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Biology and Control of *Rosellinia necatrix* causing White Root Rot Disease: A Review

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Common in all five continents temperate, subtropical and tropical regions, *Rosellinia* species are recorded all over the world including the herbaceous plants, many economical as well as ornamental and fruit trees. This pathogenic fungus has very wide host range and characteristic symptoms of this disease are rotting of roots, yellowing and falling of leaves, wilting and finally death of the tree. It is the severity of the disease caused by *Rosellinia necatrix* which makes it a most detrimental fungal pathogen of economical and fruit tree species. This pathogen is capable of surviving and remaining active for many years on the residues of susceptible crops like olive, grapevine, almond etc. It has been proved difficult to control root rot infecting fungi particularly the white root rot causing fungi *R. necatrix* in temperate and subtropical region. Although there are number of techniques available to control *Rosellinia* infection includes soil fumigation, soil solarisation to induce disease suppressive activity of the soil, and biological control using the antagonistic fungus/bacteria. A relatively new approach has been proposed to control fungal diseases, which is based on the fact that mycoviruses have the capacity of induce hypovirulence in plant pathogenic fungi. *R. necatrix* was also reported to be a tough pathogen to transform via several known and well established methods of fungal transformation, which is required for better understanding the molecular basis of pathogenesis of this devastating fungus. The present review focuses on the complete and up-to-date information on biology and control strategies of *R. necatrix*, a major concern for the farmers.

Key words: *Rosellinia necatrix*, Apple, White root rot, mycovirus, pathogenesis.

A well known serious pathogen for many plant species *Rosellinia necatrix* is reported from different countries of the world[1]. It has been reported that this pathogen can infect over 170 plant species from 63 genera and 30 families. A range of plant species including herbaceous plants were also infected by *R. necatrix* and could be seen as a potential source of inoculums[2]. This pathogen has been recognised to cause losses in many economical as well as ornamental and fruit trees, including apple trees in Japan and India[3]. *R. necatrix* was also reported as one of the most dangerous agents of root rot in poplars[4]. Likewise apple and wild cherry are badly affected in France; along with Jasmine which is important in perfume industry, together with carnation, naricissus and peony[5].

Vulnerability of the host plants by this fungus is very wide, particularly with regards to those temperate plants such as apple, plum, pear etc. which are now cultivated worldwide, indicating that there is cause for future concern regarding possible infection and economic losses. Early recognition of *Rosellinia* disease is difficult since the fungus is based on a combination of disease

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symptoms like the presence of the *Dermatophora* Hartig anamorphic state and the white fan-like mycelia growth immediately beneath the epidermis of the infected roots. However, these characteristics are sufficiently stable and reliable to enable confident identification to be made[6].

White root rot disease whose causal agent is a filamentous ascomycete *Rosellinia necatrix* Prillieux leads to host plant infection, which quickly wither and die[7]. This pathogen causes destructive damage to numerous woody and herbaceous plants, especially to fruit trees, such as avocado (*Persea americana*) and mango (*Mangifera indica* L.), which are particularly susceptible, throughout the world[8, 9, 10, 11]. The infected tree showed rotting of roots and are characterized by yellowing of the leaves that eventually wilt and ultimately result in the death of the tree within a few weeks after the appearance of the first foliar symptoms[10]. Moreover, the pathogen is capable of surviving and remaining active for many years on the residues of susceptible crops such as olive (*Olea europaea* L.), grapevine (*Vitis vinifera* L.) and almond (*Prunus amygdalus* Batsch.)[12]. Although, it is commercially important; still little is known about the molecular and cell biology of this fungus. However, comparison of the structure of cytochrome C (Cyt C) gene is useful for the estimation of fungal phylogeny and taxonomy, including horizontal transfer of mobile genetic elements[13]. Trails have been made on the biological control of white root rot, but only few experiments have been successful under field conditions. Biological control with double-stranded RNAs (dsRNAs), which are fungal viruses, seems promising. Since, these dsRNAs reduce the virulence of the fungal pathogen, a phenomenon referred to as hypovirulence[14].

