VirusDis. (April–June 2018) 29(2):134–140
https://doi.org/10.1007/s13337-018-0438-4

REVIEW ARTICLE

Mycovirus associated hypovirulence, a potential method for biological control of *Fusarium* species

Mohit Sharma[1,3] · Shiwani Guleria[1,2] · Kirti Singh[1,4] · Anjali Chauhan[5] · Saurabh Kulshrestha[1]

Received: 10 January 2018 / Accepted: 6 February 2018 / Published online: 17 February 2018

Abstract *Fusarium* is a large genus of filamentous fungi belongs to the division Ascomycota and was first described as *Fusisporium*. Innumerable members of this genus act as pathogens, endophytes and saprophytes and can be recovered from plants and soils worldwide. Many of these members are known to be phytopathogens. It is among the most diverse and widely dispersed phyto-pathogenic fungi which cause economically important blights, rots, wilts and cankers of many ornamental, field, horticultural and forest crops both in agricultural commodities and natural ecosystems. Some species, e.g. *F. graminearum* and *F. verticillioides* have a narrow host range and mainly infect the cereals, whereas *F. oxysporum* has effects on both monocotyledonous and dicotyledonous plants. Attempts have been made to control the diseases caused by *Fusarium* sp. and to minimize crop yield losses. Till date, effective and eco-friendly methods have not been devised for the control of this devastating pathogen. A new potential of using mycovirus associated hypovirulence as biocontrol method against *Fusarium* species has been proposed. The present review taking into account of worldwide researches to provide possible insights for *Fusarium*-mycovirus coevolution.

Keywords *Fusarium* · Mycovirus · Hypovirulence · Biocontrol · Phytopathogen

✉ Saurabh Kulshrestha
saurabh_kul2000@yahoo.co.in;
sourabhkulshreshtha@shooliniuniversity.com

[1] Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan, Himachal Pradesh 173229, India

[2] Department of Microbiology, Lovely Professional University, Jalandhar, Punjab, India

[3] Department of Molecular Biology and Genetic Engineering, Lovely Professional University, Jalandhar, Punjab, India

[4] National Institute of Plant Genome Research, Aruna Asaf Ali Marg, PO Box No. 10531, New Delhi, India

[5] Department of Soil Science and Water Management, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Introduction

Fungi are a group of heterotrophic eukaryotic organisms, which are highly versatile and occupy almost all the natural habitats. Less than 10% of the ~ 100,000 known fungi species are strict saprophytes with the ability to colonize plants. Many economically important crops are prone to diseases by a small fraction of these saprophytes. Fungal species have become a serious factor in the economy of crops as they are responsible for causing widespread and devastating epidemics and also significantly affect the annual crop yields. It is because of these reasons, they have attracted the attention of farmers as well as plant breeders and scientists [24].

Fusarium is a devastating phytopathogenic fungus belonging to Division: Ascomycota, Class: Sordariomycetes, Order: Hypocreales and Family: Nectriaceae. This filamentous fungus can strike any crop since it has a broad host range which includes rice, wheat, almost all horticultural crops, ornamentals and almost all other agricultural commodities [21, 28, 45, 46, 53, 54, 61]. Many economically important diseases are caused by *Fusarium* species such as *Fusarium* rot on apples by *Fusarium* species [45], sugarcane wilt by *Fusarium sacchari* [28], pokkahboeng in sugarcane by *Fusarium moniliforme* [54], bakanae in rice by *Fusarium fujikuroi* [21], oil palm wilt by

Fusarium oxysporum f. sp. *elaidis* [46], panama disease by *Fusarium oxysporum* f. sp. *cubensis* [61] etc.

Fusarium species are present in soil as well as on aboveground and subterranean plant parts, plant debris, and other organic substrates. They are commonly present in tropical and temperate regions and are also found in extreme climatic conditions like deserts, alpine and arctic regions. Fertile and cultivated land soils show the presence of many *Fusarium* species in comparison to forest uncultivated soil where it is less prevalent. *Fusarium* species are mostly abundant in soil and are associated with roots in the form of parasites and saprophytes. In the aerial plant parts, the species have active and passive means of dispersal and hence are able to cause many diseases of economic impact. Airborne *Fusarium* species are rarely found in the cultures obtained from soil or the roots of plants. *Fusarium* species has worldwide distribution because of their efficient use of a variety of substrates and dispersal mechanisms [36].

Biological control of *Fusarium* species

A promising strategy against *Fusarium* wilt of tomato employs *Trichoderma harzianum*, *T. asperellum*, and *T. virens* which having efficient antagonistic capacity against *Fusarium* species (*F. solani* and *F. oxysporum*). The severity of root wilt is decreased by administering the soil with biocontrol agents. A combination of *T. harzianum*, *T. asperellum*, and *T. virens* has the highest capacity of control (80–87%), followed by a binary combination of *Trichoderma* species (79–82%), while *T. virens* alone has the lowest control rate of 65% [1].

