent Lactobacillus rhamnoses bacteremia and complications in an immunocompromised patient with history of probiotic use: a case report," Cureus, 2024.

cureus.com

137. A. Anadón, I. Ares, M. R. Martínez-Larrañaga, "Probiotics: safety and toxicity considerations," Nutraceuticals, 2021. [HTML]

138. S. Farooq, "A review on pharmacokinetics, mechanism of action and side effects of probiotics," International Journal of Molecular Microbiology, 2024. psmjournals.org

139. B. A. Tegegne and B. Kebede, "Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature," Heliyon, 2022. cell.com

140. C. Barba-Ostria and S. E. Carrera-Pacheco, "Evaluation of biological activity of natural compounds: current trends and methods," Molecules, 2022. mdpi.com

141. N. Vaou, E. Stavropoulou, C. Voidarou, Z. Tsakris, "Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects," *Antibiotics*, 2022. mdpi.com

142. S. F. Ahmed, M. S. B. Alam, M. Hassan, M. R. Rozbu, "Deep learning modelling techniques: current progress, applications, advantages, and challenges," *Artificial Intelligence*, vol. 2023, Springer. springer.com

143. H. Alaoui Mdarhri, R. Benmessaoud, H. Yacoubi, L. Seffar, *et al.*, "Alternatives therapeutic approaches to conventional antibiotics: advantages, limitations and potential application in medicine," *Antibiotics*, vol. 2022. mdpi.com

144. A. A. Wani, "Comprehensive analysis of clustering algorithms: exploring limitations and innovative solutions," PeerJ Computer Science, 2024. peerj.com

145. K. Venkatachalam, K. Goswami, V. Madka, and C. V. Rao,

"A Review of Potential Agents for Colon Cancer Interception in FAP Patients: Evidence from Preclinical Studies in APCMin/+ Mice," Targets, 2025. mdpi.com

146. Q. Chen, J. Lei, J. Zhou, S. Ma, Q. Huang, "Chemopreventive effect of 4′-hydroxychalcone on intestinal tumorigenesis in ApcMin mice," *Oncology*, vol. 2021, 2021. spandidos-publications.com

147. M. H. Kucherlapati, "Mouse models in colon cancer, inferences, and implications," Iscience, 2023. cell.com

148. M. W. Rasheed, J. Tang, A. Sarwar, S. Shah, and N. Saddique, "Soil moisture measuring techniques and factors affecting the moisture dynamics: A comprehensive review," Sustainability, vol. 2022. mdpi.com

149. A. Balliu, Y. Zheng, G. Sallaku, J. A. Fernández, N. S. Gruda, "Environmental and cultivation factors affect the morphology, architecture and performance of root systems in soilless grown plants," Horticulturae, vol. 7, no. 2, 2021. mdpi.com

150. B. J. Huntley, "Soil, water and nutrients," Ecology of Angola: Terrestrial biomes and ecoregions, 2023. springer.com

151. P. Gupta, H. Dhar, Y. S. Bagal, and S. Jaglan, "Smart nano-fertilizers: a path to sustainable agriculture," Environmental Geochemistry and..., 2025. [HTML]

152. C. S. F. de Oliveira and F. K. Tavaria, "Therapeutic textiles: A promising approach for human skin dysbiosis?," Experimental Dermatology, 2024. wiley.com

153. H.A. López-Aguilar, A. Pérez-Hernández, *et al.*, "Biogas Production and Evaluation of the Potential of Sargassum Digestate as an Agricultural Substrate," Resources, 2025. mdpi.com

154. T. Lu, F. Yu, Y. Yu, and L. Zhang, "Intelligent pear variety classification models based on Bayesian optimization for deep learning and its interpretability analysis," Scientific Reports, 2025. nature.com

155. II Ajayi, TH Fatoki, AS Alonge, TC Balogun, "In Silico ADME, Molecular Targets, Docking and Molecular Dynamics Simulation of Key Phytoconstituents of Lobelia inflata," Journal of …, vol. 2024, World Scientific. [HTML]

