

# Review Article

## MODE OF ATTACK OF MICROBIOLOGICAL CONTROL AGENTS AGAINST PLANT PATHOGENS FOR SUSTAINABLE AGRICULTURE AND FOOD SECURITY

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### ABSTRACT

Plant diseases accounts for huge losses in agriculture. To ensure food security and sustainability, an agricultural yield must be improved to meet the growing world population. Due to growing awareness of the effect of pesticides and herbicides on human health and the environment, an alternative safe method of controlling phytopathogens has become a subject of intense research. Biological control of plant diseases is the use of living organisms to suppress or inhibit plant pathogens. Microbiological control agents (MBCAs) employ microorganisms to protect crops from destruction by phytopathogens through different modes of action. They may act by direct interaction with the pathogens through hyperparasitism. Indirect antagonistic interaction with the pathogen through induction of host resistance and priming is another mode of attack by MBCAs. Competition for nutrients and space is another important indirect mode of attack by which MBCAs suppress the growth of pathogens through nutrient deprivation. The MBCAs can also interact with the plant through secretion of antibiotics or antimicrobial secondary metabolites with inhibitory effect against the pathogens. A clear understanding of the mode of action of MBCAs is vital to achieving a successful biocontrol operation as well as improving the biocontrol process that is devoid of risks to humans and the environment. Such improvement could be achieved through the use of microbial consortia to enhance the stability and efficiency of the biocontrol process. Further studies on aspects of mass production and formulation to produce more effective, stable, safer and cost effective MBCAs are needed.

*Keywords: Microbiological control agents, Phytopathogens, Antibiosis, Microbial consortia, Direct and Indirect antagonism*

### 1. INTRODUCTION

Phytopathogens could be referred to any of the organisms such as bacteria, fungi, viruses, nematodes and protists that cause plant diseases. They are of interest for a number of reasons, ranging from the concerns about fragile ecosystems to the desire to protect the food supply. Plant pathogens are responsible for major crop losses, thus reducing the farmer's or grower's ability to produce crops and can infect almost all types of plants. Though many several things can be linked to a decrease in crop productivity, the losses caused by pests and pathogens **play** a crucial role in the damages globally [1]. Plants are

sometimes attacked by different groups of pathogens individually or sometimes by more than one pathogen producing complex and more severe disease. Pathogens attack plants in several ways which include; colonization of the tissue in the plant and settling on the surface of the plant. Some pathogens may go for specific areas such as the roots, stems and leaves. Pathogens commonly cause problems like tissue death, browning, a decrease in fruiting, problems with setting flowers and so forth. In extreme cases, they can **invade and** kill the host plant.

Effective control of plant diseases is of paramount important to ensure quantitative and qualitative food, feed and **fiber** production around the world [2]. With a view to increase the global food production by at least 70% in 2050, there is growing concern for global food security, which has become one of the major international issues [3, 4]. It is estimated that the demand for food and global food security will continue to increase for the coming 40 years with the glowing human population and consumption [1]. An efficient management and control of plant pathogens is necessary due to the increasing world population. Crop protection through **the** application of biocontrol microorganisms plays a significant role in defending crop productivity against invasion by pathogens [5].

The control of phytopathogens by the use of chemical pesticides and by cultural practices (in some cases) is a common practice [6]. However, the widespread application of agrochemicals has become an issue of public concern and surveillance, due to the inherent hazardous effects on the environment, their undesirable effects on non-target organisms and likely carcinogenicity of some of the chemicals used in agriculture [7, 8]. Moreover, evolution of resistant species of pathogens, continuous removal and phasing out of some available pesticides and the unwillingness of a number of chemical companies to develop and assess new agro chemicals due to the problems with **the** registration process and cost, are other problems militating the use of chemical pesticides [7, 6].