The ability of bacteria belonging to genera *Sphingomonas* and *Bacillus*, and yeasts belonging to genera *Cryptococcus*, *Rhodotorula* and *Saccharomyces,* respectively to be used as biocontrol agents was tested under laboratory conditions. Yeast isolates showed an inhibitory effect against *F. sporotrichioides* while *Sphingomonas* S11 isolate was antagonistic against *F. avenaceum*, *F. culmorum*, *F. tricinctum* and *F. graminearum* [55].

F. monoliforme is a facultative endophytic fungus which produces fumonisins during its biotrophic endophytic association with maize and also during the saprophytic growth. The fungus is transmitted vertically or horizontally. The use of fungicides can reduce the horizontal transmission, unlike vertical transmission which cannot be reduced with fungicides and hence, acts as a reservoir for infection and toxin biosynthesis in each generation [4]. A biological control system has been developed which uses *Bacillus subtilis* endophytically. It gave significant results for reducing the accumulation of mycotoxin during endophytic growth phase (vertical transmission). The bacterium is considered as an ideal ecological homologue to *F. moniliforme* as it occupies a similar ecological niche within the plant like fungal pathogens and its inhibitory mechanism is based on 'Competition'. In addition to the bacterium, a fungal isolate of *Trichoderma* showed antagonistic ability against *F. monoliforme* and proved to be significant in the post-harvest control of the growth of *F. monoliforme* and toxin accumulation in corn seeds during storage [4].

Plant growth promoting rhizobacteria (PGPR) strains from eight different species (*Bacillus subtilis*, *Bacillus pumilus*, *Burkholderia cepacia*, *Pseudomonas putida*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus macerans* and *Flavobacter balastinium*) were tested for antifungal activity against *F. sambucinum*, *F. oxysporum*, and *F. culmorum* causing dry rot in potato. These strains exhibited potential anti-fungal activity both under in vitro (on petri plates) and in vivo (on potato tubers) conditions. Out of these eight species, the highest antagonistic effect against *Fusarium* species was shown by *Burkholderia cepacia* which has great possibility to be used as a biocontrol agent against *Fusarium* dry rot of potatoes under storage conditions [44, 47, 48].

Till date, no effective and eco-friendly methods have been devised for the control of fungus. Therefore, utilizing a biological control method is a tactful choice. In the field, there are various strains within a species of fungi. Some of them are virulent whereas others are hypovirulent. The hypovirulent isolates are of two kinds: some are genetically hypovirulent while others become hypovirulent because of infection by mycoviruses. From a phytopathological perspective, both of these can be used as biocontrol agents [53].

Hypovirulence

Hypovirulence refers to the decline in the ability of a pathogen to cause the disease. Hypovirulent pathogens cannot produce a severe disease. Mycovirus mediated hypovirulence is a phenomenon in which the disease-causing capability of phytopathogenic fungi is reduced as a consequence of infection by mycovirus [39]. Even though mycoviral dsRNAs have been reported to be present in *F. solani* [38], *F. oxysporum* [23], *F. poae* [9] and *F. graminearum* [8], their amalgamation host-hypovirulent traits was suggested only for some isolates including *F. graminearum* strain DK21 [7].

Hypoviruses of family *Hypoviridae*, are an exception and result in significant morphological and physiological variations, including cytological alterations, changes in colony morphology and growth rate, and persistently attenuated virulence-related novel phenotypes [40].

Hypoviruses lack usual virions and their dsRNAs are enfolded in host-encoded vesicles [11].

Hypovirulent or debilitated strains of plant pathogenic fungi, carrying transmissible viruses, greatly noticed by researchers because of their potential utilization as biocontrol agents, as well as their use as probes for deciphering the mechanisms of fungal pathogenesis. Many examples of mycovirus-associated hypovirulence/debilitation are known which include the mitoviruses in *Ophiostoma novo-ulmi* [20] and *Sclerotinia homoeocarpa* [12] and also the unclassified mycoviruses which cause infection in *Diaporthe ambigua* [43], *Fusarium graminearum* [7] and *Botrytis cinerea* [6].

Fungi cause catastrophic diseases in all major crops which considerably affected human lives. For example, the bread baskets of the world are threatened by the recent recurrence of the deadly wheat black stem rust fungus [52]. When resistant cultivars are not available or lacking, application of chemical fungicides is the major method for controlling fungal diseases of economically important crops. However, efficient, environment-friendly alternative methods have to be adopted to avoid dependence on fungicides. Hypovirulence is thought to play a major role in counter-balancing the occurrence of plant diseases in nature [5], and has been successfully employed to control chestnut blight caused by the fungus *Cryphonectria parasitica*. Lee et al. [26] transmitted FgV1–DK21 virus in *C. parasitica* through protoplast fusion. The recipient strain had shown reduced growth rates, virulence and altered pigmentation. The successful application of FgV1–DK21 as hypovirulent factor had showed that it can be used as a biological control agent [26].

