RESEARCH PAPER

The Evolution of Biotechnology: Historical Discoveries, Major Milestones, and Recent Advances: A Comprehensive Review

Nalini T J.,[1] Dr Shubha.,[2] Prathibha K Y.,[3] Gurumayum Gracey Sharma.,[4] Meghana B S.,[5] Marhoob Banu.,[6*] Aathiya Vanekar.,[7] Lakshmi R.,[8]

1, Associate Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

2, Professor and Principal, Department of Botany, Government First Grade College, Vijayanagar, Bengaluru, Karnataka, India, 560104

3, Professor and Chairperson, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

4,5,6,8, M.Sc. students, Department of Botany, Bangalore University, Bengaluru, Karnataka, India, 560056

7, M.Sc. in Biotechnology Student, Indian Academy Degree College, Bangalore North University, Bengaluru, Karnataka, India, 560043

Corresponding Author: Marhoob Banu

Email: marhoobbanu@gmail.com

ABSTRACT

Biotechnology has evolved from ancient fermentation practices and the domestication of plants and animals into a multidisciplinary field that integrates molecular biology, genetics, engineering, and computational sciences. This review presents a comprehensive overview of the historical evolution of biotechnology, highlighting the major scientific discoveries, technological milestones, and recent advances that have shaped its development. Early contributions by Robert Hooke, Antonie van Leeuwenhoek, Louis Pasteur, Gregor Mendel, and Karl Ereky established the foundations of cell biology, microbiology, genetics, and industrial biotechnology. Subsequent breakthroughs, including the elucidation of the DNA double helix, recombinant DNA technology, recombinant human insulin, the Human Genome Project, and CRISPR-Cas9 genome editing, revolutionized biological research and enabled transformative applications in medicine, agriculture, and industry. The review further discusses advances in genome engineering, viral and non-viral gene delivery systems, synthetic biology, regenerative medicine, tissue engineering, bioprinting, biofortification, cellular agriculture, and sustainable biomanufacturing. Recent innovations involving high-throughput sequencing, multi-omics technologies, messenger RNA therapeutics, artificial intelligence, protein structure prediction, and computational protein design have accelerated biological discovery and expanded the capabilities of precision medicine and biotechnology. The review also examines the emergence of biotechnology regulation, the development of the bioeconomy, and the growing importance of ethical, safe, and sustainable implementation of biotechnology. Collectively, these developments demonstrate the remarkable progression of biotechnology from traditional biological practices to advanced genome engineering and AI-driven innovation, emphasizing its critical role in addressing global challenges in healthcare, agriculture, environmental sustainability, food security, and industrial development.

Keywords: Biotechnology, Recombinant DNA technology, Genetic engineering, CRISPR-Cas9, Genome editing, Human Genome Project, Artificial intelligence, mRNA technology, Gene therapy.

How to cite this article: Nalini TJ, Shubha, Prathibha KY, Sharma GG, Meghana BS, Banu M, Vanekar A, Lakshmi R. The Evolution of Biotechnology: Historical Discoveries, Major Milestones, and Recent Advances: A Comprehensive Review. Int J Drug Deliv Technol. 2026;16(6): 855-871 DOI: 10.25258/ijddt.16.6.126

INTRODUCTION

Biotechnology began thousands of years ago with serendipitous fermentation practices. Ancient civilizations unknowingly utilized microorganisms to produce leavened bread, cheese, wine, and beer, while also domesticating crops and livestock. Robert Hooke first described the cell in 1663, laying the foundation for cell biology (1). Antonie van Leeuwenhoek discovered bacteria in 1677, establishing microscopy as a critical tool for microbiology (1). M. Preysz, a Hungarian chemist, reported a procedure for preserving wine by heat treatment at a session of the Hungarian Society of Natural Sciences on 13 November 1861, representing one of the earliest documented applications of pasteurization (2). Louis Pasteur demonstrated in 1862 that microorganisms are responsible for fermentation, disproving the theory of spontaneous generation and transforming biotechnology from an empirical practice into a scientifically validated discipline (1,2). Gregor Mendel established the fundamental laws of genetic inheritance in 1865 through his experiments on pea plants, laying the foundation of modern genetics (1).

Fig 1: Gregor Johann Mendel (1822–1884) *Courtesy: Public domain portrait of Gregor Johann Mendel.*

Karl Ereky coined the term **"biotechnology"** in 1919 to describe the industrial application of biological processes in agricultural production (1,21,22). Alexander Fleming discovered penicillin in 1928, leading to the large-scale industrial production of antibiotics through fermentation during World War II (1). James D. Watson and Francis H. C. Crick elucidated the double-helical structure of DNA in 1953, marking the beginning of modern molecular biology (3). Stanley N. Cohen, Annie C. Y. Chang, Herbert W. Boyer, and Robert B. Helling developed recombinant DNA technology in 1973 by constructing biologically functional bacterial plasmids containing foreign DNA, initiating the era of genetic engineering (4). The emergence of biotechnology companies further accelerated the application of recombinant DNA technology in medicine. David Goeddel and colleagues at Genentech successfully produced recombinant human insulin in *Escherichia coli*, creating the first animal-free recombinant therapeutic protein for diabetes treatment. This breakthrough provided a safer and more effective alternative to animal-derived insulin and became a landmark achievement in biopharmaceutical production. The approval of recombinant human insulin, marketed as Humulin, represented the first commercial pharmaceutical product produced using recombinant DNA technology and marked the beginning of large-scale biotechnology commercialization (12, 13, 14). The United States Food and Drug Administration approved recombinant human insulin (Humulin) in 1982 as the first commercially available pharmaceutical product produced using genetically engineered bacteria (1). The International Human Genome Sequencing Consortium published the initial sequencing and analysis of the human genome in 2001, providing the first comprehensive draft of the human genome (5). The Human Genome Project was completed in 2003, providing the reference sequence of the human genome and accelerating advances in genomics and personalized medicine (1). Jennifer Doudna, Emmanuelle Charpentier, and colleagues developed the CRISPR-Cas9 genome-editing platform in 2012 by adapting the bacterial immune system into a programmable gene-editing tool, enabling precise modification of DNA sequences (6).

Fig 2: Karl Ereky (1878–1952)

Courtesy: Brain Kart – Principles and Processes of Biotechnology

During the past four decades, biotechnology has evolved into a sophisticated discipline that applies molecular biology and genetic engineering to develop innovative products and processes, revolutionizing medicine, agriculture, industry, and environmental sustainability (7). Modern biotechnology gained momentum with the discovery of penicillin by Alexander Fleming, who observed that a mold later identified as *Penicillium notatum* inhibited the growth of *Staphylococcus* colonies on a culture plate. Fleming isolated the active compound and named it penicillin, demonstrating its antibacterial activity against staphylococci and several other Gram-positive bacteria. Although initial attempts to purify penicillin were unsuccessful, his findings highlighted its immense therapeutic potential and laid the foundation for the antibiotic era. During the Second World War, the urgent demand for effective treatment of wounded soldiers and civilians encouraged scientists and engineers to collaborate in developing methods for the large-scale cultivation of *Penicillium* species. These efforts significantly advanced industrial fermentation technology and enabled the widespread availability of penicillin for medical use (8,9).

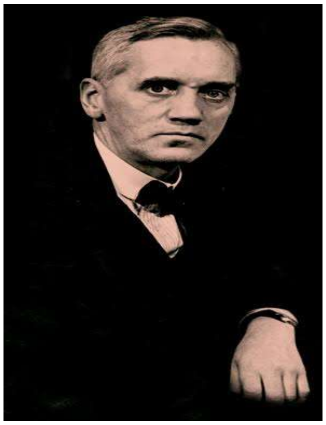

Fig 3: Sir Alexander Fleming (1881–1955)

Courtesy: Public domain portrait of Sir Alexander Fleming.