Biocontrol of plant pathogens is an eco-friendly and viable alternative mechanism to control plant diseases. It is the suppression, inhibition or elimination of the growth or populations of plant pathogens by living organisms [9]. Various biocontrol programs make use of biological agents viz: preying insects and mites, insects that **feed** on other insects or nematodes, set as **a** target against insects and mite pests [10]. However, various microbial agents (bacteria, fungi and viruses) have been advanced and used in arthropod **biocontrol** strategies. Microbial **biocontrol** agents (MBCAs) protect crops from damage by pathogens through different modes of attack [11]. Direct interaction with the pathogen through hyperparasitism or predation. Indirect mode of attack involves induction of host resistance against infection by a pathogen in plant tissues [12, 13] and competition for nutrients and space [14]. Another important mode of attack of MBCAs is the secretion of antibiotics or antimicrobial secondary metabolites with inhibitory effects against plant pathogens [15]. It is essential to understand the mode of action of MBCAs to be able to identify probable risks for humans or the environment and the possibility for resistance development against the MBCA [11]. Understanding the mechanisms of action of MBCAs is also vital in order to achieve optimum disease control and ensure food safety and sustenance.

The use of microbiological control agent in agriculture is an effective, eco-friendly and sustainable means of management of plant diseases. Thus has naturally assumed greater influence in agriculture and it is attainable through the use of microorganisms/microbial products or their genes that can keep the pathogen load under control [16]. This article thus reviews the mechanisms by which microbiological control agents are employed for the management of plant pathogens for sustainable food production and safety. Emphasis was also made on the use of microbial consortia for enhanced biocontrol process.

## 2. PLANT PATHOGENS

Bacteria, fungi, viruses, nematodes, viroids, phytoplasmas and parasitic higher plants are all plant pathogens. These pathogens cause diseases in plants using different modes of nutrient uptake [17], which have immediate result on how the plant behave in response. Understanding the pathogen behavior is of paramount important in order to assist the plants in defending themselves [1]. Plant pathogens are often classified according to their lifestyle and mode of infection into biotrophs, necrotrophs and the more recently hemibiotrophs [18].

### 2.1 Biotrophs

Biotrophic pathogens obtain nutrients and energy from living cells by maintaining host viability. They are found on or in living plants and can have very complex nutrient requirements and do not easily kill the host plant. Examples of biotrophic plant pathogens include but not limited to; *Cladosporium fulvum*; the causative agent of tomato leaf mould, *Puccinia graminis*; the causative agent of black stem rust of cereals, *Phytophthora infestans*; the causative agent of potato late blight and *Ustilago maydis*; which causes maize smut. The biotrophs and their host plants have highly specialized relationship structurally and biochemically [19]. Biotrophic fungi penetrate the host cell wall and colonize the intracellular space using feeding structures such as haustoria, to absorb nutrients and suppress host defenses without disrupting the plasma membrane [20, 21]. However, the pathogen is unable to initiate a parasitic relationship if the host cells die in the process of invasion by the pathogen.

Penetration resistance (PR) and programmed cell death (PCD)-mediated resistance are two main defense strategies that plants use to restrict biotrophic pathogen invasion and growth. In penetration resistance, plants strengthen cell walls and membranes to halt spore germination and prevent the formation of the haustorium. The PCD-mediated response is applied inside the penetrated epidermal cell, which terminates nutrient supply to the pathogen to halt further development by induction of invaded program cell death [19]. Another defense mechanisms include; reactive oxygen species (ROS) production and systemic acquired resistance (SAR). The activation of these defense responses are regulated by the salicylic acid (SA)-dependent pathway.

### 2.2 Necrotrophic pathogens

Necrotrophs derive nutrients from dead cells. They invade and kill plant tissue rapidly and then live saprotrophically on the dead remains. Examples of necrotrophic plant pathogens include but not limited to; *Pythium ultimum*; the causative agent of damping off in seedlings, *Fusarium oxysporium* that causes vascular wilt, *Cochliobolus heterostrophus*; the causative agent of leaf blight, *Botrytis cinerea*; implicated in grey mould and *Sclerotinia sclerotiorum*; the causal agent of soft rot. It is important that plants recognize necrotrophs early enough to counteract the initial oxidative burst and prevent hypersensitive response (HR) and PCD [1]. Necrotrophic pathogens benefit from death of host cells, so they are not limited by this defense, but by responses activated by jasmonic acid (JA) and ethylene signaling (ET), both of which are involved in the defense against necrotrophic pathogens.

