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Review

Sustainable Agriculture through the Enhancement of Microbial Biocontrol Agents: Current Challenges and New Perspectives

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Abstract: The future of pesticide usage in agriculture is uncertain due to its unsustainability, adverse environmental impacts, and its association in enhanced phytopathogen resistance. Hence, this situation urges the development of new sustainable practices in agriculture. A promising approach involves endophytes, which are non-pathogenic microorganisms inhabiting the interior parts of plants. However, due to the vast diversity and complexity of plant microbiomes, a major gap has formed with regards to endophytic research and its application in phytopathogen biocontrol. The gap has mainly been increasing due to the difficulty of isolating underrepresented endophytes and due to limitation of previous genetic tools availability to further research and understand plant-microbe interaction, endophytic biocontrol capabilities and their biocontrol compounds. This review highlights the current challenges being encountered in this research field. Additionally, the research advances through utilization of specialized techniques (CRISPR/Cas9 system, nanoparticles and multi-omics) are highlighted to assist in elucidating the mechanism revolving around plant-microbe interactions and to generate model systems demonstrating improved biocontrol capabilities of endophytes. The ultimate goal of this review is to provide improved approaches that could be implement in an array of microorganism that will enhance the phytopathogen biocontrol field in order to create a sustainable agricultural sector.

Keywords: endophyte; biocontrol; metabolites; phytopathogens

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1. Introduction

Over the last two decades, industry's dependency on pesticides has amplified the associated genetic resistance of phytopathogens, reducing the implementation or opportunity for natural biocontrol adaptation [1]. At present there exist multiple factors which are driving the adoption of novel sustainable approaches. Microbial antagonists exemplify a promising application as they could possess the ability to control, suppress or inhibit phytopathogens. Microorganisms which reside in the internal parts of plant tissues and do not cause harm are termed endophytes, which present the ability to act as phytopathogen antagonists [2]. Research involving endophytes has gained traction in recent years due to their innate characteristics which have made them targets for medical and agricultural research, specifically with regards to sustainable agriculture research for protection practices [3,4]. Endophytes have been shown to promote nutrient uptake and enhance host growth [5]. They might increase the plant's capacity to withstand different abiotic and biotic stresses and boost the plant's resistance to insects and other pests. They create phytohormones and other bioactive substances with potential biotechnological applications in different fields [6]. They are particularly useful in agriculture, as reports have shown their application as crop growth promotion and phytopathogen biocontrol agents. Therefore, research has been hurtling towards the substitution of synthetically derived plant protection chemicals, with the introduction of biological control agents, which are environmentally safer alternatives and sustainable in nature. However, with the increase in research endeavors, new challenges and limitations have been identified. These challenges include the difficulty of isolating endophytes, as some plant-associated microorganisms may not be fully represented as part of the family of endophytes; this could be due to the low cultivability of some microorganisms on research growth media. Thus, scientists have begun using specialized techniques in an effort to overcome these challenges while simultaneously elucidating endophytes mechanisms of biocontrol and elucidating the bioactive compounds produced by endophytes, which confers them these unique characteristics as viable agricultural tools. Consequently, this review aims to (1) highlight the challenges currently being encountered in this research field and (2) showcase how scientific technologies have been used in order to improve endophytic research and enhance microbial biocontrol activity. The term endophyte, for the purposes of this review, will only focus on microorganisms (bacterial or fungal) which reside within plants for most of their lifecycle, and which are able to be cultured. We focus on these microorganisms for their ability to be cultured as the research and techniques we will be discussing hinge on the fact that these organisms can be inoculated onto plants or their cultures can be used to produce biocontrol products (secondary metabolites or biogenic nanoparticles).

2. Challenges Associated with Isolating Microbial Biocontrols

Microbial biocontrol has proven to be a technique with a broad activity spectrum against various pathogens and pests [7]. Microbial biocontrol can support plant growth, enhance stress tolerance, support plant nutrition, and antagonize plant pathogens (Figure 1) [8]. The need to sustainably intensify agricultural productivity using endophytic research to combat biotic (pathogens and pests) and abiotic stresses has inherent challenges due to the complexity of microbiomes associated with plants. Hence, the Integration of plant biocontrol agents is an efficient approach that presents a promising solution for boosting agricultural productivity. However, the efficient commercial use of endophytic organisms as biocontrol agents or growth promoters would require addressing several challenges associated with isolating these microorganisms. Therefore, the use of novel isolation and screening approaches are required to obtain native microbial biocontrol with effective antagonistic activity. The development of these isolation and screening approaches is often driven to solve a certain threat to agricultural productivity [9].

