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The EuroBiotech Journal
REVIEW
Enviromental Biotechnology

Impacts of Biotechnologically Developed Microorganisms on Ecosystems

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Abstract
Climate change has imposed a significant struggle for survival most of the Earth's species, highlighting the urgent need for a healthy and secure environment. Recent scientific investigations have primarily concentrated on the development and use of microorganisms as powerful biotechnological tools to address the escalating pollution that poses a severe threat to life. But this microorganisims long-term effects on biodiversity and ecosystems remain a subject of inquiry. In this comprehensive review, we aim to thoroughly evaluate the effects of microorganisms on the general ecosystem and critically assess the use of existing biotechnological tools developed to combat climate-related challenges. By shedding light on the potential implications, this review strives to contribute to a deeper understanding of the intricate interplay between microorganisms, ecosystems, and climate change mitigation.

Keywords: Biodiversity, Environmental Health, Biotechnological Microorganisms, Climate Change

Introduction
The proliferation of microorganisms with enhanced access to carbon and nitrogen is triggered by the increase in greenhouse gases resulting from human activities. Factors such as combustion of coal, oil, and other fossil fuels, putrefaction of plants, and biomass burning contribute to the gradual rise in greenhouse gas concentrations, including carbon dioxide, methane, and nitrous oxide. The Intergovernmental Panel on Climate Change (IPCC-2022) defines climate change as "changes in the average conditions of a region's climate or in the variability of its properties over an extended period." (1). While pollution remains a primary driver of global warming, the IPCC report recommends that climate change can be influenced by both anthropogenic and natural factors, such as solar cycles, volcanic eruptions, and continental drift.

Each era characterized by unique life forms, reflecting the global environmental conditions of that period. Although new species can be traced back to their evolutionary ancestors, biodiversity and biodegradation vary across different eras. Thus, the current phase of global warming differs from previous warming periods. In the present era, the persistence of environmental pollutants resulting from human activities adversely affects ecosystems in multiple ways, intensifying the current phase of global warming and making it more aggressive.

The industrial age has witnessed significant increases in greenhouse gas emissions, which are responsible for approximately 98% of the observed global warming. Various industries release gases such as carbon dioxide (CO_2), methane (CH_4), nitrogen oxides, hydrofluorocarbons (HFCs) and sulfur hexafluoride (SF_6). Over time, the concentrations of these gases in the atmosphere increase leading to significant global warming. Microorganisms play a dual role in the production and consumption of greenhouse gases. They are critical in all biodegradation processes ranging from breakdown of dead organic matter to conversion of waste into forms that can be reused by other organisms.

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DOI: 10.2478/ebtj-2023-0015

Therefore, microorganisms exhibit rapid responses to climate change. Pathogenic microorganisms pose threats to marine and terrestrial ecosystems while also facilitating microbial consumption under different circumstances. For instance, methane-consuming microorganisms can remove atmospheric methane even at very low concentrations (2).

Collaboration among microorganisms for pollution remediation or biodegradation (glossary in Text Box 1) is widespread. The metabolites produced by one organism sustain another, resulting in mutual nutritional requirements (3). Direct use of the energy generated by a microorganism's own metabolism is not feasible; thus, shared metabolism is the primary mechanism for pollutant degradation (4).

Heavy metals, due to their large numbers, biological resistance, and stable structures, rank among the most toxic pollutants (5). Both organic and inorganic pollutants contaminate the environment and pose significant health risks to humans and other organisms (6). They exert teratogenic, genotoxic, and mutagenic effects on living organisms, contaminating soil and water (7). Even at low concentrations, these substances can cause endocrine and neurological problems(8) and lead to chromosomal anomalies(9).

The complications arising from pollutants and their toxicity toward existing microbial populations pose challenges to biodegradation (10). To overcome these challenges, synthetic microorganisms with greater bioremediation potential have been developed as biotechnological tools in recent years (11,12) . Microorganisms are widely acknowledged to play a significant role in determining the atmospheric concentrations of greenhouse gases, and their importance is increasingly recognized (13-16). Numerous studies have reported the potential of microorganisms (15) as crucial biotechnological agents in combating climate change and its impacts (17-23). Current research develops biotechnological solutions to address climate-related problems. However, there is a lack of knowledge regarding the potential effects of these highly effective technologies on the overall ecosystem when integrated into the environment.