### 2.3 Hemibiotrophic pathogens

Hemibiotrophic pathogens have an initial period of biotrophy with the host, followed by necrotrophy [22]. For example, *Phytophthora infestans* that causes potato late blight exhibit characteristics of both biotrophs and necrotrophs. Other important hemibiotrophs include; *Cladosporium fulvum* (also called biotroph) that causes tomato leaf mould,

*Magnaporthe grisea* (also called necrotroph) that causes rice blast and *Mycosphaerella graminicola*, causing septoria leaf blight.

### 3. MICROBIAL INTERACTIONS THAT AID IN BIOCONTROL

For better understanding of the mode of action of biocontrol agents, it is essential to highlight the various ways microorganisms interact. These interactions are termed symbiosis regardless of whether the interactions are positive or negative. Positive interactions include mutualism, proto-cooperation (synergism) and commensalism while negative interactions include predation, parasitism, amensalism and competition. The advantages of using these interactions for biotechnological applications are many-fold. The use of naturally existing plant-microbe symbiosis for plant growth and biocontrol reduces synthetic fertilizer and pesticide treatments, leading to cost effectiveness and less impact by nutrients [23] and pesticides [24] on surrounding fauna and flora. In addition, the production of useful compounds with pharmaceutical and industrial relevance using plant-microbe symbiosis is energy efficient [25, 26] and eliminates the need to add expensive precursors and catalysts. However, for interaction to take place, microorganisms must have some form of direct or indirect contact with the host plant. Important plant-microbe interactions that assist in biocontrol are summarized below.

Mutualism is an obligatory relationship in which both partners (microorganisms and plants) are metabolically dependent on each other. The mutualistic interaction between plants and beneficial microorganisms often lead to either an improvement in plant nutrition and/or promotion of the plants ability to withstand biotic and abiotic stress. This presents a competitive advantage leading to enhanced growth and plant proliferation [27]. A diversity of endophytic bacteria and free living rhizobacteria on the root surface and rhizosphere utilize materials released as exudates from the plant roots and in turn secrete metabolite substances to the soil that assist in biological control of plant diseases caused by bacteria and fungi [28, 29]. The interaction between plants and biocontrol microbes also indirectly enhance plant growth by suppressing pathogen growth and activity [30, 31]. The mutualistic plant-microbe interaction can be beneficial in directly providing nutrients to the plant or increasing the bioavailability of compounds such as iron and phosphate [32]. Free living plant growth-promoting bacteria also produce compounds that directly affect plant metabolism or modulate phytohormone production [32] and suppress harmful pathogens. Furthermore, the mutualistic interaction between *Rhizobium* bacteria and leguminous plants can also contribute to biological control by providing plant with improved nutrition or by stimulating host defense mechanism [33, 6].

Proto-cooperation or synergism is a form of mutualism but the association is not obligatory. Thus the organisms involved are not metabolically dependent on each other. Pal and McSpadden [2] opined that many of the microorganisms that have been isolated and categorized as biocontrol agents (BCAs) of plant pathogens can be considered facultative mutualists involved in proto-cooperation. This is because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions.

Competition arises when different organisms **try** to acquire the same resource, whether physical location or a particular limiting nutrient. Competition within and between species can lead to a decreased growth, activity and/or productivity of the interacting organisms [7]. Biocontrol can occur when non-pathogens compete with pathogens for nutrients and sites in host plant.

Parasitism is a symbiotic relationship in which one of the pair benefits and the other, usually the host is harmed. If the host is also a parasite, for example, a plant pathogen, the interaction is regarded as hyperparasitism [11]. *Rhizoctoniasolani* is a parasite of *Mucor* and *Pythium*, which is important in biocontrol processes. The activities of various hyperparasites, for example, those agents that parasitize plant pathogens, can result in biocontrol [34].