One of the challenges associated with isolating different microbial biocontrol candidates is mycotoxin contamination [10]. However, mycotoxin detoxification, transformation and adsorption can reduce mycotoxin contamination in the form of yeast, bacteria, fungi and enzymes [11]. These agents can degrade or adsorb mycotoxins before their direct destructive effect on the harvest. Nevertheless, these strategies are affected by environmental factors during interactions, and thus there is an increasing risk of sexual recombination between toxigenic lines and microbial biocontrol strains. This occurrence eventually leads to the emergence of hypervirulent toxigenic strains. Thus, to address this problem, characterization of microbial biocontrol agents at genus level, using sensitive methods, are required for better functional characterization at species level to address this problem [12]. Bio-detoxification of mycotoxins using probiotic lactic acid bacteria has effectively removed contaminants by biodegradation or bio-adsorption pathways [13]. Bio-adsorption involves direct binding of the toxin to the bacteria, in which binding is affected by the bacterial affinity toward the toxin [14]. However, biodegradation, which is more durable and irreversible compared to bio-adsorption, can modify toxin structure and result in unwanted metabolites (such as aflatoxicol from aflatoxin B1), which could be detrimental to the host [15], thus in itself reducing the implementation or opportunity for natural biocontrol adaptation.

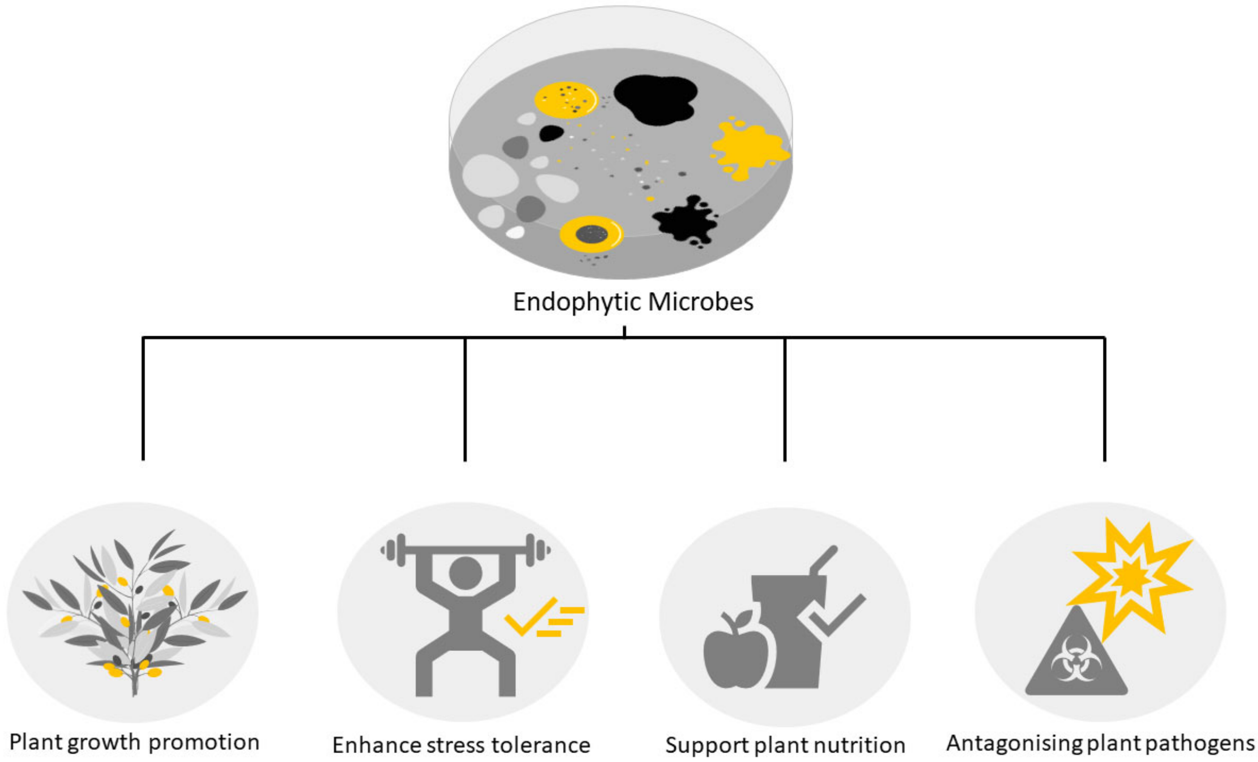

Figure 1. Advantageous microbial activity spectrum for potential beneficial endophytes. Microbial biocontrol has the potential to improve agricultural productivity by exhibiting one or all four of the activities depicted in the image.