Effect of Microorganisms on Ecosystems

Climate change exerts profound and multifaceted impacts on microorganisms inhabiting terrestrial and marine ecosystems, as well as on their pivotal role in the transmission of infectious diseases. These effects are driven by an array of interconnected factors, including microbial degradation, methanogenesis, industrial waste accumulation, microbial biomass dynamics, photosynthetic processes, agricultural practices, and livestock management. As global temperatures rise and weather patterns undergo alterations, microorganisms and their associated ecological processes face significant disruptions (Fig. 1).

Terrestrial Ecosystems: Soil microorganisms play a pivotal role in regulating the storage and release of organic carbon in soil, thereby exerting indirect influence on carbon sequestration in plants and soils through the provision of essential macronutrients such as nitrogen and phosphorus (24-26). Carbon transfer from the atmosphere to the soil occurs via carbon-fixing autotrophic organisms, encompassing photosynthetic plants and photo and chemoautotrophic microorganisms. These microorganisms use atmospheric carbon dioxide as a metabolic substrate, thereby synthesizing organic matter. Consequently, they contribute to the removal of atmospheric carbon dioxide and the production of organic matter that nourishes terrestrial ecosystems. However, temperature perturbations disturb the equilibrium between these processes, thus impacting the terrestrial biosphere's capacity to capture and store anthropogenic carbon emissions (27).

Soil pollution represents a significant global climate concern, and soil health constitutes an indispensable attribute for supporting agricultural sustainability. Carbon regulation within the soil system assumes a pivotal role in the dynamics of climate change (28). Conversely, the escalating persistence of pesticides in the environment engenders perilous repercussions for humans, plants, and animals. The World Health Organization (WHO) has documented three million instances of agricultural-chemical-related harm in nonindustrialized countries (29). Prolonged and indiscriminate use of agrochemicals adversely affects soil biodiversity, agricultural sustainability, and sanitation (30).Studies have reported adverse impacts on biodiversity and carbon sequestration associated with the projected expansion of croplands in the future (31).

Marin Ecosystems: Microbial communities in the ocean exhibit adaptive responses to environmental changes along biogeographic transitions, leading to alt erations in the structure of species communities. Temperature increase, for instance, has a pronounced effect on the metabolic rate of prokaryotes within a short timescale (within a day) and is more sensitive compared to eukaryotes. In response to higher temperatures, microbial communities adapt by increasing their metabolic rates and specific CO_2 production per unit biomass (32). These adaptations in microbial metabolism have significant implications for biogeochemical cycles, thereby influencing overall ecosystem functioning (33).

Temperature plays a crucial role in shaping microbial communities, and fluctuations at the same level can have adverse effects on the organization of these communities. The intensification of evaporation rates across the oceans leads to shallowing of the surface layer, resulting in increased stratification. This intensified stratification poses a hindrance to the transport of essential nutrient resources that organisms rely on. Moreover, density-dependent processes contribute to a reduction in oxygen solubility in surface waters, leading to a significant decrease in oceanic oxygen levels (34).

Considering the intricate relationship between ocean biogeochemistry, microbial metabolism, and diversity becomes paramount considering climate change and its associated impacts. Marine microorganisms serve as the foundation of oceanic food webs, and any disruption to their functioning can have cascading effects throughout the marine ecosystem. Therefore, a comprehensive understanding of the interactions