The elucidation of the double-helical structure of DNA by James Watson and Francis Crick, together with the development of DNA synthesis techniques by Arthur Kornberg, transformed biological sciences and marked the beginning of molecular biotechnology. These discoveries paved the way for recombinant DNA technology and genetic engineering, allowing scientists to manipulate genes in vitro. The development of recombinant DNA methods by Stanley Cohen and Herbert Boyer enabled the insertion of foreign genes into bacterial plasmids, establishing the foundation of modern genetic engineering (10, 11). Advances in genomics subsequently led to the sequencing of the first complete genome of a living organism, *Haemophilus influenzae*, demonstrating the feasibility of whole-genome sequencing. The successful cloning of Dolly the sheep demonstrated the potential of somatic cell nuclear transfer and highlighted the possibilities of cellular reprogramming and mammalian cloning (15).

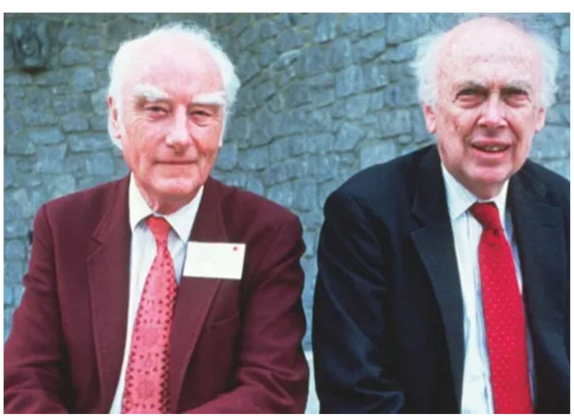

Fig 4: James D. Watson (1928) and Francis H. C. Crick (1916–2004)

Courtesy: Image of James D. Watson and Francis H. C. Crick, pioneers of molecular genetics and elucidators of the DNA double helix structure.

Fig 5: Arthur Kornberg (1918–2007)

Courtesy: Portrait of Arthur Kornberg, Nobel Prize laureate in Physiology or Medicine (1959) for his discovery of the mechanisms of DNA synthesis.

Fig 6: Stanley N. Cohen (1935–Present)

Courtesy: Portrait of Stanley N. Cohen, pioneer of recombinant DNA technology and genetic engineering.

Fig 7:Herbert W. Boyer (1936–Present)

Courtesy: Portrait of Herbert W. Boyer, pioneer of recombinant DNA technology and co-founder of modern genetic engineering.

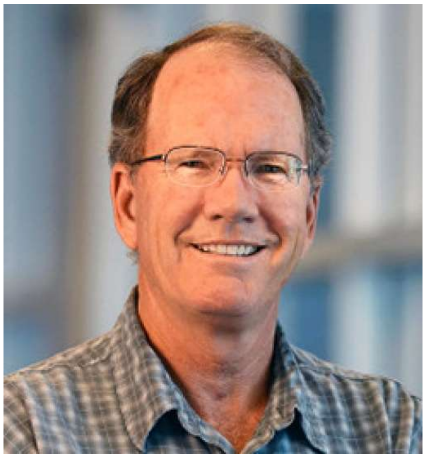

Fig 8: David V. Goeddel (1951)

Courtesy: Portrait of David V. Goeddel, molecular biologist and pioneer in recombinant protein therapeutics and genetic engineering.

The progress in recombinant DNA technology also facilitated the development of genome editing tools. Zinc finger nucleases and transcription activator-like effector nucleases enabled targeted modifications within genomes and represented the first generation of programmable gene-editing technologies. These developments were followed by the introduction of the CRISPR-Cas system, adapted from a natural bacterial defense mechanism, which provided a highly efficient and precise method for adding, deleting, or replacing specific DNA sequences in living cells and revolutionized genetic engineering and biomedical research (16). Parallel to these developments, research into messenger RNA as a therapeutic platform began to expand. Early experiments demonstrated that mRNA packaged within lipid droplets could enter living cells and direct protein synthesis. Although challenges related to instability and manufacturing costs initially limited progress, several research groups and biotechnology companies continued to explore mRNA-based therapeutics and vaccines, particularly for cancer immunotherapy. Continued advancements eventually led to the development of mRNA vaccine platforms for infectious diseases (15).

Biotechnology companies such as Moderna further advanced mRNA technology and focused on vaccine development. Following the emergence of SARS-CoV-2, researchers rapidly designed mRNA vaccines encoding the viral spike protein and initiated clinical trials within an unprecedented timeframe. Collaboration between BioNTech and Pfizer accelerated vaccine development and approval processes, resulting in highly effective vaccines based on modified mRNA encapsulated within lipid nanoparticles. These vaccines played a major role in controlling the global spread of COVID-19 and demonstrated the transformative potential of mRNA technology in modern medicine (15).

Agricultural biotechnology also experienced significant advances with the introduction of genetically modified crops. Herbicide-resistant soybean varieties were developed by introducing bacterial genes capable of conferring tolerance to glyphosate-based herbicides. These genetically modified crops enabled efficient weed management while improving agricultural productivity and became an important milestone in plant biotechnology and crop improvement (17). The observation of living systems and biological processes has existed since ancient times because of humanity's close relationship with nature and agriculture. Early societies applied biological knowledge in farming, food production, and animal domestication long before the scientific foundations of biotechnology were established (18).

The development of microscopy enabled scientists to visualize microorganisms and study biological processes at a microscopic level, providing the basis for microbiology and fermentation sciences. Scientific understanding of fermentation later transformed biotechnology from empirical practices into an evidence-based discipline (19). The concept of preserving biological products through heat treatment represented an important advancement in food biotechnology and microbial control, contributing significantly to the preservation of beverages and food products (20).

The term "biotechnology" was formally introduced by the Hungarian engineer Karl Ereky, who described the industrial use of living organisms in agricultural production systems involving meat, fat, and milk production. His work established the conceptual foundation of biotechnology as the application of biological systems for industrial purposes and is regarded as the earliest scientific use of the term (21, 22). Biotechnology subsequently evolved into a multidisciplinary field involving the application of biological organisms, cells, biological systems, and molecular processes for the production of useful products and services. It has become essential in medicine, agriculture, food production, environmental management, and industrial manufacturing. The European Federation of Biotechnology further emphasized biotechnology as the integration of natural sciences with organisms, cells, and molecular analogues to generate products and services beneficial to society (23, 24).

The emergence of recombinant DNA technology revolutionized biological sciences by demonstrating that DNA molecules could be enzymatically cleaved, inserted into autonomously replicating plasmids, and introduced into bacterial cells for propagation. These studies further demonstrated that genes from unrelated bacterial species and even animal cells could be successfully cloned and expressed in bacterial hosts. This discovery established the foundation of modern genetic engineering and enabled numerous applications in medicine, agriculture, and industrial biotechnology (25).

Biotechnology has evolved into a strategic tool for addressing global challenges in healthcare, agriculture, food security, energy production, environmental sustainability, and economic development. The concept of the bioeconomy emerged to describe the economic activities generated through biotechnology and biomanufacturing processes, highlighting the increasing contribution of biological sciences to industrial growth and workforce development (26). The importance of biotechnology became particularly evident during global public health emergencies, where advances in biotechnology and biomanufacturing enabled the rapid development and production of diagnostic tools, therapeutics, and vaccines. These achievements demonstrated the crucial role of biotechnology in protecting public health and strengthening healthcare systems worldwide (27).

Beyond healthcare, biotechnology has expanded its applications to climate change mitigation, renewable energy production, sustainable agriculture, food security, and supply chain resilience. Continued investment in genetic engineering technologies, biological data analysis, artificial intelligence, and scalable biomanufacturing processes has become essential for accelerating innovation and translating laboratory discoveries into commercial products and industrial solutions (27).

Recent advances in synthetic biology have further enhanced the ability to engineer biological systems with predictable functions, enabling scientists to program cellular processes in a manner analogous to computer programming. The integration of computational biology, artificial intelligence, and high-throughput biological data analysis is expected to drive the next generation of biotechnology innovations and support the continued growth of the global bioeconomy (27).