Another challenge of microbial biocontrol agents is the antagonistic mechanisms that control or suppress pathogens and their intricate interactions among the host, pathogen, and the environment. These interactions can be affected by multiple factors which include the environmental conditions and the presence of microbial colonizers in the biological system [16]. However, intensified efforts toward a better understanding of the mechanism and mode of actions in the area of direct antibiosis, hyperparasitism, induction of resistance and competition for space and nutrients would facilitate the optimization of control and allow the use of more efficient strains in the correct environment [17].

The use of microbial biocontrol agents within the agriculture industry hinges on the regularization and commercialization of the microorganisms while allowing them to still remain competitive with synthetic chemical pesticides and fumigants [18]. It is also expedient to evaluate the toxicology of these agents [19], while bold efforts should be made toward proper preservation methods and commercial-scale production strategies [20].

3. The Use of the CRISPR/Cas9 System in Endophytic Research for Enhanced Biocontrol or Improved Plant Growth

Genome editing research underwent a re-emergence with the development of the CRISPR/Cas9 system [21]. The system allowed for more precise genome editing, which has revolutionized the biomedicine and agricultural industries [22,23]. In recent years, researchers have used the CRISPR/Cas9 system in order to further understand the plant-microbe interaction, the role of endophytic microorganisms and their bioactive compounds to serve as potential biocontrol agents. This system provided a promising approach to understanding endophytes mode of action for colonization, growth promotion of plants [24] and understanding how they produce biocontrol agents in responses to phytopathogens to improve plant pathogen tolerance [24,25]. Hence, firstly to understand the interaction between endophytes and their hosts, the CRISPR/Cas9 system has been used to alter or knock out key genes to understand the role they may play. A study by Huang et al. (2020) [26] used the CRISPR/Cas9 system to disrupt the *PmKKA* gene associated with the beneficial fungal endophyte *Phomopsis liquidambaris*. The introduction of the *PmKKA*-deficient mutant strain in *Oryza sativa* (rice) seedlings resulted in increased lytic (chitinase and glucanase) enzyme activity, which the authors associated with a resistance response in rice [26]. The findings of the study indicated that the *PmKKA* gene (in the fungal endophyte) may be required for the rice seedlings to recognize *Phomopsis Liquidambaris*. It was further noted that the disrupted *PmkkA* gene encoded the CWI MAPK pathway, which may have been required for improved symbiosis (Huang et al., 2020). The findings in Huang et al. (2020) highlighted the importance of using techniques, such as the CRISPR-Cas9 system, to investigate the genes responsible for host colonization, as these discoveries would play a critical role in whether the adoption of endophytic technology within agricultural practices can and will be implemented.

Secondly, the CRISPR/Cas9 system has also been successfully implemented to generate endophytic mutants with increased bioactive compound production [27,28] and enhanced enzymatic expression [29]. The rationale behind manipulating the expression of the enzymes associated with these endophytic microorganisms is to understand their role in growth promotion, the plant's defense response to abiotic stress, biocontrol responses to invading pathogens and to further improve the endophytes beneficial qualities. A study by Zhu et al. (2022) [29] produced two mutant strains OE-Chi and IN-Chi by plasmid transformation and CRISPR/Cas9-mediated integration of the chitinase enzyme into the genome of *Phomopsis liquidambaris*, respectively. Both mutant strains showed the ability to inhibit the expansion of wheat pathogen *Fusarium graminearum* in plate confrontation assays in vitro [29]. However, the OE-Chi strain showed a better colonization of wheat, and it was hypothesized that the higher rate of colonization led to improved resistance to the Fusarium pathogen [29]. This study highlights one of the drawbacks of the current CRISPR/Cas9 system, as integrating the chitinase enzyme led to a decreased colonization potential of the mutant strain [29]. This is an important finding, as the premise of using endophytic microorganisms for beneficial application often hinges on the fact that the endophyte is able to colonize its host.

Endophytic microorganisms have been identified as a good source of bioactive compounds with less side effects when compared to their synthetic counterparts. Research involving bioactive compounds from endophytic microorganisms has increased, which has allowed for their different applications in the field of medicine [30] and agricultural industry [30]. In an effort to expedite the discovery of new bioactive compounds from endophytes, researchers have embraced CRISPR technology. A study by Xu, Huang and Yin (2021) [27] used the polyethylene glycol mediated protoplast transformation method to introduce the CRISPR/Cas9 system into the endophytic fungi *Pestalotiopsis fici* (Figure 2). The researchers used their new system to produce edits in the genome which included site specific gene insertions, dual locus mutations and long DNA fragment deletions (Figure 2B). In conclusion, the study showed that the system could not only be used in *Pestalotiopsis fici* but also in other fungal endophytic organisms, allowing for the excavation of more bioactive compounds [27]. Having the power to perform specific alterations in the genome of endophytes will allow future studies to target specific metabolic pathways in order to identify or improve the acquisition of bioactive compounds. The improvement of bioactive compound production would ultimately improve the biocontrol activity against various phytopathogens which are currently negatively influencing the quality of your crops.