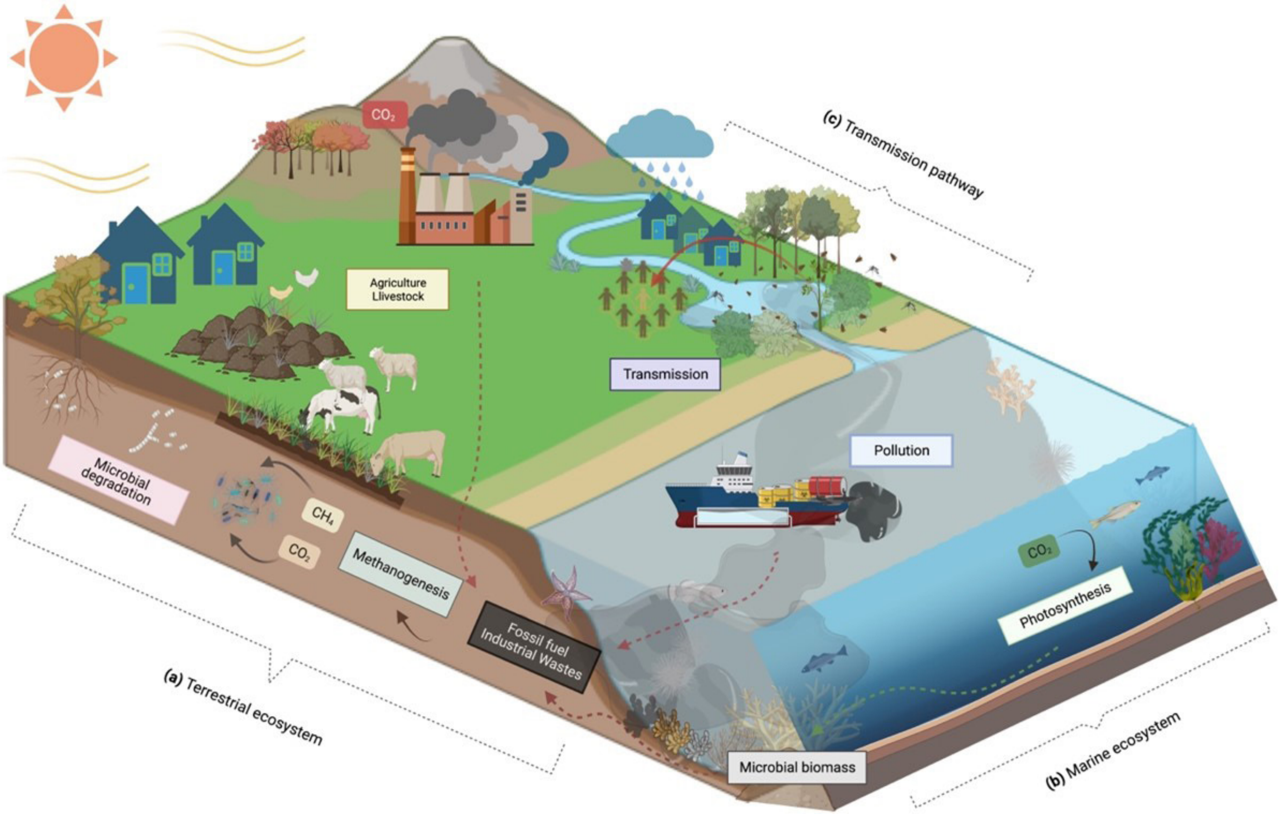

Figure 1. Impacts of climate change on microbial processes, disease transmission, and environmental pollution in the overall ecosystem. **(a)** In terrestrial environments, climate change disrupts microbial degradation mechanisms, leading to altered breakdown of organic matter in soil and disruptions in nutrient cycling dynamics. These perturbations result in diminished soil fertility and reduced agricultural productivity. Escalating temperatures intensify methanogenesis, the biological production of methane by microorganisms, exacerbating greenhouse gas emissions and amplifying the adverse effects of climate change. **(b)** In marine ecosystems, climate change also impacts microbial communities, influencing key processes such as photosynthesis and nutrient cycling. These disruptions have far-reaching consequences for marine ecosystem functioning and biodiversity. **(c)** Environmental pollution, including alterations in temperature and precipitation patterns, plays a significant role in the dissemination of infection diseases. It compromises air and water quality, facilitating the transmission of pathogens and increasing the vulnerability of populations to infections.

between microbial communities, temperature fluctuations, and biogeochemical cycles is of primary importance for assessing and addressing the consequences of climate change in the marine environment.

Infectious diseases: Climate change has significant impacts on the distribution, abundance, and activity of hosts, their resistance to infection, the physiology of host-virus interactions, virus evolution rates, and host adaptation, thereby affecting the frequencies and durations of viral outbreaks (35,36). For instance, more than a billion microorganisms are found in a liter of seawater (37). Among them, viruses contribute to 8.6% of the carbon cycle, 1.4% in marine ecosystems, 6.7% in terrestrial ecosystems, and 17.8% in freshwater ecosystems (38).