***Rosellinia necatrix* a white root rot causing fungus**

Rosellinia species are recorded from temperate, subtropical and tropical regions of the world[15]. Some live endophytically, mostly as saprobes, only a few species are known to occur as root pathogens and most well known among them are *R. necatrix* Prill. and *R. desmazieresii* (Berk. Br.) Sacc. (*R. quercina* Hart.), mostly known from temperate zones, and *R. bunodes* (Berk. Br.) Sacc., *R. pepo* Pat. and *R. arcuata* Petch, known only from the tropics.

Although root disease caused by *Rosellinia* spp. occur on a wide variety of commercially important crops, trees and ornamentals but few *Rosellinia* species appear to be of real economic importance. However, data on losses caused by *Rosellinia* species are greatly lacking. In India, during the year 1963[16], estimated the losses caused by *R. necatrix* in apple (*Malus* sp.) in the state of Himachal Pradesh, India, to be at least $272,000 and this conservative estimate converts to about $1.6 million dollars in 2002[17] and is much more at the present time.

Biology of *Rosellinia necatrix*

Taxonomy

de Notaris, in 1844[18] named the genus *Rosellinia*, but because the stromatic character of its fructification, its systematic position within the pyrenomycetes has not always been clear. This genus belongs to the family *Xylariaceae*, which includes more than one hundred species, among them economically important root rot pathogens such as *Rosellinia arcuata* Petch, *Rosellinia desmazieresii* (Berk. Br.) Sacc. and *Rosellinia pepo* Pat[15].

It was 1883, when Hartig[19] first made the scientific studies on the fungus; *D. necatrix*; previously known as *Rhizomorpha necatrix*. Later on, on the basis of morphological descriptions of the ascocarps of the fungus *R. aquila*, Berlese[20] included *D. necatrix* in the genus *Rosellinia*, which subsequently got confirmation when perithecia from fruit tree roots infected with *D. necatrix* were obtained and telomorphs were named by Prellieux as *R. necatrix*[21].

The young mycelium of *R. necatrix* is initially white and cottony (Fig. 1A) but with growing age it starts becoming pigmented (Fig. 1B). Mostly, the pigment is located on the cell wall and depends on the metabolic activity. Generally, the mycelium of *R. necatrix* is fast growing and covers the entire culture medium when incubated at 20-24ºC in dark. The characteristic feature of *R. necatrix* hyphae which is generally used to identify the species is the existence of pear shaped or pyriform swellings immediately above septum (Fig. 2)[22, 23].

Ecology

The first and foremost ecological factor required by all *Rosellinia* species to flourish is moisture[15]. Soil rich in organic matter and acidity contributes towards the growth of this root

pathogen[24, 25]. Fungus expands vegetatively through the soil from diseased roots to healthy roots. However, the role of ascospores as propagules remains unclear, presumably due to the scarcity of teleomorph production. Although, ascospores isolates were only confirmed to be pathogenic to apple trees[26, 9, 27].

Host range and symptoms

This pathogenic fungus has very wide host range and is destructive to many fruit trees, including tropical and subtropical species[28]. Premature defoliation is common. According to Anselmi and Giocelli [29] *R. necatrix* spreads readily on loose soil with high sand content. Almost 63 genera including 170 plant species are vulnerable to the attack of *R. necatrix*. Characteristic symptoms of this disease are rotting of roots, yellowing and falling of leaves, wilting and finally death of the tree[23, 10].

Isolation, culturing and storage

Isolation is very easy from the surface of the infected roots which are covered with the strands of white mycelium or from under the bark, where a fine, continuous layer of the mycelium is usually present[30]. It is also possible to isolate *R. necatrix* using the leaf disc or twigs from the soil samples. The leaf disc method can also be used to assess the relative levels of pathogen population[31].

Generally, the use of acidified Potato Dextrose Agar (PDA) is recommended, since the direct transference to PDA will not be effective due to contamination with strong competitors and mycoparasites that inhibit pathogen growth[32, 33].

An isolate of *R. bunodes* proved fairly difficult to store for periods longer than 6 months. However, refrigeration at 4°C on different substrates proved possible for upto two years for *R. necatrix* and *R. pepo*. Though storage in liquid nitrogen is an obvious solution, however, specifically subtropical and tropical isolates may be sensitive to cold storage[34].