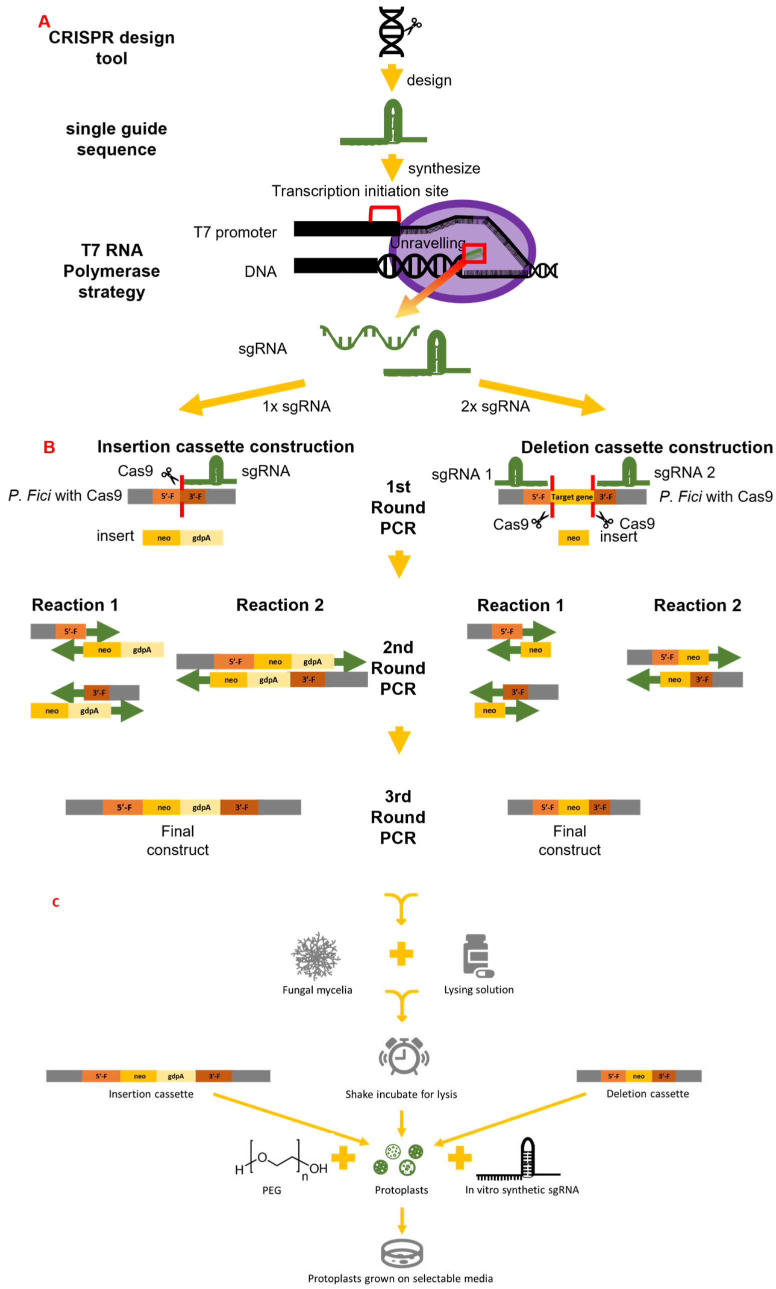

Figure 2. An effective CRISPR/Cas9-based genetic manipulation (gene insertion and/or deletion) approach in the endophytic fungus *P. fici*. (**A**) The generation of the single guide sequence (sgRNA). The single guide sequence was firstly identified using the CRISPR design tool, and thereafter this design was used to construct the sgRNA using the T7 RNA polymerase approach (in vitro transcription-based). (**B**) The DNA cassette construction and genetic manipulation. The double-joint PCR approach was used to construct the insertion (left) and deletion (right) cassettes. One sgRNA was required for the insertion cassette construction and two sgRNAs were required for the deletion cassette construction. The marker gene neo was used in the construction of both cassettes. The insertion cassette was constructed by using *gdpA* sequence and a target site amplified from the genomic DNA of *P. fici*. The deletion cassette was constructed by using target genes amplified from the genomic DNA of *P. fici*. The final construct are schematics of the insertion (left) and deletion (right) cassettes. (**C**) Polyethylene glycol (PEG)-mediated protoplast transformation approach. This 3-step approach (**A**–**C**) yielded improvement of the efficiency of gene manipulation up to 48% and furthermore represented a time-efficient transformation approach.