The gradual acceleration of virus spread and replication leads to an accelerated carbon cycle (39,40). For example, exposure of corals to heat shock has been shown to trigger the formation of virus-like particles that induce cell disintegration in nonstressed corals, which is evident in zooxanthellae and surrounding seawater (41,42). Viruses transmitted by vectors such as insects, bacteria, and protozoa. Climate change influences vector-borne diseases (43). The vector, pathogen, vector-host interaction, host immunity, and pathogen evolution are all dependent on climate. As a result, they can adapt to climate fluctuations, altering the geographical ranges of new disease cases and expanding them (44).

Warming affects the behavior, physiological traits, life histories of vectors and pathogens as well as host populations and behaviors (45,46). The water above the seafloor where nototenioids reside, for example, is rapidly warming and becoming less saline. With increasing temperatures and climate change, it is expected that the regional distributions of pathogens and vector species will expand (47,48). Depending on their adaptive abilities, vectors may not carry existing

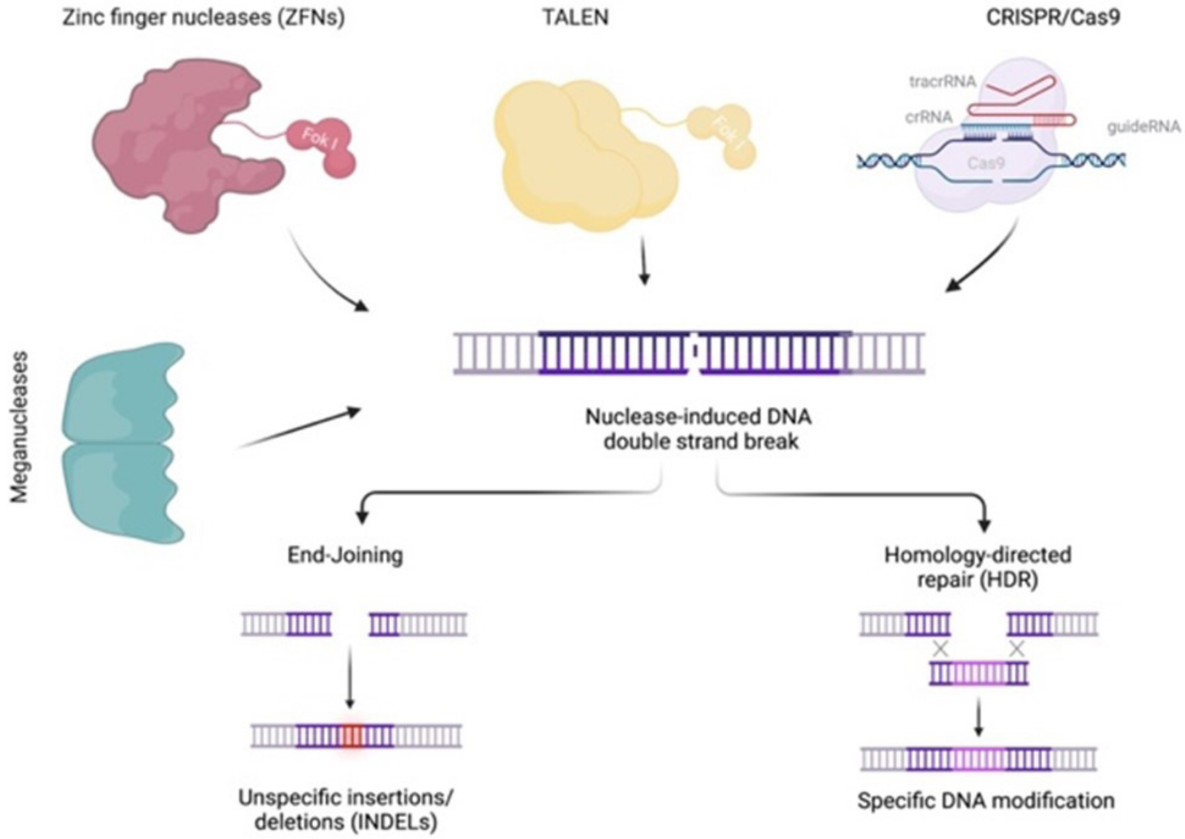

Figure 2. The most commonly used tools for gene editing.Biotechnological tools include transcription activator-like effector nucleases (TALENs) and nucleases associated with clustered regularly interspaces short palindromic repeats (CRISPR) (81). The DNA binding module of TALEN is sequence-specific to the host genome (82) . When TALEN binds to DNA and leaves sticky ends for stability, double-stranded breaks (DSBs) are generated. Zinc finger nucleases (ZFNs) have a DNA binding domain of 30 amino acids, and the Fok1 cleavage domain brings the DSBs to the targeted location in the host genome. Hybrid nucleases containing TALENs and ZFNs are used (83). CRISPR method can simultaneously edit multiple genes with high precision.Crispr-derived RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) are combined by the gRNA (guide RNA) in the CRISPR/Cas system (84). The Cas9 enzyme is directed by the gRNA sequence to create a DSBs at the target DNA sequence.

pathogens, but climate-mediated ecosystem changes can facilitate the transmission of new ones by bringing together different pathogens, vectors, and hosts (49). For instance, insect and mammal vectors allow for range expansion, facilitating changes in animal species and the spread of microorganisms through new pathways (50).