Biotechnology is commonly classified into several sectors based on its area of application. White biotechnology focuses on industrial applications and sustainable manufacturing processes, green biotechnology emphasizes agricultural improvements and crop production, while blue biotechnology explores marine and aquatic resources for industrial, pharmaceutical, and environmental applications. These specialized branches collectively demonstrate the broad and multidisciplinary nature of modern biotechnology (26).

The rapid expansion of biotechnology products and the increasing ability to manipulate genetic material highlighted the need for comprehensive regulatory oversight to ensure their safe application in research, industry, agriculture, healthcare, and environmental management. Regulatory systems were therefore developed to provide coordinated supervision of biotechnology products through agencies responsible for public health, food safety, agriculture, and environmental protection. As biotechnology continued to advance, regulatory approaches were further refined to accommodate emerging technologies and to address new risks associated with increasingly sophisticated methods of genetic manipulation. Policy initiatives emphasized the importance of updating risk assessment strategies and clarifying the responsibilities of regulatory agencies involved in biotechnology governance. The growing diversity of biotechnology-derived products reinforced the need for adaptable legal and regulatory frameworks capable of keeping pace with scientific innovation while ensuring safety, transparency, and responsible use of biotechnology. Recent governmental initiatives have focused on strengthening biotechnology innovation and expanding the bioeconomy through investments in biomanufacturing, biological data analysis, artificial intelligence, and scalable production technologies. These initiatives aim to accelerate progress in healthcare, agriculture, food security, climate resilience, renewable energy, and supply chain sustainability while promoting the safe, ethical, and secure application of biotechnology for societal benefit (28).

The development of a programmable RNA-guided nuclease system enabled precise modification of target DNA sequences within living cells. The technology offered improved specificity, simplicity, and efficiency compared to previous genome editing methods. Its applications have expanded rapidly in biomedical research, gene therapy, agriculture, and functional genomics, making it one of the most important advancements in modern biotechnology. [32]

Health biotechnology has contributed significantly to improvements in diagnostics, vaccines, therapeutic products, and disease management strategies. The growth of biotechnology industries has strengthened healthcare systems while promoting scientific innovation and economic development. Continued investment in research infrastructure and technological development remains essential for maximizing the benefits of biotechnology in developing nations. [33]

Biotechnology plays a crucial role in the development of a sustainable bioeconomy through the utilization of renewable biological resources and environmentally friendly production systems. Its applications extend to agriculture, pharmaceutical production, biofuels, waste management, and environmental remediation. The integration of biotechnology into industrial processes contributes to resource efficiency, reduced environmental impact, and sustainable development. [34]

The understanding of biological systems at the molecular level enabled biotechnology to progress from traditional fermentation-based applications to sophisticated genetic manipulation techniques. Advances in molecular biology provided the basis for studying gene structure, function, and expression, thereby facilitating the development of modern biotechnology applications in medicine, agriculture, and industry (35).

The discovery and characterization of restriction endonucleases provided scientists with molecular tools capable of cutting DNA at specific sequences, making it possible to isolate, modify, and recombine genetic material from different organisms. These enzymes became fundamental to cloning and recombinant DNA methodologies (36).

The production of recombinant human proteins in microbial systems demonstrated the feasibility of using microorganisms as biological factories for the large-scale production of therapeutic molecules. This achievement significantly improved the safety, availability, and effectiveness of treatments for chronic diseases and established the foundation of the biopharmaceutical industry (37). The development of gene therapy introduced the possibility of correcting genetic disorders by delivering functional genes into target cells, creating new opportunities for the treatment of inherited and acquired diseases and contributing to the advancement of personalized medicine (38).

Genome-editing technologies provided greater precision and efficiency in modifying genetic material compared with conventional genetic engineering approaches. These technologies enabled targeted insertion, deletion, and modification of DNA sequences in living organisms (39). The emergence of CRISPR-based genome editing transformed biotechnology by offering a simple, efficient, and versatile system for precise genetic modifications. Its applications rapidly expanded into biomedical research, therapeutic development, agriculture, and industrial biotechnology (40). The use of recombinant DNA technology and genome engineering has facilitated the development of improved crop varieties, disease-resistant organisms, therapeutic proteins, and novel strategies for disease management and environmental sustainability (41).

The expansion of industrial biotechnology and biomanufacturing enabled the sustainable production of biofuels, enzymes, pharmaceuticals, chemicals, and biomaterials through biological processes, reducing dependence on fossil resources and promoting environmentally friendly manufacturing systems (42). Synthetic biology further extended the scope of biotechnology by enabling the design and construction of artificial biological pathways and engineered microorganisms with customized metabolic functions for industrial and medical applications (43).

The integration of bioinformatics, computational biology, artificial intelligence, and high-throughput sequencing technologies has accelerated biological discovery and improved the ability to analyze and engineer complex biological systems with greater accuracy and efficiency (44).

Recent advances in biotechnology have been driven by the convergence of molecular biology, computational sciences, and engineering approaches, enabling the development of innovative technologies for healthcare, agriculture, industry, and environmental sustainability (45). High-throughput sequencing technologies have significantly reduced the cost and time required for genome analysis, facilitating precision medicine, disease diagnosis, population genetics studies, and the identification of novel therapeutic targets (45).

Artificial intelligence and machine learning have increasingly been integrated into biotechnology workflows to analyze large biological datasets, predict protein structures, accelerate drug discovery, optimize metabolic pathways, and improve diagnostic accuracy (46).

Advances in synthetic biology have enabled the rational design and construction of biological systems with customized functions, allowing the development of engineered microorganisms capable of producing pharmaceuticals, biofuels, enzymes, and other high-value products (47).

The development of messenger RNA technologies has transformed vaccine design and therapeutic development by enabling rapid production, high specificity, and adaptability to emerging infectious diseases and personalized treatments (48).

Genome editing technologies continue to evolve with the development of next-generation approaches such as base editing and prime editing, which allow highly precise genetic modifications without introducing double-stranded DNA breaks, thereby improving efficiency and reducing unintended genetic alterations (49).

Single-cell sequencing technologies have provided unprecedented insights into cellular heterogeneity by enabling the analysis of gene expression, epigenetic modifications, and cellular interactions at the individual cell level, contributing significantly to cancer biology, immunology, and developmental studies (50).

Advances in regenerative medicine and tissue engineering have facilitated the development of stem cell therapies, organoids, and bioengineered tissues for disease modelling, drug screening, and potential organ replacement therapies (51).

Bioprinting technologies have emerged as promising tools for fabricating complex biological structures through the layer-by-layer deposition of cells and biomaterials, offering new opportunities for tissue regeneration and personalized medicine (52).

Industrial biotechnology has increasingly adopted sustainable biomanufacturing processes that utilize renewable feedstocks and engineered microorganisms for the production of chemicals, biomaterials, and bioenergy, contributing to the development of a circular bioeconomy (53).

The integration of multi-omics technologies, including genomics, transcriptomics, proteomics, metabolomics, and epigenomics, has enabled a systems-level understanding of biological processes and accelerated the discovery of biomarkers and therapeutic targets for complex diseases (54).

Genetic modification technologies have become essential tools for understanding gene function, investigating disease mechanisms, and developing experimental models for biomedical research. Various genome engineering strategies enable precise manipulation of the genome to study the effects of specific genetic alterations and their biological consequences (55,56).

Gene knockout approaches involve the deletion or disruption of specific DNA sequences to eliminate gene expression and determine the physiological role of a gene. Smaller targeted deletions can also be used to remove regulatory elements or modify protein structure and activity without completely abolishing gene function (55).

Gene knockin strategies involve the insertion of exogenous DNA sequences into specific genomic locations. These insertions may include fluorescent reporter genes, epitope tags, or other marker sequences that facilitate the visualization, localization, and monitoring of gene and protein expression in living systems (55).

Gene replacement techniques enable endogenous DNA sequences to be substituted with modified or foreign genetic material, allowing the introduction of disease-associated mutations, replacement of large genomic regions, or simultaneous gene inactivation and transgene insertion for functional studies and disease modelling (55).