4. The Use of Nanotechnology for the Improvement of Endophytic Biocontrol Agents

Nanotechnology, which involves study of molecules or compounds at nanoscale, is a novel field of study with development and applications in various fields such as energy, medicine and agriculture [31]. Nanotechnology has shown its potential as a novel tool for the treatment of diseases [32], thus improving human health. Consequently, it has been suggested that these same techniques could serve the same purpose and have a positive effect on plant health [33]. Recently, nanomaterials have been applied in combination with microorganisms to aid and create new properties for dealing with diseases. Nanomaterials can be synthesized through physical, chemical, and green routes. Green synthesis involves the use of biological materials such as plants, bacteria and fungi to synthesize nanomaterials [32]. An exponential increase in accounts of nanoparticles in phytopathology has been noted over the last few decades [34]. In a study by Zhao et al. (2022) [35], copper oxide nanoparticles (CuO-NP) were biosynthesized from marine endophytic actinomycetes because of its antibacterial and antifungal properties. The NPs used were able to inhibit biofilm production from *Escherichia coli* and *Proteus mirabilis* [35]. One notable finding of this study was that the biogenic nanoparticles exhibited a broad spectrum of antibacterial activity against human pathogenic bacteria. *P. mirabilis* and *E. coli*, which are bacteria that mostly affect humans, but this study shows the ability of biogenic NPs to possess antifungal and antibacterial traits which could possibly be applied in the agricultural industry for the improvement of biocontrol agents in an effort to enhance phytopathogen tolerance [36].

Another nanoparticle that has displayed antifungal activity against pathogenic microorganisms is silver (Ag). A study by Elbahnasawy et al. (2021) [33], showed that Ag NPs synthesized from actinobacterium, *Rothia endophytica*, had antifungal activity against *Candida albicans*. The study further explained the antibacterial mechanisms of the nanoparticles, which were attributed to its ability to reduce cell viability, promote cellular leakage of sugar and proteins and increase lipid peroxidation in *C. albicans* (Figure 3). With the premise of using nanoparticles synthesized from endophytic microorganisms against human pathogens, more studies need to explore the potential this technique can have on the health and growth of plants, specifically commodity crops. Hence, Elbahnasawy et al., 2021 [33] depicted the potential of the application of *Rothia endophytica's* Ag-NP as a biocontrol agent for inhibiting various phytopathogens growth within plants. It has also been reported that biogenic zinc nanoparticles were observed to be effective against some phytopathogenic fungi [37]. A study by Abdelaziz et al., 2022 [38] showed that Zn nanoparticles worked through multiple mechanisms to increase the plant's antioxidant and defense system against fusarium species. These mechanisms included the increase in phenolic compound production which have been cited in literature to have good antioxidant capabilities as well as the activation of the host defense mechanisms which were attributed to an increase in soluble proteins [38]. Another study also explored the antifungal activity of biogenic zinc oxide nanoparticles synthesized from three endophytic streptomyces species; *Streptomyces capillispiralis* Ca-1, *Streptomyces zaomyceticus* Oc-5, and *Streptomyces pseudogriseolus* Acv-11 against four phytopathogens (*Alternaria. alternate*, *Fusarium oxysporum*, *Pythium ultimum*, and *Aspergillus niger*) [39]. The study highlighted the ability of the nanoparticles to inhibit the phytopathogens in a concentration dependent manner. It also reported that the antioxidant activity of the nanoparticles was equivalent to or higher than known antioxidant compounds such as ascorbic acid [39]. The results of the study showed that the biogenic nanoparticles may enhance phytopathogen tolerance in plants as well as plant reactive oxygen species (ROS) homeostasis, leading to overall improvement in plant health. Accordingly, these are just a few studies representing the positive implications of implementing nanotechnology in order to improve biocontrol agents, against an array of phytopathogens, by not only controlling the phytopathogen's growth, but by efficiently inhibiting its growth within organisms. Thus, most of these studies suggest using plant-derived metalloids/metallic oxide nanoparticles as fungicides in order to inhibiting phytopathogens.