Predation is an association between the predator and the prey, whereby the predator engulfs or attacks the prey, which normally results in the death of the prey. Several of the best examples of predatory bacteria include *Bdellovibrio*, *Vampirococcus* and *Daptobacter*, each of them having a unique mode of attack against susceptible bacteria [35]. Thus, they therefore assist in the biocontrol of plant pathogens to ensure food security and sustenance.

Moreover, amensalism or antagonism is a term used to describe the negative effect one organism has on another. It is a unidirectional relationship based on the release of specific compound by one organism which has a negative effect on another organism. An excellent example is the production of antibiotics or other metabolites by microorganisms which kill or inhibit the growth of susceptible bacteria, thus assisting in biocontrol.

#### **4. MODE OF ATTACK OF MICROBIAL CONTROL AGENTS**

The microorganisms employed in biocontrol of plant pathogens have different modes of action [36, 37]. These mechanisms of action were categorized into; direct antagonism, indirect antagonism and mixed path antagonism by several authors [2, 36, 38]. Direct antagonism may include hyperparasitism/predation [39] while indirect interaction includes induction of host resistance or prime enhanced resistance [12, 13] and competition for nutrients and space [14]. Mixed path interaction (antibiosis) involve metabolite secretion, production of lytic enzymes and other volatile antagonistic compounds [40]. The various mode of attack of MBCAs against plant pathogens are discussed below.

##### **4.1 Direct antagonism**

###### **4.1.1 Hyperparasitism/predation**

Hyperparasitism is the ability of biocontrol microorganisms to parasitize other microorganisms (pathogens) and utilize them as a source of food and energy. The term hyperparasitism may also be referred to as mycoparasitism when fungi are used to parasitize plant pathogens or other fungal plant pathogens. Mycoparasitism involves a sequence of events such as initial contact and recognition of the host pathogen, attack and subsequent penetration and killing of the host pathogen [41]. Many chemical compounds such as lectins, implicated during the initial contact and recognition of the pathogen and cell wall-degrading enzymes (CWDEs) are involved in these processes. Other chemical compounds include  $\beta$ -1,3-glucases, chitinases, proteinases and lipases which are involved in the attack and penetration of the host pathogen [42]. The study on biological control of *Botrytis cinerea* (the powdery mildew pathogen) by yeast antagonist *Pichiaguilliermondii* showed that lectin-like interaction resulted in firm attachment of antagonist's cell to *B. cinerea* [43]. Cell wall lysis of the fungi pathogen also occurred as a result of the action of extracellular  $\beta$ -1,3-glucanase enzyme released by the antagonistic biocontrol yeast *P. guilliermondii*.

The mode of action of hyperparasites in biocontrol of phytopathogens is most widely studied and understood in *Trichoderma* species. Morphological changes like coiling and formation of appressorium-like structures used to penetrate the host pathogen is observed in mycoparasitism. Using the cell-wall carbohydrates, *Trichoderma* adhere to the pathogen

lectins. Once attached, it winds the hyphae around the pathogen and thus forms the bulb-like appressoria. This is then followed by the production of CWDEs and peptaibols [44] that aid in both the entry of *Trichoderma* hyphae inside the lumen of the fungal pathogen and ingestion of the cell wall contents. This attack by MBCAs such as *Trichoderma* results in further increase in permeability and breakdown of the host cell walls and subsequently death of the pathogen. A synergistic transcription of different genes associated with cell wall degradation was also reported in *Trichodermaatroviride* in interplay with *Phytophthora capsici* and *B.cinerea* [45].

## 4.2 Indirect antagonism

### 4.2.1 Competition for nutrient and space

Competition for limiting nutrients and space represents an important mode of action by which MBCAs suppress the growth of pathogens. Nutrient deprivation frequently results in the death of microorganisms, thus competition for available resources will eventually result in biological control of phytopathogens [41]. It was reported that non-pathogenic symbiotic plant microbes shield the plant from effective colonization by exhausting the limiting nutrients so that none will be accessible for the pathogen proliferation [6]. For instance, the mechanism by which *Enterobacter cloacae* employs in suppressing the growth of *Pythium ultimum* has been established via effective nutrient uptake in the spermosphere [46, 47].