156. T. Sevimoglu, E. Kızılpınar, Z. O. Çalışkaner, and N. Zengin, "Uncovering potent natural phytochemicals targeting SARS-COV-2 spike protein variants: molecular dynamics insights," Scientific Reports, 2025. nature.com

157. J. Zhang, L. Zhang, C. Wang, H. Liu, S. Dai, and L. Sun, "The Fritillaria alkaloid peiminine acts as a chemosensitizer to potentiate oxaliplatin efficacy against gastric cancer," *Research and Practice*, vol. 2025, Elsevier. [HTML]

158. S. Alanazi, "Recent Advances in Liquid Chromatography-Mass Spectrometry (LC-MS) Applications in Biological and Applied Sciences," Analytical Science Advances, 2025. wiley.com

159. H. Y. Magee, M. M. Maurer, A. Cobos, S. Adhikari, *et al.*, "Impact of LC-MS/MS quantification methods on analytical results by example of nationwide US environmental samples," *Science of the Total Environment*, vol. 2023, Elsevier. ssrn.com

160. B. P. N. Yuen, K. S. Wong, Y. M. So, W. H. Kwok, *et al.*, "Gene doping control analysis of human erythropoietin transgene in equine plasma by PCR-Liquid chromatography high-resolution tandem mass spectrometry," *Analytical Chemistry*, vol. 2024, ACS Publications. chemrxiv.org

161. A. Assouguem, S. Annemer, K. Mohammed, and A. Lazraq,

"Innovative approaches in the extraction, identification, and application of secondary metabolites from plants," Phyton, 2025. [HTML]

162. M. A. Acquavia, R. Pascale, L. Foti, G. Carlucci, and L. Scrano, "Analytical Methods for Extraction and Identification of Primary and Secondary Metabolites of Apple (Malus domestica) Fruits: A Review," *Separations*, vol. 2021. mdpi.com

163. N. Gatti, M. Maghrebi, G. Serio, C. Gentile, "Seaweed and yeast extracts as sustainable phytostimulant to boost secondary metabolism of apricot fruits," Frontiers in Plant, 2025. frontiersin.org

164. H. R. Roth, M. Lewis, and L. Hancock, "Laws, regulations, standards, certifications, and ecolabels," in *The Green Building Materials Manual: A …*, 2021, Springer. [HTML]

165. M. K. Misra, A. Harries, and M. Dadlani, "Role of seed certification in quality assurance," Seed Science and Technology, vol. 2023. oapen.org

166. A. Bujang and S. Bakar, "Halal packaging: halal control point in manufacturing of packaging materials and halal labeling," in *Innovation of food products in halal supply chain*, 2023, Elsevier. [HTML]

167. L. Inbathamizh and D. Prabavathy, "Quality assurance of nutraceuticals and their approval, registration, marketing," in *... and applications*, 2022. [HTML]

168. S. D. Jain, S. K. Shrivastava, A. Agrawal, *et al*., "WHO guidelines for quality control of herbal medicines: From cultivation to consumption," *International Journal of …*, 2024. researchgate.net

169. D. Bhaduri, D. Sihi, A. Bhowmik, and B. C. Verma, "A review on effective soil health bio-indicators for ecosystem

restoration and sustainability," *Frontiers in …*, 2022. frontiersin.org

170. D. A. Nikitin, M. V. Semenov, T. I. Chernov, "Microbiological indicators of soil ecological functions: a review," Eurasian Soil Science, vol. 2022, Springer. researchgate.net

171. N. D. Ananyeva, K. V. Ivashchenko, and S. V. Sushko, "Microbial indicators of urban soils and their role in the assessment of ecosystem services: a review," Eurasian Soil Science, 2021. [HTML]

172. I. P. Nwakoby and I. H. Iheukwumere, "Use of probiotics as food additives and its legal basis," African Journal of..., 2025. ipsintelligentsia.com