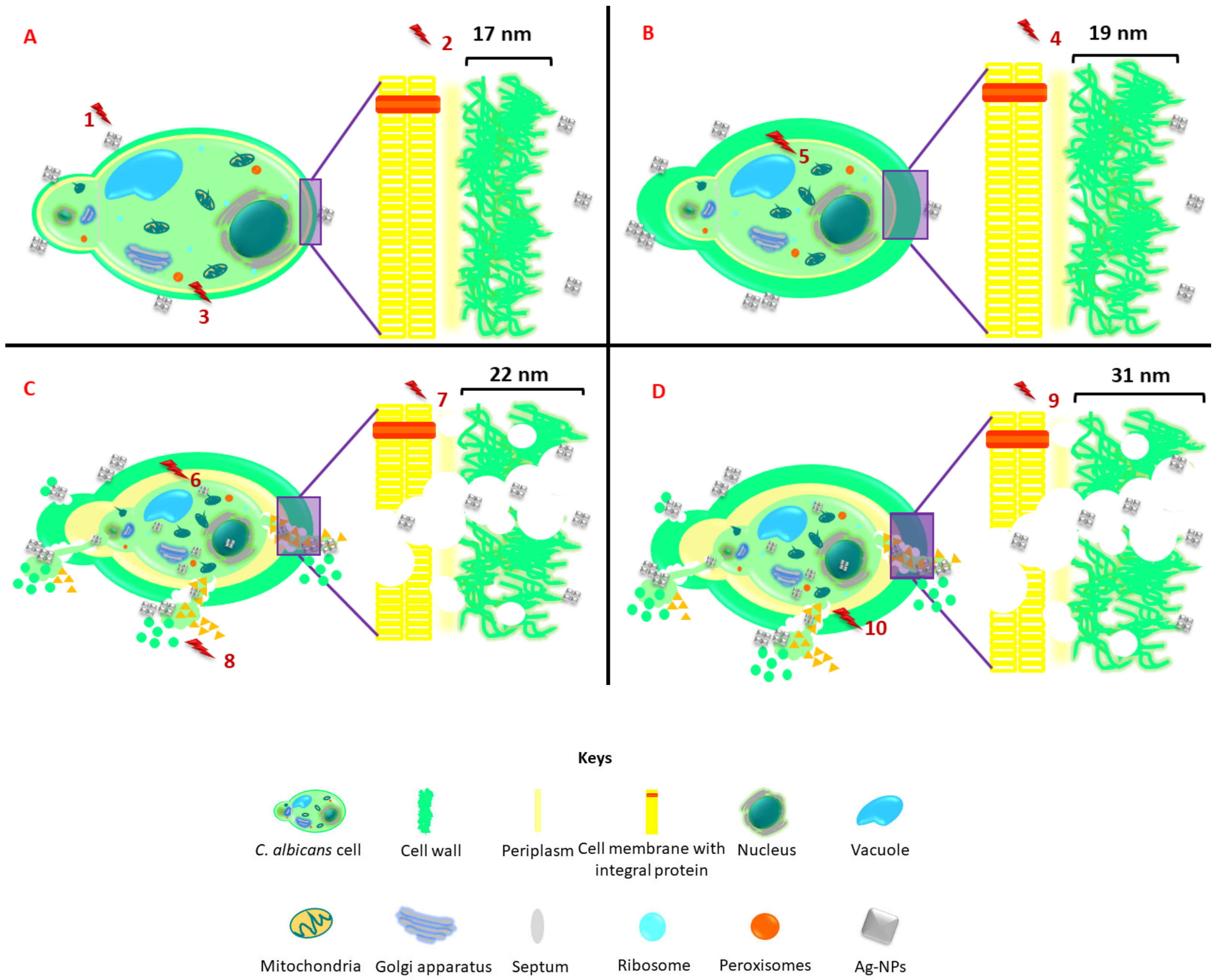

Figure 3. Schematic diagram of the mode of action of Ag-NPs against *Candida albicans*. Diagram depicts when (**A**–**D**) and what effects (1–10) Ag-NPs have on *C. albicans*. (**A**) 12 h after exposure of *C. albicans* cell to Ag-NPs, the Ag-NPs comes into contact with the cell wall of the fungal cell (1). The cell wall thickens (2), and this instigates the compression of the cytoplasmic membrane (3). (**B**) 24 h post exposure, thickening of the cell wall further is exhibited (4) and causes the cytoplasmic materials to compress to the middle of the fungal cell (5). (**C**) 36 h after exposure to Ag-NPs, the cytoplasmic membrane separates (6) and the cell wall thickens even further with degradation of the cell wall due to pore formation (7). The degradation of the cell wall leads to the cell collapsing and hence the release of cellular content (8). Finally, at 48 h (**D**), the 31 nm region exists between the outer membrane (9), and finally the broken cell wall and cytoplasmic membrane result in severe damage to the *C. albicans* cell, leading to cell death (10).