Identification of fungal pathogenesis

The gene products necessary for virulence are seen as candidate targets to devise proper control strategy. So, the identification of novel targets involved in various aspects of fungal biology would be a rational approach to increase our antifungal arsenal.

Alternatively, gene products those are essential for fungal growth both *in vivo* and *ex vivo* would be attractive alternative as antifungal targets[35]. In order to produce disease free propagative material, it is necessary to produce methods for the identification and detection of pathogen[36]. A reliable technique for the identification and detection of fungal pathogen is PCR. Internal transcribed spacer (ITS1 and ITS2) regions within ribosomal gene clusters are widely utilised to design species specific PCR primers[37]. However, due to post amplification procedures necessary for amplified fragments, use of conventional PCR for large scale application is limited[38]. When the probe forms a stable hybrid with complementary internal sequence of amplicon it leads to the generation of a specific fluorescence signal in Real-time PCR, which is also widely used in medicine for the diagnosis of viral and bacterial infections [39, 40, 41, 42, 43]. Attempts are also being made to generate amplification systems in which the amplicon detection is based on fluorescence resonance energy transfer (FRET) such as Taq-Man[44] and Molecular Beacons[45]. Satisfactory results have also been reported by Scorpion PCR[46] which uses a uni-molecular approach. In this the probe target binding is kinetically favoured over duplex reannealing which consequently thermodynamically favour over intrastrand secondary structures. These peculiar characteristic features favour over intrastrand secondary structure making Scorpion PCR rapid and sensitive technique for the detection of pathogens[47, 48]. It has been widely used to detect antagonistic[42] or pathogenic fungi[41] and viruses[47].

Currently, there is little information available regarding the mechanism of infection of fruit tree roots by *R. necatrix* that go beyond pathologic-anatomical observations and light microscopy visualisation of infected roots grown under axenic conditions. In this sense, fungal invasion of young mulberry tree roots has been reported to take place by boring and dissolving cork cell and, on rare occasions, by wedging them. Alternatively, invasion of adult roots into the inner tissues appears to occur primarily through the suberized closing layers of the lenticels, generally as hyphal strands[49]. In addition, penetration of *R. necatrix* into apple roots has been reported to occur in various phases, each involving different forms of hyphal aggregates[50]. Nevertheless, a detailed description of the infection process of *R.*

necatrix has not been reported till date for any fruit tree. A valuable tool to study the behavior of microbes in their natural environments, such as in soil, in a living plant, or in an animal host, is the use of reporter auto-fluorescent proteins (AFPs). In contrast to other reporters that depend on cofactors or additional substrates for activity, it is possible to visualise AFP expression *in vivo* within individual cells or throughout the entire organism interacting with their hosts. The vast majority of studies utilising AFP technology in fungi have used modified forms of the green fluorescent protein (GFP)[51], such as synthetic green fluorescent protein (SGFP)[52] and enhanced green fluorescent protein (EGFP)[53], which confer higher levels of fluorescence in filamentous fungi with no obvious effects on fungal growth or pathogenicity.

A number of transformation systems have been developed for filamentous fungi, including plant pathogens. However, to the best of our knowledge, only two studies have reported the transfer of exogenous genes to *R. necatrix*. Kanematsu and co-workers[54] reported the transformation of *R. necatrix* protoplasts with plasmids pSH75[55] and pAN7.1[56]. In addition, *Agrobacterium tumefaciens* - mediated transformation of *R. necatrix* has also been reported[57] but only with limited success.

Valuable knowledge about the molecular basis of the pathogenicity of *R. necatrix* could be gained from genetic studies such as gene insertional mutagenesis. However, understanding the genetic basis of its pathogenicity has been limited by the lack of a suitable transformation system. *Agrobacterium tumefaciens* mediated transformation (ATMT), which has long been a workhorse in plant science, has been exploited for fungal transformation. *A. tumefaciens* has the ability to deliver its T-DNA into chromosomes of the budding yeast, *Saccharomyces cerevisiae* and diverse filamentous fungi. Besides ascomycetes and basidiomycetes, this technique has been successfully applied to transform zygomycetes also. In comparison with restriction enzyme mediated integration (REMI), ATMT does not require protoplasts and allows a broad spectrum of starting material to be transformed. Protoplasts, hyphae, spores and even blocks of mushroom mycelia tissues were transformed through ATMT with a higher efficiency than through REMI. DNA transfer from *A. tumefaciens* has been used for both gene knockout and gene transformation studies in filamentous fungi and is being developed as a system for insertional mutagenesis in filamentous fungi[58].