Approximately 58% of all known infectious diseases that affect humans are climate-related. It has been reported that these pathogens causing infectious diseases can lead to disease outbreaks through more than a thousand different transmission routes (51). Storms, heavy rainfall, and floods create stagnant water, providing breeding and growth areas for mosquitoes and the pathogens they transmit (e.g., leishmaniasis, malaria, yellow fever, St. Louis encephalitis, dengue fever, and West Nile fever)(52-54). The increase in the pathogen virulence capacity is triggered by climate-related hazards (55).

Infections in hosts, along with pathogens (bacterial, fungal, or viral), enable the sharing of virulence genes through horizontal gene transfer or the emergence of highly antibiotic-resistant "superbugs" (56,57). As zoonotic and opportunistic pathogens adapt to a warmer environment, they can cause increased infectivity and pathogenesis (58). For example, it has been reported that the spread of viruses to human populations, resulting in increased virulence, creates a natural selective pressure against "heat-resistant" viruses, as the human body's main defense mechanism, fever, can better cope with them (59). On the other hand, drought-related food shortages weaken bat autoimmune defenses, leading to increased virus transmission and Hendra virus outbreaks (60). Ocean warming accelerates the spread of harmful algal blooms and diseases caused by *Pseudonitzschia* sp., cyanobacteria, and dinoflagellates (61).

Climate-related threats have been observed in marine systems, such as Vibrio species, anisakiasis, and jellyfish poisoning (62,63). For example, *Vibrio* spp. (*Vibrio parahaemolyticus* and *Vibrio cholerae*) are aquatic bacteria found in warm estuarine and coastal waters with low to moderate salinity. *Vibrio cholerae* is responsible for cholera outbreaks, while other Vibrio species (*V. parahaemolyticus*,

Table 1. Biotechnological tools and target organisms used in the overall ecosystem

Ecosystem / Target Organism	Method	References
Marine Ecosystem		
Chlamydomonas reinhardtiimarin	CRISPR	96
Chlorella sorokiniana, Chlorella vulgaris	CRISPR	97
Ascidian Ciona intestinalis	CRISPR	98
Coral	CRISPR	99
Yellow catfish	ZFN	100
Streptomyces albogriseolus	CRISPR	101
Terrestrial Ecosystema[a]		
Arabidopsis thaliana	CRISPR	102
Ceratitis capitata	CRISPR	103
Helicoverpa armegera	CRISPR	104
Wheat	CRISPR, TALEN	105,106
Rice	TALEN, CRISPR	107,108
Maize	CRISPR	109
Peanut	TALEN	110
Tomato	CRISPR	111
Maize	ZFN	112
Terrestrial Ecosystem[b]		
Goat	TALEN	113
Chicken	CRISPR	114
Cattle	ZFN	115
Cattle	TALEN	116
Sheep	ZFN	117
Infectious Diseases		
Malaria	CRISPR	118
Zika	CRISPR	119
Dengue	CRISPR	120
Vibrio parahaemolyticus	CRISPR	121
West Nile virus-induced cell death	CRISPR	122