5. Integrating Multi-Omics Approaches for Studying Endophytic Research and Improving Biocontrol Activity

Plants and endophytes coexist in nature, and their genes and proteins involved in metabolic pathways can influence plant development and health, either directly or indirectly [40]. In this section, we will review work focused on biocontrol research and evaluating the use of multi-omic approaches with an emphasis on genomics and proteomics. Previously, it was considered that plants or their associated endophytes were responsible for the production of certain secondary metabolites that had a beneficial effect on the growth or health of a plant. However, advances in genomic technology have allowed for the discovery of various homologous gene clusters present in both plants and their endophytes [41]. These findings highlight the ability of endophytes to directly influence plant metabolic processes by producing the gene products recognized by the plant. Howitz and Sinclair (2008) [42] hypothesized that homologous gene clusters present in plants and microorganisms may be cross activated by stress induced molecules, under certain conditions. A study by Pitakbut, Spiteller and Kayser (2022) [43] observed through gene analysis that endophytes associated with *Gymnosporia heterophylla* were responsible for the formation of maytansine through the expression of their AHBA synthase and halogenase genes. Alongside the expression of these genes and activation of the maytansine biosynthesis, the authors also observed an increase in the expression of the friedeline synthase genes in the plant. By using gene analysis, researchers can evaluate the roles genes play in the endophyte–crop interaction, which has the potential to improve biotic stress tolerance/biocontrol agents and improve growth when augmented. However, it must be noted that it is not only genes that are homologous between plants and their endophytes that have shown importance. A study by Zou et al. (2021) [44] observed through genome analysis and LTR-RT elements analysis that the MVK genes responsible for the mevalonate kinase enzyme in plants could have alternative non-homologous copies of this gene within the endophytes when in long-term symbiosis. This finding highlights the use of comparative genome analysis to uncover how endophytes may compensate for plants or offer access to alternative genes required for plant metabolic processes, especially when under certain environmental conditions such as abiotic and biotic stress. By understanding the non-homologous gene pairs shared by endophytes and their host plant, this technology could be used to enhance the production for more effective biocontrol agents.

Gene and genome information has enabled researchers to create a plethora of novel omics-based technologies, that are extremely valuable for assessing biological responses and interactions between plants and microbes in depth and at a speed that was previously unimaginable. Omics-based technologies such as proteomics has been crucial to analyzing the microbes' proteomes in order to highlight the intricate dynamics and different kinds of interactions that occur between endophytic microorganisms and their host plants [45]. This advancement in proteomics strategies has contributed to the discovery and understanding of different pathways involving a wide range of microorganisms that can be utilized mitigating the effect of biotic and abiotic stresses [46,47]. For instance, a label-free proteomics approach revealed that ACC deaminase-generating endophytic bacteria play an important role in conferring salt stress resistance in rice [48]. The findings of Choudhury et al. (2022) [48] found that inoculating rice with ACC deaminase-producing bacteria may improve plant physiological traits. it is inoculation also reduces stress-related organelle damage, by reroutes glucose metabolism, improving photosynthetic machinery, and increasing antioxidant activity, all of which contribute to rice growth and development. Similarly to Choudhury and colleges [48], a previous study focusing on proteomic analysis of two contrasting chickpea cultivars, revealed that salt-tolerant chickpea cultivars were effective in increasing photosynthetic machinery proteins, which are essential for photosynthesis to occur [49]. In another instance, *Burkholderia Cenocepacia* strain YG-3 was isolated from the root of a poplar tree and shown to be an effective cadmium (Cd) remover. The use of label-free quantitative proteome profile analysis revealed a sophisticated means of Cd removal, revealing that strain YG-3 binds to Cd and excretes it out of the cell via efflux pumps before activating antioxidant enzymes to reduce the impact induced by Cd [50]. This study combined genomics with proteomics, which provided additional benefits, such as assessing protein expression in response to Cd as well as helping to discover the major schematic model pathways involved in an efficient bioremediation system for toxic metals. Multi-omics investigation could be an excellent opportunity to identify mechanisms underpinning the induction of endophytic-host resistance to pathogenic infection. Their extracellular protein release could also be utilized to uncover novel secondary metabolites, which can have their usefulness in the production of bioinoculants and biocontrol agents. Unlike animal and plant cells, fungi require basic nutritional media for fermentation, making endophytic fungi a sustainable source for novel metabolites which could be used in the production of biocontrol agents.

A study by Dautt-Castro et al., 2022 [51] implicated the Trichoderma genus (soil filamentous fungi) in the production of large amounts of secondary metabolites of industrial and medical significance. Additionally, they exhibit an equal significance as biocontrol agents, owing to their performance as mycophagous and plant growth stimulators [51]. Thus, they are recognized as serving dual roles in plant protection. Furthermore, systemic disease resistance is stimulated upon colonization of plant roots by Trichoderma spp., issuing resistance against a wide variety of phytopathogens [51]. Resistance is achieved by Trichoderma spp. stimulating phytohormone homeostasis, contributing to improved assimilation of nutrients from the soil [51]. These authors demonstrated the effectiveness in utilizing a multiomics approach to identify crucial players within fungi that confer these positive biocontrol attributes. Thus, it can be proposed that by incorporating the Dautt-Castro et al., 2022 [51] approach (to establish the crucial players) and CRISPR/Cas9 systems to manipulate or alter these crucial players, these coupling techniques could potentially enhance/introduce similar attributes in other endophytes, to confer disease resistance and improve biocontrol activity in commodity crops.