Conditional genetic systems permit gene modification in specific tissues, cell types, or developmental stages while maintaining normal gene function in other tissues. Site-specific recombination technologies provide temporal and spatial control over gene expression and have become valuable tools for studying genes that are essential for survival or development (55).

Inducible genetic systems allow genes to remain inactive until activated by specific molecular signals or recombinase activity, enabling controlled gene expression, lineage tracing, reporter activation, and reversible genetic modifications under experimental conditions (55).

Alternative strategies based on DNA inversion mechanisms have further expanded the possibilities for conditional genome engineering by enabling reversible gene activation, conditional point mutations, and the modification of genes that are difficult to manipulate using conventional approaches (55).

Collectively, these genome engineering approaches have significantly advanced functional genomics, disease modelling, translational medicine, and the development of novel therapeutic strategies for human diseases (55,56).

CRISPR/Cas9 technology has transformed genome engineering by enabling precise and efficient modification of specific DNA sequences in a wide range of organisms. The system functions by generating targeted double-strand breaks in DNA, which are subsequently repaired through endogenous cellular repair pathways, allowing the introduction of desired genetic modifications (57).

One of the principal repair mechanisms is non-homologous end joining, an error-prone process that frequently generates small insertions or deletions at the target site. These mutations often result in frameshift alterations, premature termination codons, and loss of protein function, making this approach highly effective for generating gene knockout models and studying gene function (57).

A second repair pathway, homology-directed repair, utilizes an exogenous donor DNA template to introduce precise genetic modifications. This strategy enables the generation of point mutations, reporter gene insertions, conditional alleles, inducible genetic systems, and targeted modifications of regulatory regions or entire genes, thereby facilitating the creation of sophisticated disease models and functional genomic studies (57).

Successful genome editing requires careful selection of target sequences and guide RNAs, along with the use of highly specific nuclease variants to minimize off-target modifications. When precise genome engineering is required, donor DNA molecules containing homologous sequences are introduced to promote accurate repair and insertion of the desired genetic changes (57,58).

The introduction of large DNA fragments remains technically more challenging than the generation of gene knockouts because insertion efficiency decreases with increasing donor size and varies among genomic loci. To improve editing outcomes, donor vectors are frequently designed to prevent repeated nuclease cleavage and to facilitate molecular screening of edited cells and organisms (58).

Following genome editing, molecular characterization using polymerase chain reaction and DNA sequencing is essential to verify the accuracy of insertions, deletions, or sequence substitutions. Additional analysis of genomic regions surrounding the edited site is necessary to identify unintended genetic alterations that may influence gene function or phenotype (57,58).

The generation of genetically modified animal models often requires breeding strategies to establish stable and heritable genetic modifications because founder animals frequently exhibit genetic mosaicism resulting from editing events occurring after early embryonic cell divisions (58,59).

Genome editing in immortalized cell lines presents additional challenges due to differences in chromosome number, gene copy variation, transfection efficiency, and cellular heterogeneity. These factors complicate the generation of homozygous mutations and require detailed molecular characterization of edited clones (59,60).

Several delivery strategies have been developed for introducing genome editing reagents into cells, including viral vectors, transposon systems, electroporation, and transfection-based approaches. However, prolonged nuclease expression associated with some delivery methods may increase the likelihood of unintended off-target modifications (59).

Recent technological improvements, including fluorescently labelled genome editing components, paired nickase systems, and base-editing enzymes, have enhanced editing precision, reduced double-strand DNA damage, improved homology-directed repair efficiency, and simplified the identification of successfully edited cells and organisms (59,60).

Collectively, these advances have established genome editing as a versatile platform for functional genomics, disease modelling, therapeutic development, and translational biomedical research (57–60).

Efficient delivery of genetic material into target cells is essential for successful genome engineering and gene therapy applications. Viral vectors and transposon systems have emerged as important tools for the stable introduction of exogenous DNA into mammalian cells and tissues because of their high delivery efficiency and long-term expression capabilities (61).

Recombinant viral vectors are generated by removing genes required for viral replication while retaining genetic elements necessary for genome packaging and integration. These engineered vectors can deliver coding sequences, non-coding RNAs, RNA interference molecules, and genome editing components without producing infectious viral particles, thereby improving biosafety while maintaining efficient gene transfer (61).

Transposons function as mobile genetic elements capable of integrating DNA into host genomes through transposase-mediated mechanisms. Unlike viral vectors, transposons are not limited by viral packaging constraints and therefore allow the transfer of larger DNA fragments. These systems have been widely applied in the generation of transgenic mammalian cells, immune cell engineering, induced pluripotent stem cell production, and genome editing applications (61).

Some transposon systems permit the precise excision of inserted genetic elements following successful genetic modification, allowing the generation of transgene-free cells and reducing the risk of long-term genomic alterations. Transposons have also been adapted for the delivery of genome editing reagents and multiplex gene editing applications, while subsequent removal of editing components helps minimize off-target effects (61).

Retroviral vectors have become important tools for stable gene transfer because they integrate their genetic material into the host genome following reverse transcription of viral RNA into DNA. Different retroviral systems exhibit distinct integration properties, influencing their suitability for various experimental and therapeutic applications (62,63). Lentiviral vectors possess the ability to efficiently transduce both dividing and non-dividing cells and can deliver relatively large genetic cargos, including complete genome editing systems and selectable markers. Their ability to support stable transgene expression has made them widely used in functional genomics, disease modelling, and gene therapy studies (63). Despite their advantages, retroviral integration into host genomes may disrupt endogenous genes or regulatory regions, potentially causing insertional mutagenesis and unintended alterations in cellular function. The development of self-inactivating vector designs has reduced these risks and improved the safety profile of lentiviral gene delivery systems (62,63). Adeno-associated viral vectors are widely used for gene targeting because of their high delivery efficiency, low immunogenicity, and favorable safety characteristics. Different viral serotypes enable tissue-specific targeting, making these vectors valuable for both experimental and therapeutic applications (64). The limited packaging capacity of adeno-associated viral vectors restricts the size of genome editing components that can be delivered within a single vector. Consequently, smaller nucleases, multiple vector systems, or separate delivery strategies are often required for complex genome editing applications (64,65). Adeno-associated viral vectors are also extensively used for delivering guide RNAs and donor DNA templates required for homology-directed repair and precise genome editing. However, unintended integration of vector sequences at genome editing sites has been reported, highlighting the importance of thorough molecular characterization following genome modification (64,65).

Biotechnology has also contributed significantly to nutritional improvement through biofortification, a strategy aimed at increasing the concentration and bioavailability of essential micronutrients in food crops during plant growth rather than after harvest (66). Biofortification can be achieved through agronomic practices, conventional breeding approaches, and genetic engineering technologies. The integration of genomics, transcriptomics, proteomics, metabolomics, and ionomics has improved the identification of target genes involved in nutrient uptake, transport, and metabolism, thereby accelerating crop improvement programs (66). Several international initiatives have promoted the development and distribution of nutrient-enriched staple crops to combat micronutrient deficiencies and hidden hunger, particularly in developing regions. Biofortified crops enriched with vitamin A, iron, and zinc have demonstrated substantial improvements in nutritional status among populations relying heavily on staple foods (66).

Cellular agriculture has emerged as an innovative biotechnology approach that produces animal- and plant-derived food products using cell culture technologies with minimal reliance on conventional farming systems. This technology has gained attention as a sustainable alternative to traditional agriculture due to its potential to address increasing global food demands while reducing environmental impacts associated with livestock production (67). Cellular agriculture encompasses cultured meat, plant-based meat alternatives, insect-derived protein products, and other cell-based agricultural products. Compared with conventional animal farming, these technologies have the potential to reduce greenhouse gas emissions, decrease land and water consumption, minimize environmental degradation, and improve animal welfare while maintaining nutritional quality (67). Research activities in cellular agriculture have expanded rapidly, particularly in areas related to food science, biotechnology, environmental sustainability, nutrition, agriculture, and ethics. Several countries have emerged as major contributors to this field, reflecting increasing scientific and commercial interest in alternative food production systems (67). Despite its considerable promise, cellular agriculture faces challenges related to production costs, product quality, sensory characteristics, regulatory approval, food safety assessment, and consumer acceptance. Future developments are expected to focus on improving production efficiency, reducing manufacturing costs, optimizing nutritional value, and establishing supportive regulatory frameworks for commercialization (67).