Nevertheless, although hygromycin B (HygB) resistance was used as the selectable marker, a method that is widely used in fungi and is conferred by the *Escherichia coli* hygromycin B phosphotransferase gene (hph), low transformation efficiency was reported in both of the aforementioned transformation protocols[28].

Double stranded RNA virus and Hypovirulence

In filamentous fungi, the presence of double stranded RNA (dsRNA) elements has been reported. The elements are known to reduce the virulence of phytogenic fungi. Such dsRNA elements in *R. necatrix* have also been reported[59]. It has been hypothesized in 2005 by Ikeda *et al*[14] that the hypovirulent isolates were more likely to persist in soil as saprobes and thus attempts were made to isolate these hypovirulent factors from soil. Belonging to eight different mycelial compatibility groups (MCGs), sixteen isolates were obtained from two active and one abandoned pear orchards in Japan. Out of these eight MCGs two were obtained exclusively from soil. Other than these two isolates, isolates within the same MCG were similar in virulence, competitive saprophytic ability (CSA) and mycelia growth rate whether or not they carried dsRNA. Hypovirulence, weakened CSA and restricted mycelia growth on nutrient-rich media, these were the symptoms which were obtained from the other two isolates obtained from the soil having multiple dsRNA segments. Ikeda *et al*[14] observed that these isolates obtained from soil contained various dsRNAs (44%) including hypovirulence factors, more frequently than the isolates from the diseased roots in the same field (25%). Further, he suggested that the isolation of *R. necatrix* is an effective method to obtain isolates with dsRNAs, including hypovirulence factors.

Characterization of Hypovirulent factors: W8, W370 and W779

Out of 1000 isolates of *R. necatrix* obtained from Japan for the identification of hypovirulence factors many fungal isolates containing diverse types of dsRNA which were assumed to be mycovirus genomes were also

observed. Virus containing isolates of *R. necatrix* W8 and W370, showed irregular colony morphology and low virulence[60, 61].

One of these strains of *R. necatrix* W8 harbours four dsRNAs (L1, L2, M1, and M2, named according to size). Out of these four M dsRNAs have been identified as the genome of partitivirus *Rosellinia necatrix partitivirus 1*-W8 (RnPV1-W8), however the dsRNAs are thought to belong to a distinct virus. The partivirus RnPV1-W8 has isometric particles with a diameter of 25nm comprising two genomic dsRNAs (2,299 and 2,279 bp)[62].

In contrast the isolate of *Rosellinia necatrix* W70 harbored double-shelled, spherical particles 80 nm in diameter comprising equimolar amounts of 12 segmented genomic dsRNAs of 943 to 4,143 bp[62- 63, 64]. Osaki *et al*[62] obtained 12 segments from the hypovirulent strain of *Rosellinia necatrix* W370 and eight full length cDNA sequences. All of them confirmed to be the member of the family *Reoviridae* as all the eight sequences had conserved regions at the 5' and 3' termini. Morphological as well as genomic analysis of this virus particle indicated that the virus was a novel reovirus designated as *Rosellinia necatrix Mycoreovirus 3* or RnMyRV3/ W370 (MyRV3). MyRV3, along with two *Cryphonectria parasitica Mycoreovirus* spp. (MyRV1 and MyRV2), placed in a newly established genus *Mycoreovirus* in the family *Reoviridae*[65]. There is reduced virulence of host fungus *R. necatrix* when get infected with MyRV3 in addition to the altered colony morphology[54]. Likewise MyRV1 and MyRV2 infections also cause hypovirulence in *C. parasititca*[66,67].