6. Screening Techniques for Identifying Secondary Metabolites Associated with Endophytic Microbes and Biocontrol Activity

Many strategies have been developed to discover structurally novel natural products such as secondary metabolites from endophytic microorganisms, which could aid in various metabolic reactions within plants. Endophytic microorganisms store numerous novel secondary metabolites that can serve as an excellent source of plant-defense compounds against phytopathogens [52]. Due to the high number of commodity crops being affected by various soil-borne pathogens it necessitates the identification of novel compounds for crop improvement against phytopathogens. The use of chromatographic techniques in endophytic research has been explored and continues to grow due to the ongoing search for novel compounds [53]. There are four main different chromatographic techniques that have been used to study the compounds from endophytic microorganisms, which include gas chromatography, liquid chromatography, thin-layer chromatography and paper chromatography. The gas chromatography–mass spectrometry (GC-MS) technique has been used as one of the technology platforms for fingerprint analysis of secondary metabolites in both plant and non-plant species [54]. To understand the therapeutic effect of endophytic microorganisms in the plant, GC-MS has been used to identify the secondary metabolites that are responsible for the antimicrobial/antifungal activity against phytopathogens. In a study by Tchameni et al., 2020 [55], GC-MS was used to identify volatile organic compounds that were isolated from four different Trichoderma strains *Trichoderma erinaceum*(IT-58), *Trichoderma gamsii* (IT-62), *Trichoderma afroharzianum* (P8) and *Trichoderma harzianum* (P11). The study identified over 20 compounds in each extract, which included harzianolide, 6β-hydroxyfluoxymesterone, and 2,6-Bis (1,1-dimethylethyl)-4-(1-oxopropyl) phenol which the authors proposed contributed to the inhibition of the fungal growth of *Pythium myriotylum*. This study shows the successful use of GC-MS to identify secondary metabolites, which play a pivotal role in plant pathogen defense.

High-pressure liquid chromatography (HPLC) is a chromatographic technique that is widely used in research for identifying bioactive compounds from endophytic microorganisms especially endophytic fungi. The advantages of using HPLC is that the analysis of the sample moves at high speed, it has a high sensitivity, and the quantification of results is accurate and precise [56]. A study by Zhao et al., 2020 [57] reported the identification of 2,6-pyridinedicarboxylic acid from *Simplicillium* Chinense strain SneF5 using a HPLC. The identified compound exhibited a broad spectrum of activities, including antitumor, anti-inflammatory and antibacterial activities. The same study also showed the effectiveness of 2,6-pyridinedicarboxylic acid as an antagonist of the root rot nematode *Meloidogyne incognita*. This study highlights the increasing need for screening tools such as HPLC to be used to screen for potential biocontrol microorganisms, as well identifying compounds within microorganisms that can play an important role in controlling phytopathogens and improving plant health.

7. Conclusions

In conclusion, as the global requirements for food production increase alongside a varying climate, various new technologies could sustainably alleviate numerous challenges in disease management by lessening dangerous chemical inputs and endorsing swift detection and inhibition of phytopathogens. Endophytic research has demonstrated potential in the role of sustainable approaches in agriculture owing to the growth promotion and phytopathogen biocontrol proficiencies of microorganisms associated with plants. Research into these microorganisms is especially vital, as they exhibit numerous compensatory advantages (naturally occurring agents) compared to currently implemented chemical approaches. However, with advances in endophytic research came increased discovery of the challenges and limitations facing research approaches associated with endophytic research. These challenges were mainly linked to the complexity of microbiomes associated with plants, the antagonistic effects that control or suppress pathogens that are affected by (1) interaction between microbial biocontrol isolates and host plants, (2) pathogenic infection, and (3) environmental factors. Thus, recent research has been focused on specialized techniques in order to understand the mechanisms and bioactive compounds associated with endophytes and mode of action of microbial biocontrol in vivo (host). Hence the current review highlighted the implementation of the CRISPR/Cas9 systems, nanotechnology and omics approaches and how these advancements in techniques have bridged the gap regarding the challenges. Through the use of these technologies' important genes, proteins and secondary metabolite compounds were identified to understand plant–microbe interactions, but also highlights candidates which could be exploited for the improvement of biocontrol agents for the enhancement of phytopathogen tolerance. To fully optimize endophytic technologies in order to implement them in the agricultural industry, we need to understand microbial interactions with plants in order to exploit them for improved plant pathogen management.

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