Artificial intelligence has become an important component of modern biotechnology by improving the speed, accuracy, and efficiency of biological research, diagnostics, therapeutic development, and industrial bioprocesses. By analyzing large biological datasets and identifying complex patterns, AI enables predictive modelling and data-driven decision-making throughout the biotechnology workflow (68,69). Machine learning and deep learning approaches have been widely applied in genome analysis, biomarker discovery, protein engineering, multi-omics studies, and precision medicine. These computational tools support personalized healthcare by predicting therapeutic responses and improving the assessment of drug safety and efficacy parameters (68,69). One of the most significant applications of artificial intelligence is in drug discovery and development, where computational models accelerate target identification, virtual screening, molecular interaction prediction, and therapeutic molecule design. Advanced generative models have further enhanced the ability to optimize candidate compounds with improved pharmacological characteristics (68). Artificial intelligence has also transformed protein engineering and antibody discovery by enabling accurate prediction of protein structures and interactions. These technologies facilitate the rational design of enzymes, therapeutic antibodies, and protein binders with improved specificity, affinity, and stability for biomedical applications (68,70). The application of AI in nanoparticle engineering has improved the design of drug delivery systems by optimizing particle stability, targeting efficiency, and controlled drug release properties, thereby enhancing therapeutic performance and reducing adverse effects (68). Artificial intelligence further contributes to biotechnology manufacturing through optimization of fermentation processes, monitoring of bioreactor performance, prediction of production outcomes, and improvement of product quality control, resulting in increased productivity and reduced manufacturing costs (69).

Protein structure prediction has undergone a major transformation through artificial intelligence technologies capable of predicting three-dimensional protein structures directly from amino acid sequences with near-experimental accuracy. These advances have accelerated biological research and substantially reduced the time and cost associated with traditional structural biology techniques (70,71). Computational protein design technologies have enabled the creation of novel proteins with customized biological functions for applications in medicine, industrial biotechnology, and environmental remediation. These developments have expanded opportunities for vaccine development, enzyme engineering, and synthetic biology applications (70). Although artificial intelligence has significantly advanced biotechnology research, experimental validation remains essential to confirm computational predictions, particularly for complex biological systems and molecular interactions (68,69,71).

Artificial intelligence has transformed structural biology by enabling highly accurate prediction of three-dimensional protein structures directly from amino acid sequences. This advancement has significantly reduced the time, cost, and experimental effort traditionally required for structure determination using methods such as X-ray crystallography and cryo-electron microscopy (72,73). AI-based protein structure prediction systems have accelerated biological research by providing structural information for millions of proteins, thereby improving the understanding of protein function, molecular interactions, and disease mechanisms. The availability of large-scale protein structure databases has become an important resource for researchers working in medicine, biotechnology, and life sciences (72,73).

Computational protein design has further expanded the capabilities of biotechnology by enabling the creation of novel proteins with customized structures and biological functions. Engineered proteins developed using these approaches have potential applications in therapeutics, vaccine development, enzyme engineering, environmental remediation, and industrial biotechnology (72). The integration of artificial intelligence into protein engineering has accelerated the development of biologics, improved enzyme optimization, and facilitated the design of proteins with enhanced stability, specificity, and catalytic efficiency for diverse biomedical and industrial applications (72,73).

Recent developments in multimodal artificial intelligence combine biological, clinical, imaging, and molecular datasets to improve disease prediction, therapeutic development, personalized medicine, and digital healthcare applications. The integration of these diverse data sources enables more comprehensive biological analysis and supports precision biotechnology approaches (74). Despite these advances, computational predictions still require rigorous experimental validation, particularly for complex protein interactions, dynamic molecular systems, and clinical applications. Challenges related to data quality, model interpretability, ethical considerations, and regulatory acceptance remain important areas for future research and development (72,74).

Artificial Intelligence (AI) is transforming modern biotechnology by integrating machine learning (ML), deep learning (DL), and advanced computational methods into biological research and industrial applications. AI as a powerful tool for analysing large-scale biological datasets, improving research accuracy, reducing experimental time, and accelerating innovation across biotechnology. The fundamentals of AI, including supervised learning, unsupervised learning, deep neural networks, convolutional neural networks (CNNs), recurrent neural networks (RNNs), and transformer models. These techniques enable efficient analysis of complex genomic, proteomic, transcriptomic, and metabolomic datasets while improving disease classification, biomarker identification, and predictive modelling.

AI-assisted drug discovery and development, AI identifies novel drug targets, designs therapeutic molecules using generative models, predicts toxicity, and optimizes clinical trials. Commercial AI platforms such as Insilco Medicine, Ex Scientia, Atom wise, and Benevolent AI demonstrate how AI has shortened drug development timelines while improving clinical success rates and reducing costs. AI in genomics and multi-omics integration. AI-based tools such as DeepVariant and Clairvoyante improve genetic variant detection, while deep learning models analyze single-cell RNA sequencing, spatial transcriptomics, proteomics, and metabolomics data. These technologies facilitate biomarker discovery, disease diagnosis, precision medicine, and patient stratification by integrating multiple biological datasets into a unified analytical framework.

The role of AI in synthetic biology and metabolic engineering, where machine learning assists in pathway optimization, gene circuit design, CRISPR guide RNA selection, enzyme engineering, and microbial strain improvement. AI accelerates the Design–Build–Test–Learn (DBTL) cycle, increasing efficiency in industrial biotechnology and sustainable biomanufacturing. AI combined with robotics, automation, microfluidics, and lab-on-a-chip technologies enables high-throughput screening, automated experimentation, real-time quality control, and intelligent laboratory workflows. These innovations improve reproducibility while reducing manual intervention and operational costs.

AI has several applications in personalized medicine and medical diagnostics. AI analyses electronic health records, genomic information, and medical imaging to predict treatment responses, improve disease diagnosis, optimize chemotherapy dosing through digital twins, and assist clinicians in individualized patient care. Deep learning significantly enhances radiology, pathology, and diagnostic imaging accuracy (75)

Nanotechnology has revolutionized biotechnology by enabling the fabrication of nanoscale surface structures that can influence cellular behaviour. Surface-directed nanobiotechnology, which has applications in tissue engineering, cell culture, biomedical implants, biosensors, and biofouling control. Cells naturally interact with nanometre-scale structures in the body and are highly sensitive to their surroundings. Artificial surfaces used in laboratories and medical implants often possess random nanoscale features, which can significantly alter cell adhesion, orientation, migration, and growth. Research has demonstrated that even structures as small as 5 nm can guide the behaviour of certain cell types.

Several nanofabrication techniques including electron-beam lithography, microcontact printing, colloidal resists, self-assembling systems, embossing, injection molding, and particle synthesis. These methods enable the creation of controlled nanopatterns that can regulate cell responses and can be reproduced economically for large-scale biomedical applications.

The methods used to characterize nanostructured surfaces, such as Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), and Scanning Probe Microscopy (SPM). These techniques provide detailed information about surface topography and help researchers understand how nanoscale features influence biological interactions.

A major topic of debate addressed is whether cells respond primarily to surface chemistry or surface topography. Although increasing evidence suggests that nanoscale topographical features can independently regulate cell adhesion, alignment, cytoskeletal organization, and differentiation.

Some of the biotechnological applications of nanotechnology, include tissue engineering scaffolds, biosensors, drug delivery systems, nanomachines, artificial implants, and anti-biofouling surfaces. These advances in nanobiotechnology require collaboration among engineers, material scientists, physicists, chemists, and biologists to translate laboratory research into practical medical and industrial applications (76).