[a] Agriculture
[b] Animal disease

V. vulnificus, and nontoxigenic *V. cholerae*) have been reported to be pathogenic to humans, associated with sporadic gastroenteritis cases, wound infections, ear infections, and septicemia. *V. parahaemolyticus* is one of the most common bacterial causes of gastroenteritis due to contaminated seafood and causes wound infections (64-68).

According to the IPCC 2022 report, *Vibrio* spp. are increasingly being observed at higher latitudes, and *Vibrio* spp. infections are observed for longer periods within a year (69). This exacerbates the existing outbreaks of viruses by allowing vectors and pathogens to survive during winter months (48). Storms and floods have been associated with hantaviruses, hepatitis, and Cryptosporidium infections through direct or foodborne transmission due to sewage overflow (70-71).

Biotechnological Tools Developed for Combating Climate Change

Biotechnology encompasses processes such as waste treatment and prevention of environmental hazards, production of commercial chemicals, and synthesis of therapeutic compounds such as vaccines and antibiotics. Activities such as greenhouse gas emissions, desertification, deforestation, and industrial pollution have been increasing global warming and negatively impacting the environment (72). To mitigate the adverse effects caused by climate change, modern genetic manipulation techniques are employed in biotechnology to develop new microorganisms with desired traits. These advancements have facilitated the development of genetically modified microbial cleansers (synthetic microorganisms) to reduce various pollutant species (73-75). Biotechnologically developed microorganisms offer heightened specificity compared to their naturally occurring counterparts enabling effective degradation of pollutants and pathogen control.

Maintaining a healthy ecosystem necessitates the degradation and prevention of harmful pollutants from spreading (76). However, determining the species involved in biodegradation processes in a natural environment is challenging (77). Therefore, the development of an artificial microbiome containing functionally specialized species is being pursued under well-defined conditions based on interactions (78). The structure and dynamics of microbial communities are influenced by structural, functional and ecological factors (79,80). Microbial community interactions are a consequence of metabolically directed artificial microbial interaction models (81).

Genetic alterations at the level of an organism influence its phenotype. Mutations can occur randomly or intentionally, and their effects can be either harmful or beneficial (82). Plasmids, integrons, and transposons all contain genes specific to biodegradation (Box 1). Bacteria are microorganisms that can survive longer in contaminated environments due to mutations. It is possible to apply specific mutations to disrupt the structure of resilient microbes in polluted soil and water. Biotechnological degradation using microbial metabolism is applied in various ways. For example, the use of mutant enzymes stabilizes catalytic processes in the breakdown of synthetic pollutants (83). DNA molecule fragments containing one or more nucleotides can be inserted, removed, or edited (Fig. 2)(85-88)in an organism's genome using technologies in rational genetic engineering at the genome or gene level (84,89).

Various methods exist to manipulate environmental conditions in order to design collaboration between two microorganisms, such as gene removal and insertion (90,91).

Box 1 | Glossary

Ecosystem: are dynamic systems comprised of living organisms and their environment, operating as interconnected and functional units. They encompass a diverse array of components, including plants, animals, microorganisms, soil, water, and air, and are characterized by intricate energy flows and nutrient cycling processes.
Bioremediation: is a process that harnesses the capabilities of living organisms or their byproducts to mitigate or eliminate pollutants from contaminated environments. This approach utilizes the natural metabolic activities of microorganisms, plants, or enzymes to degrade, transform, or immobilize hazardous substances, thereby facilitating the restoration of ecosystems to a healthier state.
Biodegradation: is an intrinsic process driven by microorganisms or other living organisms, whereby complex organic materials are enzymatically broken down into simpler forms such as carbon dioxide, water, and biomass. This natural phenomenon contributes significantly to the recycling and renewal of organic matter in ecosystems. Through biodegradation, organic compounds are transformed into more basic components, allowing for nutrient recycling and sustaining the functioning of ecosystems.
Biotechnological tools: encompass a range of techniques that leverage living organisms, their components, or their biochemical processes to develop innovative products, enhance industrial processes, and tackle environmental challenges.
Plasmids integrons, and transposons: are genetic elements possessing the ability to mediate the transfer of genetic material. Plasmids, circular DNA molecules, exhibit presence in both bacteria and eukaryotes. Integrons, on the other hand, are integrated within bacterial chromosomes and serve to capture genes. Transposons, referred to as DNA segments, possess the capacity to mobilize within and across genomes, thereby facilitating the transmission of genes alongside them.