The complete nucleotide sequences of W370 dsRNA genome segments 1, 2, 3 & 5 were reported in 2003/04 by Wei and his co-workers[63, 64] The complete nucleotide sequence of the genome segment 1 encoded a putative RNA-dependent RNA polymerase (RDRP). With a long open reading (ORF) and 47% GC content, the nucleotide sequence of the genome segment 1 was 4143 bases long. Designated as VP1, the deduced polypeptide contained 1360 amino acid residues (29 - 4110) with a predicted molecular mass of about 153 kDa. It showed some identity to the members of genera Fijivirus and Cypovirus in the genus Coltivirus, these viral proteins belong to the *Colorado tick fever virus* (CTFV) and *European Eyach virus* (EYAV). With a 3773 bases a single long ORF of segment 2 encoded 1226 amino acid residues with a predicted molecular mass of approximately 138.5 kDa. The nucleotide sequence of the segment 5 was 2089 bases long with a single long ORF, whose deduced polypeptide contained 646 amino acid residues, with predicted molecular mass of about 72 kDa. On the other hand, the nucleotide sequence of the genome segment 3 was 3310 bases long and has a GC content a little more of about 48.6% with a long ORF. Designated as VP3, the deduced polypeptide contained 1086 amino acid residues (bases: 10 - 3270) with a predicted molecular mass of about 121.9 kDa, showing no similarity to the other viral proteins.

Another isolate of *Rosellinia necatrix* W779 was isolated from soil in Ibaraki Japan[68]. Later on in 2009 Chiba and co-workers[69] isolated particles ~50 nm in diameter from strain W779 consisted of two dsRNA elements approximately 9 and 7 kbp termed as dsRNA-1 & 2 and a major protein of 135 kDa encoded by the ORFs on dsRNA-1. It was also observed that the purified virus particles were contagious and conferred hypovirulence on vegetatively incompatible fungal strains. Sharing the conserved termini sequences at both the ends, both possessed extremely long (~1.6 kb) 5' untranslated regions (UTRs) similar to each other, two ORFs, and relatively short 3' UTRs. Though 3'-proximal ORF of dsRNA-1 encoded RdRp showing low levels of sequence identity to those of members of the families *Totiviridae* and *Chrysoviridae*. Phylogenetic analysis revealed that the W779 virus was to be placed into a separate clade from the recognized virus families. These attributes indicated that dsRNA-1 & 2 represent the genome segments of a novel bipartite virus, designated *Rosellinia necatrix megabirnavirus 1* (RnMBV1), with virolocontrol agent potential. The establishment of a new family, *Megabirnaviridae*, to accommodate RnMBV1 as the type species was proposed.

Two novel quadripartite dsRNA virus strains were identified namely *Rosellinia necatrix* quadrivirus 1 strain (W1075)[70] and *Rosellinia necatrix* quadrivirus 1 strain W1118[71]. Both quadriviruses posses quadripartite genome structure with a size range of 4.9 – 3.7 kbp, each possessing a single large ORF, spherical particle

morphology, sequence homogeneity in the extreme terminal ends, 72-82% sequence identity between the corresponding proteins and are only able to cause latent infection to *R. necatrix*.

Fig. 1. Growth of *Rosellinia necatrix* on potato dextrose agar. A). Young white and cottony mycelium of *R. necatrix* after 10 days of growth, B). Old and pigmented mycelium of *R. necatrix* after incubation of 30 days at room temperature

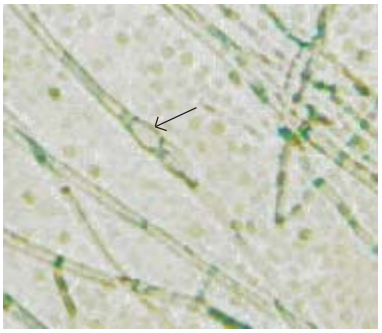

Fig. 2. Cultural and morphological characteristics of R. necatrix. Microphotograph of typical pear - shaped swelling of fungal mycelia (Shown by arrow)

After inoculating an apple orchard with two incompatible *R. necatrix* isolates strain W563 (virus free, MCG 139) and NW10 (virus infected, MCG442). Yaegashi and co-workers[72] recovered forty two sub isolates of *R. necatrix* after 2-3 years and found that all are genetically identical to W563 or NW10. However, 22 of them contained novel dsRNA. Six novel dsRNA (S1-S6) were found in which S1 was a new victorivirus; S2, S3 and S4 were new partitiviruses and S5 and S6 were novel viruses that could not be assigned to any known mycovirus family. Chiba and coworkers isolated a phytopathogenic strain of *R. necatrix* infected with a novel victorivirus named *Rosellinia necatrix* victorivirus 1 (RnVV1) and a partitivirus. RnVV1 showed moderate level of CP and RdRp sequence identity (34-58%) with other members of genus victorivirus[73].