Industrial biotechnology has not expanded as rapidly as anticipated despite its potential to produce sustainable chemicals, biofuels, bioplastics, and other bio-based products, Competition from inexpensive fossil fuels, shale gas, natural gas hydrates, rising agricultural feedstock prices, and advances in chemical manufacturing have reduced the economic competitiveness of industrial biotechnology, several limitations of current bioprocesses compared with conventional chemical processes. Microbial fermentation is generally slower, requires expensive sterilization, consumes large amounts of freshwater, produces lower product concentrations, and involves higher recovery costs. In contrast, chemical industries achieve faster production, higher substrate conversion efficiencies, continuous processing, and lower operational costs.

Polyhydroxyalkanoates (PHAs) are biodegradable and bio-based plastics, their commercial success remains limited because production costs are still too high. To overcome this challenge, industries must improve microbial strains, optimize fermentation technologies, and reduce manufacturing expenses while maintaining high product yields. For high-value applications such as biomedical materials and specialty polymers, a "low-volume, high-value" production strategy may be more practical. Emerging strategies include developing minimized microbial cells, engineering microorganisms capable of utilizing mixed substrates, removing unnecessary metabolic pathways to improve conversion efficiency, designing contamination-resistant strains, reducing oxygen demand, and developing continuous, open fermentation systems. These approaches can make bioprocesses more similar to efficient chemical manufacturing processes.

Another major recommendation is the use of inexpensive and sustainable feedstocks such as kitchen waste, activated sludge, cellulose, and seawater, instead of food-based agricultural raw materials. This approach would reduce production costs while minimizing competition with food resources and improving environmental sustainability. Integrating biological and chemical manufacturing to maximize the advantages of both technologies. Examples include microbial production of lactic acid followed by chemical polymerization to manufacture biodegradable polylactic acid (PLA). Similar hybrid approaches are suggested for producing succinic acid and other bio-based chemicals (77).

The evolution and significance of microbial biotechnology in industrial and medical applications is that microorganisms have been used for thousands of years in traditional fermentation processes to produce foods and beverages such as bread, beer, wine, and cheese. During the early 20th century, industrial fermentation expanded to produce important chemicals including acetone, butanol, glycerol, citric acid, vitamins, and antibiotics, marking the beginning of modern industrial microbiology, the revolutionary impact of molecular biology and genetic engineering in the 1970s, which transformed microbial biotechnology from conventional fermentation into a sophisticated field capable of producing recombinant biopharmaceuticals.

Recombinant microorganisms were engineered to manufacture therapeutic proteins such as human insulin, erythropoietin, interferons, and human growth hormone, greatly improving healthcare and pharmaceutical products. The diverse industrial applications of microorganisms in producing enzymes, organic acids, amino acids, biofuels, vaccines, and specialty chemicals. Advances in strain improvement, metabolic engineering, fermentation technology, and bioprocess optimization have significantly increased product yield, reduced manufacturing costs, and improved production efficiency (78).

In addition, the growing role of microbial biotechnology in environmental protection through bioremediation, waste treatment, and sustainable industrial processes. Microorganisms are used to degrade environmental pollutants, recycle waste materials, and develop eco-friendly alternatives to conventional chemical processes. Continuous advances in molecular genetics, genomics, and microbial engineering are expected to further expand its applications in medicine, industry, and environmental sustainability (78).

These emerging technologies are expected to improve microbial strain development, accelerate industrial production, enhance agricultural productivity, and address global challenges related to food security, environmental protection, and human health. Henceforth, microbial biotechnology is one of the most promising fields for sustainable scientific and technological advancement (79).

Recent advances in agricultural biotechnology for improving crop tolerance to salinity stress, demonstrates how integrated multi-omics technologies, marker-assisted breeding, genomic selection, and CRISPR/Cas genome editing have transformed modern crop improvement. It effectively combines molecular biology, genetics, and biotechnology to explain the mechanisms underlying stress tolerance and their practical applications. The importance of precision breeding for ensuring food security under climate change (80).

The evolutionary history of nucleases, enzymes that degrade DNA and RNA, from their earliest role as bacterial Défense molecules to their current importance in biotechnology and human immunity. Restriction endonucleases were among the first immune Défense systems in bacteria, protecting cells by recognizing and cleaving foreign viral DNA. These primitive Défense mechanisms later evolved into the sophisticated CRISPR-Cas adaptive immune system, with Cas9 becoming one of the most powerful genome-editing tools in modern biotechnology. The evolution of nucleases across different organisms, including archaea, fungi, protists, invertebrates, vertebrates, and mammals.

As organisms became more complex, nucleases acquired specialized functions beyond genome protection, such as DNA repair, apoptotic cell clearance, immune regulation, and maintenance of genomic stability. This evolutionary progression illustrates how nucleases adapted to support increasingly sophisticated immune systems.

The roles of DNases and RNases in human immunity like DNases (DNase I, DNase II, TREX1, TREX2, and DNase γ) degrade extracellular and intracellular DNA to prevent chronic inflammation and autoimmune diseases such as systemic lupus erythematosus and Aicardi–Goutières syndrome. RNases (RNase 1–8, RNase L, and RNase T2) degrade viral and extracellular RNA, regulate inflammation, and protect against bacterial, viral, fungal, and parasitic infections while maintaining immune homeostasis. Nuclease-generated oligonucleotides function as immune signaling molecules. DNA and RNA fragments produced during nuclease activity activate innate immune pathways such as TLR9, cGAS–STING, RIG-I, and MDA5, triggering interferon production, inflammation, and antiviral responses. These pathways enable the immune system to distinguish between self and foreign nucleic acids and mount rapid defense mechanisms against infections.

Several applications of nucleases in modern biotechnology, including restriction enzymes revolutionized recombinant DNA technology and gene cloning, while zinc finger nucleases (ZFNs), TALENs, and especially CRISPR-Cas9 have transformed genome editing. These technologies are now widely used in gene therapy, functional genomics, crop improvement, disease modeling, and precision medicine. CRISPR-based diagnostic platforms such as SHERLOCK and DETECTR, which enable rapid and highly sensitive detection of infectious diseases and genetic mutations.

Numerous emerging therapeutic applications of nucleases, including targeted cancer therapy, antiviral treatments, personalized medicine, and innovative drug delivery systems such as TOUCAN (Therapeutic Oligonucleotides Activated by Nucleases). However, nucleases have evolved from simple bacterial Défense enzymes into indispensable tools for biotechnology, immunology, and diagnostic applications.

CONCLUSION

Biotechnology has undergone remarkable transformation from ancient fermentation practices and traditional agricultural applications to an advanced multidisciplinary field driven by molecular biology, genetic engineering, computational sciences, and artificial intelligence. Throughout its evolution, key scientific discoveries—including the understanding of microbial fermentation, the elucidation of DNA structure, recombinant DNA technology, the Human Genome Project, and CRISPR-based genome editing—have revolutionized biological research and enabled innovative applications across medicine, agriculture, industry, and environmental management. Recent advances in synthetic biology, high-throughput sequencing, genome engineering, messenger RNA technologies, regenerative medicine, cellular agriculture, and artificial intelligence have further expanded the capabilities of biotechnology, providing powerful tools for disease diagnosis, therapeutic development, sustainable biomanufacturing, crop improvement, and environmental sustainability. The integration of multi-omics technologies, computational biology, and machine learning is accelerating biological discovery while supporting the development of precision medicine and next-generation biotechnological solutions. Despite these remarkable achievements, continued progress depends on responsible innovation supported by robust regulatory frameworks, ethical oversight, biosafety, and sustained investment in research, infrastructure, and technological development. As biotechnology continues to evolve, its convergence with engineering, computational sciences, and data-driven approaches is expected to drive future innovations that address global challenges related to healthcare, food security, renewable energy, climate change, and sustainable economic development.