The use of engineered models in bioremediation systems is becoming increasingly prevalent (92,93). Genetic engineering tools have been applied in the genome engineering of plants, animals, and microorganisms for the expression of specific genes (94-98). Furthermore, using recombinant DNA technology, any organism can be transformed into the desired form (99,100). Subsequently, with the addition of the relevant gene to the genome of another organism's vector (such as phage, plasmid, or virus), the production of the desired gene can be achieved in a different host (78). Modern biotechnological remediation processes developed to mitigate the effects of climate change are built upon these strategies. Below, we provide a list of biotechnological systems developed in response to climate change-related issues reported in the literature (Table.1)(101-127).

Conclusions

Increased atmospheric CO_2 concentrations, higher temperatures, and changing precipitation patterns are exerting pressure on many species and populations. From terrestrial organisms to marine life and bacteria, every ecosystem and population is being affected in different ways. Microorganisms without doubt serve as the primary actors in bioremediation and biodegradation processes.

Recombinant bacteria show potential and promising results in pollutant degradation facilitated by recombinant host bacteria. However, they still face various challenges. Only a few engineered bacteria focus on degradation and removal of toxins in complex environments with diverse substrates and numerous microbial interactions. Plasmids in a strain can slow down the development and proliferation of the strain, making it less efficient in pollutant degradation. On the other hand, certain biosafety concerns limit the assessment of gene editing efficacy in bioremediation processes (128,129).

Most water pollutants are synthetic, making their degradation or removal not always straightforward. The impact of pollution on the ecology of a water system can lead to many outcomes reflecting species adaptation, biology, and ecology. Worldwide, water system pollution is constantly increasing. Unfortunately, marine and coastal ecosystems are the most threatened and least understood ecosystems in the world, despite their significant importance as highly productive ecosystems. The integration of biotechnological tools into the marine ecosystem and their long-term effects on both marine and other organisms in the ecosystem remain uncertain.

Biotechnological tools lack comprehensive experimental data and validations due to the disadvantages associated with bioinformatics. Additionally, there exists a significant knowledge gap among various synthetic microbial groups and their responsible enzymes. Therefore, identifying suitable metabolic pathways for the degradation of identified enzymes may only be achievable through comprehensive and multidisciplinary studies. When supported by such studies in the near future, a combination of bioinformatics tools, metabolic engineering, genetics, molecular biology, and systems biology approaches could offer a viable and sustainable option for biological breakdown of numerous pollutants.

Biotechnological microorganisms are being developed as a cheaper and more environmentally friendly solution to environmental pollution and contamination problems. However, it remains uncertain whether these technologies will be sufficient to combat the destructive effects of climate change. Furthermore, there is curiosity about how these technologies will eventually affect biodiversity and ecosystems. Interference with natural systems yields consequential outcomes, and pollution can result in irreversible effects.It is evident that while biotechnological tools hold promise, they still lack sufficient and validating data despite rapidly threatening climate change and its impact on life, health, and our planet.

In conclusion, the development of prevention techniques should take precedence and urgency over the use of biotechnological tools. In fact, prevention should be the primary solution because some effects of pollution may be irreversible and result in lasting consequences. Environmental hazards threatening ecosystems due to anthropogenic activities need to be addressed rapidly and comprehensively. This is crucial because the issue provides multiple pathways for the entry of toxins, affecting the environment, marine organisms, and ultimately humans. It causes numerous public health concerns, including cancer, neurological and endocrine problems, and infectious diseases. Adopting protective and preventive measures for ecosystem biosecurity emerges as a more reliable approach. Most importantly, prevention remains the most effective biological tool and is still awaiting implementation.

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