Double-stranded RNA is an imperative non-specific indicator of the presence of RNA viruses in bacteria, fungi, and plants [13]. It represents the genome of an RNA virus or its replicative form. An accurate measurement of the size of dsRNA can provide essential information pertaining to the virus-like particle infecting a host. If it is isolated and cloned, it can be used a specific diagnostic probe without the need for purifying the virus itself [22]. Analysis of the sequence of these clones can deliver significant information for the taxonomic positioning of these virus-like particles associated with dsRNA and can also help greatly in the identification of the virus. If the mycoviral infection is found to adversely affect the pathogenicity of a fungus, then they can be used as potent means of biological control against fungal diseases.

Mycoviruses

Mycoviruses are the viruses that cause infection in fungi. Most of the mycoviruses contain double-stranded RNA (dsRNA) as their genome and are isometric particles. However, approximately 30% have positive sense, single-stranded RNA (+ ssRNA) genomes [42, 51]. A true mycovirus can infect other healthy fungi. Many dsRNA elements do not fit this description in fungi and are known as virus-like particles or VLPs. Initially, it was indicated that the phylogeny of mycoviruses was not found to be largely consistent with their hosts [16].

A significant difference between the genomes of mycoviruses to other viruses is the lack of genes for proteins related to 'cell-to-cell movement'. Therefore, it is presumed that mycoviruses progress intercellularly during cell division (e.g. sporogenesis) or via hyphal fusion. Mycoviruses may not even require an external way of infection and spread using their fungal host's life style; Plasmogamy and cytoplasmic exchange over extended periods of time, asexual spores produced in vast amount overwinter via sclerotia [29] and more or less effective dissemination into sexual spores. The process of viral transport has not been conclusively said to be active or passive. But it is generally thought that fungal viruses are transported forward by plasma streaming [49]. Theoretically, they are said to drift with the cytoplasm as it extends into the new hyphae, or get attached to the web of microtubuli, which would drag them via internal cytoplasmic space. However, many researchers have found them to be located next to the septum walls, which may imply that they got stuck and were unable to actively move forward themselves.

Phenotypic effects of mycoviral infections can vary from advantageous to deleterious. The relation between the fungal phenotype and the presence of mycovirus is not always straight forward. This may be accounted for by a number of reasons. Firstly, the lack of appropriate infectivity assays has often hindered the researchers from reaching a coherent conclusion [33]. Secondly, mixed infections or unknown numbers of infecting viruses makes it very difficult to associate a particular phenotypic change with the investigated virus. Although most mycoviruses often do not seem to disturb the fitness of their host, this does not necessarily mean they are living unrecognized by their hosts. A balanced co-existence might be the result of a long co-evolutionary process [3, 32]. Accordingly, when certain conditions of the virus-fungus-system change, symptoms of infection appear and make the system go out of balance. This effect could be environmental as well as cytoplasmic. It is still unknown yet why some mycovirus-fungus-fusions are harmful while others are asymptomatic

or even valuable. Nevertheless, harmful effects of mycoviruses are economically important, mainly if the fungal host is a phyto-pathogen and the mycovirus could be exploited as a bio-control agent. The scenario is best represented by the case of CHV1 and *C. parasitica* [15].

The main negative effects of mycoviruses are decreased growth rate, lack of sporulation, attenuation of virulence and less germination of basidiospores [35]. Phenotypes of hypovirulence do not appear to correspond with specific genome traits and probably there is no single specific metabolic pathway causing hypovirulence. In addition to the negative effects, beneficial interactions also occur. Best examples are the killer phenotypes in *Ustilago* and yeasts [30, 50]. Killer isolates secrete toxic proteins to which are toxic to the sensitive cells of closely related or same species, while the producing cells themselves are immune. Mostly toxins degrade the cell membrane. Killer isolates have many potential applications in medicine, food industry and agriculture [11].

Mycoviruses of *Fusarium* species

Several mycoviruses have been reported worldwide from *Fusarium* species, some of them are being employed as a biocontrol against diseases. Mycoviruses infecting *Fusarium* species have been identified by the presence of dsRNAs. It was reported that most of the *Fusarium* species contained RNA viruses and although infection rate was generally low. Only a minimal number of mycoviruses cause hypovirulence in *Fusarium* [17].