REFERENCES

1. Singh J, Singh A. *Major Milestones in the Evolutionary Timeline of Biotechnology.* 2023. doi:10.1729/Journal.35933.
2. El-Sayed WMM, Ibrahim HAH, Abdul-Raouf UM, et al. *Evaluation of Bioethanol Production from Ulva lactuca by Saccharomyces cerevisiae.* Journal of Biotechnology & Biomaterials. 2016;6:226.
3. Watson JD, Crick FHC. *Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid.* Nature. 1953;171(4356):737–738.
4. Cohen SN, Chang ACY, Boyer HW, Helling RB. *Construction of biologically functional bacterial plasmids in vitro.* Proceedings of the National Academy of Sciences. 1973;70(11):3240–3244.
5. International Human Genome Sequencing Consortium. *Initial sequencing and analysis of the human genome.* Nature. 2001;409(6822):860–921.
6. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. *A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.* Science. 2012;337(6096):816–821.
7. Abuduxike G, Aljunid SM. 2012; Lokko Y, et al. 2018.
8. American Chemical Society. *Discovery and Development of Penicillin*. American Chemical Society National Historic Chemical Landmarks. Available at: https://www.acs.org/education/whatischemistry/landmarks/flemingpenicillin.html
9. Renneberg, R., & Loroch, V. (2016). *Biotechnology for Beginners* (3rd ed.). Academic Press.
10. Watson, J. D., & Crick, F. H. C. (1953). Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature, 171*(4356), 737–738.
11. Cohen, S. N., Chang, A. C., Boyer, H. W., & Helling, R. B. (1973). Construction of biologically functional bacterial plasmids in vitro. *Proceedings of the National Academy of Sciences, 70*(11), 3240–3244.
12. Liao, Y., & Wang, X. (2023). Historical development of biotechnology companies and recombinant therapeutics.

13. Falcetta, F., et al. (2022). Recombinant human insulin and its impact on diabetes treatment.
14. The Dong-A Science. (2015, October 18). *The 1973 experiment that launched the era of recombinant DNA technology*. https://www.dongascience.com/
15. Bifulco, M., Di Zazzo, E., Affinito, A., & Pagano, C. (2025). The relevance of the history of biotechnology for healthcare: Teaching students how biotechnology and medicine have been closely entwined during the past century highlights how both fields have inspired and driven each other. *EMBO Reports, 26*(2), 303–306. https://doi.org/10.1038/s44319-024-00355-8
16. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213), 1258096.
17. Monsanto. Development of Roundup Ready soybean technology and herbicide-resistant crops.
18. Yalikun, Y., Kanda, Y., & Morishima, K. (2016). A Method of Three Dimensional Micro-Rotational Flow Generation for Biological Applications. *Micromachines, 7*(140).
19. Reder Christ, K., & Bendas, G. (2011). Biosensor Applications in the Field of Antibiotic Research—A Review of Recent Developments. *Sensors, 11*, 9450–9466.
20. Venzin, C. M., Jacot, V., Berdichevsky, A., et al. (2016). Biocompatibility of Pegylated Fibrinogen and Its Effect on Healing of Full-Thickness Skin Defects: A Preliminary Study in Rats. *Journal of Biotechnology & Biomaterials, 6*, 233.
21. Gábor, M. G., & Kralovánszky, U. P. (2005). The father of the term "biotechnology" was Hungarian: The forgotten works of Károly Ereky. *Acta Agraria Debreceniensis, 17*, 107–110.
22. Ereky, K. *Biotechnologie der Fleisch-, Fett- und Milcherzeugung im landwirtschaftlichen Grossbetriebe*.
23. Tylingo, R., Mania, S., Panek, A., et al. (2016). Isolation and Characterization of Acid Soluble Collagen from the Skin of African Catfish (*Clarias gariepinus*), Salmon (*Salmo salar*) and Baltic Cod (*Gadus morhua*). *Journal of Biotechnology & Biomaterials, 6*, 234.
24. Sheikh, Z., Najeeb, S., Khurshid, Z., et al. (2015). Biodegradable Materials for Bone Repair and Tissue Engineering Applications. *Materials, 8*, 5744–5794.
25. Cohen, S. N. (2013). DNA cloning: A personal view after 40 years. *Proceedings of the National Academy of Sciences of the United States of America, 110*(39), 15521–15529. https://doi.org/10.1073/pnas.1313397110
26. Barcelos, M. C., Lupki, F. B., Campolina, G. A., Nelson, D. L., & Molina, G. (2018). The colors of biotechnology: General overview and developments of white, green and blue areas. *FEMS Microbiology Letters, 365*(21), 239.
27. Stasi, A. (2023). *Background and Overview of Biotechnology*. In *Biotechnology Law and Regulation*. https://doi.org/10.1007/978-981-99-2135-5_1
28. Executive Office of the President. *Advancing Biomanufacturing and Biotechnology Innovation for a Sustainable, Safe, and Secure Bioeconomy*.
29. Singh, J., & Singh, A. (2023). *Major milestones in the evolutionary timeline of biotechnology*. https://doi.org/10.1729/Journal.35933
30. Watson, J. D., & Crick, F. H. C. (1953). *Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid*. *Nature, 171*(4356), 737–738.
31. International Human Genome Sequencing Consortium. (2001). *Initial sequencing and analysis of the human genome*. *Nature, 409*(6822), 860–921.
32. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). *A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity*. *Science, 337*(6096), 816–821.
33. Abuduxike, G., & Aljunid, S. M. (2012). *Development of health biotechnology in developing countries: Can private-sector players be the prime movers?*
34. Lokko, Y., Heijde, M., Schebesta, K., Scholtès, P., Van Montagu, M., & Giacca, M. (2018). *Biotechnology and the bioeconomy—Towards inclusive and sustainable industrial development*.
35. Verma AS, Singh A. *Biotechnology in the Realm of History*. Journal of Pharmacy and Bioallied Sciences. 2011;3(3):321–323.
36. Bujnicki JM. *Crystallographic and Bioinformatic Studies on Restriction Endonucleases: Research Perspectives*. Current Protein and Peptide Science. 2003;4(5):327–337.
37. Riggs AD. *Making, Cloning, and the Expression of Human Insulin Genes in Bacteria: The Path to Humulin*. Proceedings of the National Academy of Sciences. 2021;118(51):e2021612118.