173. R. A. Britton, D. E. Hoffmann, and A. Khoruts, "Probiotics and the microbiome—how can we help patients make sense of probiotics?," Gastroenterology, 2021. gastrojournal.org

174. M. E. Boyte, N. Akhtar, B. Koshy, *et al.*, "A review of probiotic ingredient safety supporting monograph development conducted by the United States Pharmacopeia (USP)," Journal of Dietary, 2025. tandfonline.com

175. R. Sweet, P. A. Kroon, and M. A. Webber, "Activity of antibacterial phytochemicals and their potential use as natural food preservatives," *Critical Reviews in Food Science and Nutrition*, vol. 2024, Taylor & Francis. [HTML]

176. QA Al-Maqtari, A. Rehman, A. A. Mahdi, W. Al-Ansi, "Application of essential oils as preservatives in food systems: challenges and future prospectives-a review," *Phytochemistry*, vol. 2022, pp. 1-15, Springer, 2022. researchgate.net

177. C. Novais, A. K. Molina, R. M. V. Abreu, "Natural food

colorants and preservatives: A review, a demand, and a challenge," *Journal of Agricultural*, vol. 2022, ACS Publications. acs.org

178. S. Misra and S. Raghuwanshi, "Safety concerns, regulatory guidelines, current market trends, and future directions toward the use of probiotics in gut-brain-skin axis," in *Research in Therapeutics: Volume 3: Probiotics*, 2021, Springer. [HTML]

179. D. Merenstein, B. Pot, G. Leyer, A. C. Ouwehand, *et al.*, "Emerging issues in probiotic safety: 2023 perspectives," Gut, vol. 2023, Taylor & Francis. tandfonline.com

180. S. Udayakumar, D. M. D. Rasika, H. Priyashantha, *et al.*, "Probiotics and beneficial microorganisms in biopreservation of plant-based foods and beverages," *Applied Sciences*, vol. 12, no. X, pp. Y-Z, 2022. mdpi.com

181. B. Sionek and A. Szydłowska, "Probiotics and Prebiotics in the Aspect of Health Benefits and the Development of Novel Plant-Based Functional Food," Applied Sciences, 2025. mdpi.com

182. M. Ali, K. Shabbir, S. Ali, M. Mohsin, A. Kumar, "A New Era of Discovery: How Artificial Intelligence has Revolutionized the Biotechnology," *Journal of Biotechnology*, 2024. nepjb.com

183. A. Holzinger, K. Keiblinger, P. Holub, K. Zatloukal, "AI for life: Trends in artificial intelligence for biotechnology," *New Biotechnology*, vol. 2023, Elsevier. sciencedirect.com

184. H. Takeda, "The Impact of Artificial Intelligence on Biotechnological Research and Development," Journal of Commercial Biotechnology, 2025. [HTML]

185. Z. Jiang, Y. Liu, and Z. Ke, "Recent Progress in Autonomous Laboratories for Chemical Synthesis," Communications in Computational..., 2025. global-sci.org

186. Y. Tang, W. Wan, H. Chen, and M. Matsushita, "Zero-Shot Recognition of Test Tube Types by Automatically Collecting and Labeling RGB Data," in *Proceedings of the IEEE International Conference on Robotics and Automation*, 2025. [HTML]

187. H. Zhu, Z. Wang, L. Li, L. Dong, Y. Li, B. Xue, "Revolutionizing supramolecular materials design with artificial intelligence," Supramolecular Materials, 2025. sciencedirect.com

188. Y. Lu, X. Luo, D. Yang, Y. Li, T. Gong, B. Li, and J. Cheng, "Effects of probiotic supplementation on related side effects after chemoradiotherapy in cancer patients," *Frontiers in …*, 2022. frontiersin.org

189. R. Danis, M. Mego, M. Antonova, *et al*., "Orally administered probiotics in the prevention of chemotherapy (±Radiotherapy)-induced gastrointestinal toxicity: a systematic review with meta-analysis of …," Integrative Cancer Research, 2022. sagepub.com