Highly antagonistic microorganisms are promising candidates as bio-control agents due to their ability to exploit competition for nutrients and space as a mode of action. In order to adopt this mechanism in disease control, a thorough awareness of the spread and control of plant diseases is crucial. This will assist in identifying the areas where nutrient limitations and space will alter pathogen growth [11]. Such situations may include; availability of nutrients in wounds of fruits that stimulate pathogen infection of the fruit, *Botrytis cinerea* infection of flowers may be triggered by the existence of worn herbaceous fiber and dead or decaying tissues may serve as the main habitat for pathogens, thus their presence will stimulate pathogen invasion [48, 14]. Antagonistic MBCAs must be able to out-compete pathogens in such areas like plant surfaces and in plant residues, to survive and consume nutrients like sugars, pollen and plant exudates. Thus, pathogens will be unable to grow and infect the host plant. Fast growing yeasts and molds employ this effective mechanism to shield wounds in fruits from pathogen attack [14]. Di Francesco *et al.* [49] studied the wound protection of peaches by *Aureobasidium pullulans* against the pathogen *Monilinia laxa* by incubating *A. pullulans* and *M. laxa* in in vitro assay in peach juice, with asparagine as nitrogen source. Result of the experiment showed that complete metabolism of the nitrogen source, asparagines, consequently inhibited the growth of *M. laxa*. Thus, contention for sugar in carbohydrate rich wound environment in conjunction with competition for limited nitrogen sources is essential in antagonistic interactions of MBCAs with pathogens [14].

Apart from carbohydrate and nitrogen sources, iron is one of the most important micro-nutrient and its limited availability for microbial growth is due to poor solubility of Fe<sup>3+</sup> ions, thus competition for iron has been identified as a vital method of attack against pathogens [14]. Numerous microorganisms possess the ability to secrete medium-sized molecules known as siderophores with high specificity for binding or chelating iron. This gives them a competitive advantage over other microorganisms with less ability to produce siderophores. Raaijmakers *et al.* [50] studied this mode of action for *Pseudomonas* spp and revealed that pathogen growth in rhizosphere was reduced via siderophore mediated iron competition. Certain fungal antagonists also employ this mode of action against pathogens. For instance, a few *Trichoderma* species produce siderophores that chelate iron and thereby inhibit the

growth of other plant pathogenic fungi such as *Pythium* and *Fusarium* [51]. *T. harzianum*T35 suppress the growth of *Fusariumoxysporium* through competition for both nutrients and rhizosphere colonization, with biocontrol becoming more effective with decrease in nutrient concentration [52]. A siderophore known as fusigen produced by *Aureobasidiumpullulans* was reported to have inhibitory effect against bacteria pathogens [53]. The characteristic red colour of the yeast *Metschnikowapulcherrima* colonies was as a result of the formation of pulcherriminic acid that binds iron [54, 55] and iron deprivation of the fungal pathogens viz; *B. cinerea*, *A. alternata* and *P. expansum* has been suggested as one of the several mechanisms by which this yeast antagonizes plant pathogenic fungi [54, 56, 57].

Besides competition for nutrients, biofilm formation may be highly regarded as a successful mode of competition for space. Biofilm formation in the wounds of fruits is well known in *Pichiafermentans* [55]. Biofilm formation of *P. fermentans* in apple wounds protects it against postharvest diseases, but on Peaches, *P. fermentans* changes from yeast-like to hyphal growth pattern, thus leading to the spoilage of the fruits in the absence of a plant pathogen [58, 59, 60]. Based on this “Jekyll & Hyde” pathogenic behaviour of *P. fermentans* on peach fruit, the ability to differentiate hyphae and pseudohyphae particular conditions of growth has been recommended as a possible biohazard factor for biological control yeasts [61]. Apart from *P. fermentans*, several reviews have reported the involvement of *A. pullulans*, *Kloeckeraapiculata*, *Wickerhamomycesanomalus*, *Pichiakudriavzevii*, *M. pulcherrima* and *Saccharomycescerevisiae*, in the protective and biocontrol exercise, through biofilm formation [62, 63, 64, 65, 66]. It was also reported that biofilm cells of *S. cerevisiae* were far more efficient in colonizing the interior surface of apple wounds than the planktonic cells, and by that were able to control *P. expansum*, the causative agent of blue mold in apple [66, 67].