38. Wirth T, Parker N, Ylä-Herttuala S. *History of Gene Therapy*. Gene. 2013;525(2):162–169.
39. Carroll D. *Genome Editing: Past, Present, and Future*. Yale Journal of Biology and Medicine. 2017;90(4):653–659.
40. Gostimskaya I. *CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing*. Biochemistry (Moscow). 2022;87(Suppl 1):S8–S19.
41. Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, Hou H. *Role of Recombinant DNA Technology to Improve Life*. International Journal of Genomics. 2016;2016:2405954.
42. Zhang YHP. *Biomanufacturing: History and Perspective*. Journal of Industrial Microbiology and Biotechnology. 2017;44(4-5):773–784.
43. Schindler D, et al. *Genetic Engineering and Synthetic Genomics in Yeast to Understand Life and Boost Biotechnology*. FEMS Yeast Research. 2020;20(8):foaa059.
44. Ishino Y, Krupovic M, Forterre P. *History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology*. Journal of Bacteriology. 2018;200(7):e00580-17.
45. National Human Genome Research Institute. Advances in DNA Sequencing Technologies and Their Applications in Genomic Medicine. Available through PubMed Central resources.
46. Arshadi AK, Webb J, Salem M, et al. Artificial Intelligence for Drug Discovery and Development. *Nature Reviews Drug Discovery*. Accessible through PubMed and PubMed Central.
47. Liu X, Stewart CN Jr. Synthetic Biology for Sustainable Development and Biotechnology Innovation. *Plants*. MDPI Open Access.
48. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA Vaccines — A New Era in Vaccinology. *Nature Reviews Drug Discovery*. Accessible through PubMed.
49. Anzalone AV, Koblan LW, Liu DR. Genome Editing with CRISPR-Cas Nucleases, Base Editors, Transposases and Prime Editors. *Nature Biotechnology*. Accessible through PubMed.
50. Svensson V, Vento-Tormo R, Teichmann SA. Exponential Scaling of Single-Cell RNA Sequencing Technologies. *Nature Protocols*. Accessible through PubMed Central.
51. Kim J, Koo BK, Knoblich JA. Human Organoids: Model Systems for Human Biology and Medicine. *Nature Reviews Molecular Cell Biology*. Accessible through PubMed.
52. Matai I, Kaur G, Seyedsalehi A, McClinton A, Laurencin CT. Progress in 3D Bioprinting Technology for Tissue and Organ Regeneration. *Biomaterials*. Accessible through PubMed.
53. Zhang YHP. Biomanufacturing: History and Perspective. *Journal of Industrial Microbiology and Biotechnology*. Accessible through PubMed.
54. Hasin Y, Seldin M, Lusis A. Multi-omics Approaches to Disease. *Genome Biology*. Accessible through PubMed Central.
55. Gurumurthy, C. B., O'Brien, A. R., Quadros, R. M., et al. *Methods to Genetically Modify the Mouse Genome*. **Genes**. 2020;11(3):291. https://doi.org/10.3390/genes11030291
56. Robert, J. S., & Baylis, F. *Genetic Engineering*. In: Heggenhougen HK (Ed.), **International Encyclopedia of Public Health**. Academic Press; 2008:35–39. https://doi.org/10.1016/B978-012373960-5.00133-7
57. Ansori AN, Antonius Y, Susilo RJ, Hayaza S, Kharisma VD, Parikesit AA, Zainul R, Jakhmola V, Saklani T, Rebezov M, Ullah ME, Maksimiuk N, Derkho M, Burkov P. Application of CRISPR-Cas9 genome editing technology in various fields: A review. *Narra J.* 2023;3(2):e184. https://doi.org/10.52225/narra.v3i2.184
58. Yoshimi K, Kuno A, Yamauchi Y, Hattori K, Taniguchi H, Mikamo K, Iida R, Ishida S, Goto M, Takeshita K, Ito R, Takahashi R, Takahashi S, Mashimo T. Genome editing using type I-E CRISPR-Cas3 in mice and rat zygotes. *Cell Reports Methods.* 2024;4(8):100833. https://doi.org/10.1016/j.crmeth.2024.100833
59. Chenouard V, Remy S, Tesson L, Ménoret S, Ouisse LH, Cherifi Y, Anegon I. Advances in Genome Editing and Application to the Generation of Genetically Modified Rat Models. *Frontiers in Genetics.* 2021;12:615491. https://doi.org/10.3389/fgene.2021.615491
60. Corrado A, Aceto R, Miglietta S, Silvestri R, Dell'Anno I, Lepori I, Ricci B, Romei C, Giovannoni R, Poliseno L, Evangelista M, Vitiello M, Cipollini M, Elisei R, Landi S, Gemignani F. Strategies for single base gene editing in an immortalized human cell line by CRISPR/Cas9 technology. *3 Biotech.* 2024;14(2):45. https://doi.org/10.1007/s13205-023-03878-4

61. Vargas JE, Chicaybam L, Stein RT, Tanuri A, Delgado-Cañedo A, Bonamino MH. Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives. *Journal of Translational Medicine.* 2016;14(1):288. https://doi.org/10.1186/s12967-016-1047-x
62. Anson DS. The use of retroviral vectors for gene therapy—what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery. *Genetic Vaccines and Therapy.* 2004;2(1):9. https://doi.org/10.1186/1479-0556-2-9
63. Jaballah SA, Ali LM, Jehad MA, Akhlaq S, Rizvi TA, Mustafa F. Retroviral vector technology for gene therapy: History, current landscape, and future prospects. *Journal of Molecular Biology.* 2025;437(24):169473. https://doi.org/10.1016/j.jmb.2025.169473
64. Naso MF, Tomkowicz B, Perry WL III, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs.* 2017;31(4):317–334. https://doi.org/10.1007/s40259-017-0234-5
65. Khan IF, Hirata RK, Russell DW. AAV-mediated gene targeting methods for human cells. *Nature Protocols.* 2011;6(4):482–501. https://doi.org/10.1038/nprot.2011.301
66. Ofori KF, Antoniello S, English MM, Aryee ANA. Improving nutrition through biofortification—A systematic review. *Frontiers in Nutrition.* 2022;9:1043655. https://doi.org/10.3389/fnut.2022.1043655
67. Nyika J, Mackolil J, Workie E, Adhav C, Ramadas S. Cellular agriculture research progress and prospects: Insights from bibliometric analysis. *Current Research in Biotechnology.* 2021;3:215–224. https://doi.org/10.1016/j.crbiot.2021.07.001
68. Liu YT, Zhang LL, Jiang ZY, Tian XS, Li PL, Wu PH, Du WT, Yuan BY, Xie C, Bu GL, Zhong LY, Yang YL, Li T, Zeng MS, Sun C. Applications of Artificial Intelligence in Biotech Drug Discovery and Product Development. *MedComm.* 2025;6(8):e70317. https://doi.org/10.1002/mco2.70317
69. Gomes A, Gonçalves B, Inglês B, Silvério S, Pinto CA, Saraiva JA. Potential impacts of artificial intelligence (AI) in biotechnology. *Applied Sciences.* 2024;14(24):11801. https://doi.org/10.3390/app142411801
70. Genc AG, McGuffin LJ. Beyond AlphaFold2: The Impact of AI for the Further Improvement of Protein Structure Prediction. *Methods in Molecular Biology.* 2025;2867:121–139. https://doi.org/10.1007/978-1-0716-4196-5_7
71. Mihai A. Nobel Prize in Chemistry: Protein folding and AI. *Lindau Nobel Laureate Meetings.*
72. Genc AG, McGuffin LJ. *Beyond AlphaFold2: The Impact of AI for the Further Improvement of Protein Structure Prediction.* Methods in Molecular Biology. 2025;2867:121–139. doi:10.1007/978-1-0716-4196-5_7.
73. Mihai A. *Nobel Prize in Chemistry: Protein Folding and AI.* Lindau Nobel Laureate Meetings.
74. Bhushan A, Misra P. *Unlocking the Potential: Multimodal AI in Biotechnology and Digital Medicine—Economic Impact and Ethical Challenges.* npj Digital Medicine. 2025;8:619. doi:10.1038/s41746-025-01786-4.
75. Phillip, A. (2026). *The role of artificial intelligence in modern biotechnology: A comprehensive review*. Journal of Applied Biotechnology Reports, 13(1), 1899–1921. https://doi.org/10.30491/jabr.2025.523309.1873
76. Curtis, A., & Wilkinson, C. (2001). *Nano techniques and approaches in biotechnology*. Trends in Biotechnology, 19(3), 97–101. https://doi.org/10.1016/S0167-7799(00)01511-9
77. Chen, G.-Q. (2012). *New challenges and opportunities for industrial biotechnology*. Microbial Cell Factories, 11, Article 111. https://doi.org/10.1186/1475-2859-11-111
78. Demain, A. L. (2000). *Microbial biotechnology*. Trends in Biotechnology, 18(1), 26-31. https://doi.org/10.1016/S0167-7799(99)01400-6
79. Maurya, D. K., Kumar, A., Chaurasiya, U., Hussain, T., & Singh, S. K. (2021). *Modern era of microbial biotechnology: Opportunities and future prospects*. In M. K. Solanki, P. L. Kashyap, R. A. Ansari, & B. Kumari (Eds.), *Microbiomes and Plant Health: Panoply and Their Applications* (pp. 317–343). Academic Press. https://doi.org/10.1016/B978-0-12-819715-8.00011-2
80. Raza, A., Tabassum, J., Fakhar, A. Z., Sharif, R., Chen, H., Zhang, C., Ju, L., Fotopoulos, V., Siddique, K. H. M., Singh, R. K., Zhuang, W., & Varshney, R. K.

(2022). *Smart reprograming of plants against salinity stress using modern biotechnological tools*. Critical Reviews in Biotechnology. Advance online publication. https://doi.org/10.1080/07388551.2022.2093695

81. Hernandez, F. J. (2025). *Nucleases: From primitive immune defenders to modern biotechnology tools*. Immunology, 174, 279–286. https://doi.org/10.1111/imm.13884