Control

It has been proved difficult to control root rot infecting fungi particularly the white root rot causing fungi *R. necatrix*. However, in 1987 Sztejnberg and co-workers[36] proposed that solarisation of soil prior to planting or exposure of infected roots to air, light and summer heat coupled with treatment of the soil with 0.1-0.2% suspensions of benomyl/ or thiabendazole compounds seems to offer the best chance of control white root rot.

Boesewinkel[74] and Anselmi and Giocelli[75] also emphasized to remove remains of dead trees, especially their roots, and organic material which has been in contrast with *R. necatrix*. Members of the genus *Bacillus* are among the first successful biocontrol agents used against insects and pathogens. *B. subtilis* have been marketed commercially as biocontrol agents for fungal diseases of crops[76]. In Europe, a successful biocontrol agent with hypovirulent strains against chesnut blight disease inspired a group of Japanese researchers to conduct an extensive search of a large collection of ~1,000 field fungal isolates for mycoviruses that might serve as virocontrol. A virocontrol means the one form of biological control utilising viruses that infect organisms pathogenic to useful organisms[59]. 20% of the collections were found to be dsRNA positive *R. necatrix* and presumed to be infected by mycoviruses. However Kanematsu and Sasaki, (unpublished results) reported that the agarose gel profiles of dsRNA suggested infections by members in the families

Totiviridae, *Partitiviridae*, *Reoviridae*, and *Chrysoviridae*. And as previously discussed among these many dsRNAs remained uncharacterized only the genomic segments of *Mycoreovirus 3* (MyRV3) and *Rosellinia necatrix partitivirus 1* (RnPV1) were well characterized.

Following the characterization many artificial virion introduction protocols have been developed for specific viruses infecting the white root rot fungus. One of these is PEG mediated method, as was established for MyRV1 and MyRV2 infecting *C. parasitaca*. Subsequently, the cause-effect relationship was established: MyRV3 was demonstrated to confer hypovirulence (attenuated virulence) on an isogenic strain and a few vegetatively incompatible virulent strains of *R. necatrix*, while RnPV1 was shown to be associated with symptomless infection. Another way out was to use protoplast fusion for introduction of partitiviruses and uncharacterized viruses into recipient fungal strains those are vegetatively incompatible with virus-containing ones. In addition the advanced DNA transformation, systems are available for foreign gene expression in *R. necatrix*. According to Chiba and co-workers[69], these technical advances have made the *R. necatrix* mycovirus systems attractive for studies of virus-host interactions and virocontrol.

Heterogenic incompatability is a major defence mechanism in plant pathogenic fungi including *R. necatrix* against mycoviruses. Ikeda and co-workers[68] succeeded in transmission of a hypovirulence conferring mycovirus to a strain with different genetic background. They used various chemical reagents known for their effect in program cell death pathway or in cell wall modification. Amongst them they found that treatment with zinc compounds aid in transmission of mycoviruses to a strain with different genetic backgrounds.

Buck[77] states that the sexual reproduction functions as a mechanism to eliminate dsRNA associated with the hypovirulence in *Cryphonectria parasitica* (Murrill) Barr and other fungi. In order to develop the effective biocontrol agents the interactions established at different tropic levels be considered. In this respect, biocontrol research has focused almost exclusively on understanding how bacterial antagonists impact fungal pathogen survival and disease-causing activity[78].