Apart from competition for nutrients and space, MBCAs may modify the growth conditions for the pathogens. For instance, the use of *Bacillusbrevis* as BCA lead to the fast drying of leaf surfaces, thus lowering the growth of *B. cinerea* by 68%, which was similar to the application of a standard fungicide in Chinese Cabbage [68]. Moreover, antagonistic fungi may modify the pH of the growth substrate during competitive interaction with bacteria pathogens without any direct interaction between the antagonist and the pathogen. For instance, *A. pullulans* antagonistic to *Erwiniaamylovora*, the causative agent of fire blight in pome fruit, inhibits the growth of the pathogen through lowering the pH of the growth substrate to four (4) [69], which is unfavorable for the growth of the pathogen.

#### **4.2.2 Induction of host resistance and priming**

Plants have devised a way of responding to external stimuli produced by BCAs which can trigger host defense mechanisms against pathogen infection. Induction of host resistance can be localized or systemic depending on the origin, type and magnitude of the stimulant [70, 71]. The various channels of induced resistance excited by biocontrol agents and other non-pathogenic microorganisms have been characterized by plant pathologists. The first channel known as systemic acquired resistance (SAR), mediated by salicylic acid (SA) leads to the expression of pathogenesis related (PR) proteins and a variety of enzymes [71]. The second type known as induced systemic resistance (ISR) is mediated by jasmonic acid (JA) and/or ethylene, produced directly after treatment with some non-pathogenic rhizobacteria [36, 70]. This type of resistance relies on improved protective capability of the entire plant to different pathogens [13]. Due to the fact that the SA and JA reliant defense pathways can sometimes be antagonistic, some pathogens utilize that opportunity to overwhelm the SAR [6]. This is the case in pathogenic *Pseudomonas syringae*, which produces a compound; coronatine, similar to JA which it employs to prevail over the SA-mediated pathway [71]. In this way, the strength and period of host defense initiation may change over time.

However, some biocontrol active species of root-colonizing microbes such as *Pseudomonas* sp. and *Trichoderma* sp. are well recognized for their strong induction and/or ability to provoke host defense responses [72, 73]. In addition, it has been shown that the introduction of plant growth promoting rhizosphere (PGPR) was effective in combating numerous disease causing fungal pathogens. However, a variety of synthetic elicitors of SAR and ISR including SA, lipopolysaccharides, siderophore and 2,3-butanediol can be produced by the PGPR species upon introduction [74].

Apart from induction, priming of plants by stimulus prepares the plant for enhanced resistance not only in the presence of the stimuli but also for a long lasting and stronger defense strategy [75]. Multi-generational priming has been reported elsewhere [13]. Induction of host responses may include: formulation of reactive oxygen species (ROS), phytoalexins, phenolic compounds, pathogenesis related proteins or the formulation of physical barriers such as cell wall and cuticles modifications by the induced plant [76]. This type of metabolism is energy dependent and longer periods of stimuli will lead to energy cost for the plant to actively sustain induced defense mechanisms [11]. Contrary to induced resistance, priming gives plants the opportunity to respond to stimulus later, in a speedy and healthy manner with low energy costs [75].

### **4.3 Mixed path antagonism (antibiosis)**

Antibiosis is synonymous with ammensalism type of microbial interaction whereby a particular microorganism produces products such as antibiotics or other metabolites that are deleterious to other interacting microorganisms. Several microorganisms produce these metabolites and discharge to their surroundings in minute amounts. Some important antimicrobial metabolites are discussed.

#### **4.3.1 Antibiotics**

Vast amounts of antibiotics produced by actinomycetes, bacteria and fungi were reported [77, 11]; while a greater number of antibiotics that are produced in situ in the environment, have not been characterized [15]. It has been shown that some antibiotics produced by microorganisms are very effective in suppressing the growth of the target pathogen in vitro and/or in situ [78, 79, 6]. However, for greater effectiveness, the antibiotics must be produced in sufficient dose near the pathogen.

Biocontrol bacteria such as *Bacillus*, *Agrobacterium*, *Pseudomonas*, *Pantoea*, *Serratia*, *Streptomyces*, *Stenotrophomonas* and several other species, has the ability to produce a wide range of antimicrobial metabolites with broad spectrum activity [11]. Research has revealed that the production of these metabolites by biocontrol agents can sometimes lead to suppression of plant pathogens [6]. *Pseudomonas* species are known to inhibit the growth of fungal pathogens through the production of antimicrobial metabolites such as 2,4-diacetyl phloroglucinol (DAPG), pyrrolnitrin, phenazine, pyocyanin, hydrogen cyanide, kanosamine, among others [16]. The production of kanosamine, iturin A, bacillomycin, zwittermycin A, plipastarins A, among others, by biocontrol *Bacillus* species have been investigated [80]. However, fungal antagonist such as *Trichoderma* and *Clonostachys* has the potential to produce antimicrobial metabolites such as 6-n-pentyl-2H-pyran-2-one (6-PAP), viridian, gliotoxin, gliovirin, among others [39]. Often, antimicrobial metabolites are regarded as the most effective weapon against pathogens, giving antibiotics producing microorganisms competitive advantage in resource-scarce environments [15].

Genomic analysis of microorganisms showed large numbers of hidden antibiotic gene clusters encoding yet unknown antibiotics [11]. Identification of these antimicrobial metabolites implicated in biocontrol enables copying of the genes that encode their biosynthesis and using these genes to enhance a particular biocontrol agent. With regard to antibiosis, bio-control could be enhanced either by supplying it with genes that encrypt the biosynthesis of antibiotics usually produced by other bacteria and fungi or through improving the **number** of antimicrobial metabolites that the microorganism produces via genetic recombination [16].

#### 4.3.2 Lytic enzymes

Lytic enzymes are microbial metabolites that can hydrolyze polymeric compounds such as cellulose, proteins, hemicellulose, chitin and DNA [81, 82, 83]. Release of some of these metabolites by MBCAs can at times directly lead to the elimination of plant pathogens. Many fungal and bacterial antagonists produce extracellular cell wall degrading enzymes viz; chitinases, cellulases,  $\beta$ -1,3 glucanases and proteases, which play a crucial role in the biocontrol of plant pathogenic fungi [84]. Antagonistic *Trichoderma* species have been distinguished for their potential to hydrolyze the cell wall of plant pathogens through secretion of chitinases, glucanases, and proteases [85]. It was reported that N- $\beta$ -acetylglucosaminidase and  $\beta$ -1,3-glucanase enzymes plays important role in biocontrol of *Sclerotinia sclerotiorum* by *Trichoderma* species [86]. It was also discovered that *Serratiamarcescens* has the potential to inhibit the growth of *Botrytis* spp, *R. solani* and *F. oxysporium* through secretion of chitinases [87]. Other bacteria BCAs implicated in the control of plant pathogenic fungi through production of multiple chitinases include: *Pseudomonas*, *Streptomyces*, *Bacillus* and *Aeromonas* [88, 89]. Considering the fact that a lot of the lytic enzymes secreted by MBCAs are encoded by a single gene, the biocontrol action of these agents can be improved by simply isolating a number of these genes and transferring them to other biocontrol agents [16].