Among the strains tested in biocontrol assays, *Pseudomonas pseudoalcaligenes* AVO110 and *Pseudomonas alcaligenes* AVO73 exhibited similar mechanisms that could contribute to biocontrol, such as production of cellulase, indole-3-acetic acid and siderophores, as well as swimming and twitching motility. Role of Cytochalasin E, a known secondary metabolite secreted *in vitro* by *R. necatrix*, was studied for pathogenecity on Japanese pear by creating mutants defective in Cytochalasin E biosynthesis[79]. Results demonstrated that the pathogenecity of these mutants was same as the wild type strain, although these mutant strains produced CE only about 4-7% of the parent strain *in vitro* and less than 5% level *in planta*.

Control of avocado *Dematophora* root rot poses difficulties, because any preplanting treatment must have a long-term effect and any postplanting treatment must not adversely affect the crop[36]. Several approaches have been used over the past two decades for the control of *D. necatrix* before planting, such as i) soil fumigation using methyl bromide[80], ii) soil solarization[81], which may induce disease suppressive activity of the soil by increasing microbial activities, resulting in protection of the plants[82], and iii) biological control using the antagonistic fungus *Trichoderma harzianum*[36]. Biological control of avocado diseases has not been investigated, although recently, an approach for isolating bacterial biocontrol strains for the control of avocado root rot caused by *Phytophthora cinnamomi* was reported[78,83].

Diverse antagonistic *B. subtilis* strains isolated from healthy avocado rhizoplanes have shown promising biocontrol abilities, which are closely linked with the production of antifungal lipopeptides and good colonization aptitudes. In the context of biocontrol, a successful exploratory attempt to repress avocado root rot caused by *R. necatrix* by using antagonistic pseudomonads has been reported[12]; however, the use of bacterial strains as biocontrol agents against avocado soil-borne phytopathogens remains an issue to be further explored. The success of biocontrol strategies will depend to a large extent on the seeking and selection process of potential biological agents, which consider the pathogen to be the target and the cropping system[12].

Industrial Applications of the *R. necatrix*

Lignin is a structurally complex aromatic biopolymer which is recalcitrant to degradation. The degradation of lignin is an important step in the mineralization of carbon in nature. It has been demonstrated that the extracellular enzymes of some white rot fungi including *R. necatrix* are able to degrade lignin extensively. These extracellular enzymes include lignin peroxidases, manganese peroxidases, and laccases. In addition to their industrial application in delignification, laccases are also known to polymerize phenolic compounds. This characteristic of laccases make them of potential interest for industrial applications involving the polymerization of phenolics in liquids, the oxidation of dyes and dye precursors, and polymerization of lignin and lignosulfonates. In fungi, besides a role in delignification, laccases appear to be involved in sporulation, pigment production, and plant pathogenesis[84]. Laccase have been successfully used for the environmental pollution control such as Acid orange-7 bio-decolourization, delignification of *Eucalyptus sp*. and also in the degradation of cypermethrin[85].

CONCLUSION

R. necatrix causing the white root rot disease is common in temperate, tropical as well as subtropical zones. This pathogen is capable of surviving for many years in host as well as causing the destructive damage to all potential plant species including many economical and ornamental plants. Himachal Pradesh and other hill states of India are most vulnerable sites for the infection of *R. necatrix* because they posses most favourable conditions for *R. necatrix* growth and proliferation, which leads to significant losses to farmers. Mostly in hill states curing by solar solarisation won't work because of low temperature round the year and fungicide treatment is not preferred because of the environmental and health concerns. Here the most potent control mechanisms of this devastating pathogen are by the means of biological methods of control. Among them using antagonistic bacteria to inhibit fungal growth caters great attention of the researchers in the past. A relatively new approach of controlling fungal infections by virtue of hypovirulence capabilities of certain mycoviruses has been reported. There is a need to look into this biological control mechanism especially in case of economically important fungal diseases. Another way to develop safer and more effective disease control mechanisms is by targeting specific infection stages during pathogenesis. For this it is important to first workout the complete mechanism of infection of *R. necatrix* by understanding genes involved in different stages of pathogenesis. In order to achieve this it is again important to develop faster and more effective methodologies to create random and target mutations in the *R. necatrix* as the previously known and studied methods have several limitations. Controlling devastating fungal diseases including *R. necatrix* infection is the need of the time if we want to increase the productivity, to feed the growing population and to add prosperity to the well being of the farmers.

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