#### 4.3.3 Volatile organic compounds (VOC)

Volatile organic compounds produced by MBCAs can be divided into different categories viz; alcohols, aldehydes, esters, lactones and ketones [90]. Several of these VOCs produced by microbial antagonists have proven effective in the control of postharvest fungal pathogens [55]. For instance, Di Francesco *etal.* [91] reported that VOC produced by *Aureobacillus pullulans* has proven effective in controlling the proliferation and infection by *B. cinerea*, *Colletotrichum acutatum*, *Penicillium expansum*, *Penicillium italicum*, and *Penicillium digitatum*, both in the lab and field experiment. In a similar way, VOC produced by *Candida sakei* minimized the occurrence of apple rot disease implicated by *P. expansum* and *B. cinerea* [92]. Parafati *etal.* [93] attributed the biocontrol efficiency of varying food yeasts (such as *Wickerhamomyces anomalus*, *S. cerevisiae*, *Metschnikowiapulcherrima* and *A. pullulans*) against *B. cinerea* in vitro and in planta, to the release of VOC. Huang *etal.* [94] attributed the antagonistic activity of *Sporidiobolus pararoseus* on mycelial growth and spore germination of *B. cinerea*, to the production of a VOC, 2-ethyl-1-hexanol. VOCs produced by *Pichia kluyveri*, *Pichia anomala* and *Hanseniaspora uvarum*, suppressed the growth of *Aspergillus ochraceus* and release of ochratoxin A during coffee preparation and storage [95]. VOC like ammonia released as exudate by *Enterobacter cloacae* was reported to control the damping-off of cotton seedling caused by *Pythium multivium* [96, 6]. Despite several reports on the use of VOCs as biocontrol agents of plant pathogens, the efficient exploitation of microbial VOCs remains a problem, thus more research needs to be carried out in this regard [97].

## 5. USE OF MICROBIAL CONSORTIA FOR IMPROVED BIOCONTROL ACTIVITY

Combination of different biocontrol agents with high levels of disease suppression has been examined for improved biocontrol of various plant pathogens [98]. It was reported that different species of *Pseudomonas* and in combination with other bacteria or fungi provided more effective disease control than the use of a single *Pseudomonas* specie [99]. It was also noted that a mixture of different *Pseudomonas* species was more effective in disease suppression than the use of a single specie [100, 101].

The application of mixture of BCAs with different disease control mechanisms that are complementary to each other is another approach to obtaining a successful microbial biocontrol consortium [16]. In a study by Wu *et al.* [102], a mixture of *Trichodermaasperellum* GDFS1009 and *Bacillusamyloliquefaciens* ACCC1111060 was more effective in the control of *B. cinerea*, “the causative agent of gray mold disease” than the use of a single strain. In another study, the combination of *Trichoderma virens* G1006 with *Bacillus velezensis* Bs006, proved more effective in controlling *Fusarium* wilt of cape gooseberry [103]. Moreover, bacterial consortium consisting of *Chitionophaga* sp. 94 and *Flavobacterium* sp. 98 provided better protection against root rot of sugar beets by *Rhizoctoniasolani* than the individual species [104]. Thus a consortium of MBCAs are capable of providing more effective control of phytopathogens than single strains [105].

## 6. CONCLUSION

A clear understanding of the mode of action of microbial biocontrol agents is vital to achieving a successful biocontrol operation as well as improving the biocontrol process that is devoid of risks to humans and the environment. Various biocontrol agent (BCA) consortia consisting of two or more microbial strains could be assembled to improve the stability and efficiency of disease suppression. As a result of the challenge the use of chemical pesticides poses on human health and the environment, the search for alternative and safe method of biocontrol of plant pathogens becomes imperative. Moreover, there is increasing interest and demand for biological based pest management practices for its safety to humans and the environment. Thus, the application of microbial biocontrol agents (MBCAs) in the control of plant pathogens is very essential for sustainable agriculture and food security. Microbiological control of plant pathogens is however, one of the most important aspects of Integrated Pest Management (IPM) strategies for sustainable agriculture and food security. Further studies are needed on practical aspects of mass production and formulation to make new biocontrol products more stable, effective, safer and more cost-effective.

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