Strain SUF704 out of 34 strains of *Fusarium solani* from Japan contained dsRNA fragments of sizes 1.9 and 1.7 kbp, respectively [37]. Together these dsRNA fragments produced a single polypeptide of 38 kDa on SDS-PAGE gels. This mycovirus, named as *Fusarium solani virus 1* (FsV1 or FusoV), was consequently proven to contain two different dsRNA segments with RNA-dependent RNA polymerase (RdRp) domain in one of the dsRNA segments [38].

Fifty-five isolates of *Fusarium poae* isolated from wheat collected worldwide contained dsRNAs and encapsidated virus-like particles. This was astounding because previous studies had indicated that mycoviruses caused infection in low percentage in *Fusarium* isolates. Furthermore, the dsRNA patterns were different in all *F. poae* isolates but after repeated subculturing, the patterns got stable [14]. No morphological changes have been observed in *F. poae* isolates harboring dsRNAs. This indicates that the host was not harmed by mycoviruses. One mycovirus, which was obtained from *F. poae* isolate A-11, was named *Fusarium poae* virus 1 (FpV1 or FuPO-1) [9].

Only a few of the detected mycoviruses have been exposed to cause morphological variations in the host. Similarly, very few studies have been able to identify hypovirulence associated with *Fusarium* mycoviruses. Three mycoviruses found to confer hypovirulence in *Fusarium oxysporum* [23]. Kilic and Griffin [23] found that out of 57 isolates of *Fusarium oxysporum* in the United States, only six contained dsRNAs, and six isolates contained four dsRNA segments with sizes of 2.2, 2.7, 3.1, and 4.0 kbp, respectively.

Fusarium graminearum virus 1 (FgV1) was the first *Fusarium* mycovirus to be characterized. It led to the decreased pathogenicity of fungus and morphological variations, including increased pigmentation and lesser mycelial growth [60]. It has been seen that if mango is inoculated with a VLP-infected and VLP-free isolate of *Fusarium moniliforme,* only the VLP-free isolate caused shoot malformation. This suggests that the mycovirus contained in *F. moniliforme* may be responsible for suppressing mango shoot malformation.

Fusarium proliferatum, cause infection in maize and sorghum in the United States. Only four isolates of *F. proliferatum* out of 100 were reported to contain dsRNA [18]. The dsRNAs ranged from 0.7 to 3.1 kbp in size. One isolate consisted of a single form of dsRNA, whereas other isolates consisted of multiple forms of dsRNAs. The segments of dsRNAs in one isolate were related with mitochondria [18].

A survey by Herrero et al. [19] testified 103 isolates of endophytic fungi belonging to 53 species. Out of these, 12 isolates contained dsRNAs and one isolate of *Fusarium culmorum* contained two dsRNAs of sizes 3 and 4.4 kbp, respectively. However, it has not been determined that the two dsRNAs are a part of one mycovirus or of two different mycoviruses.

Most of the mycoviruses have been identified and studied in *Fusarium graminearum* [2, 7, 8, 10, 59]. 19 dsRNA fragments from *F. graminearum* isolates procured from diseased plants of barley and maize in Korea and one of the dsRNA virus has been detected as *Fusarium graminearum virus-DK21*(*FgV1-DK21*) [7, 25, 58]. The detected mycoviruses contain 2-5 segments of dsRNAs of 1.7–10 kbp in size [8]. Two isolates of *Fusarium graminearum*, JB33, and JNKY19, shown infection by two different viruses. Additional mycoviruses infecting *F. graminearum* have also been identified. Darissa et al. [10] isolated a mycovirus, cited as *Fusarium graminearum virus-ch9* (FgV-ch9), containing five dsRNAs. It was identified from ten *F. graminearum* strains in China. Aminian et al. [2] detected at least three different dsRNAs, of 0.9–5 kbp in size, from twelve *F. graminearum* isolates of wheat in Iran. The dsRNAs of those twelve isolates caused less serious disease than dsRNA-free isolates and

developed substantially less quantities of the mycotoxin deoxynivalenol (DON) on susceptible wheat in the greenhouse. Wang et al. [56] in China isolated *F. graminearum* hypovirus 1 (FgHV1) from *F. graminearum* strain HN10, of approximately 13 kbp and closely related to *Cryphonectria* hypovirus 1 (CHV1) and *Cryphonectria* hypovirus 2 (CHV2) in family *Hypoviridae*. The 3′ Open reading frame B (11118nt) was predicted in FgHV1 encoding a large polypeptide which is sharing 32% amino acid similarity with CHV1 and CHV2, respectively. Marvelli et al. [31] in the USA isolated two mycoviruses namely *F. virguliforme* dsRNA mycovirus 1 and *F. virguliforme* dsRNA mycovirus 2, of approximately 9.3 kbp in size related to family *Totiviridae*. On grouping of 44 isolates of *F. virguliforme* on the basis of dsRNA profiles, isolates containing large dsRNA were significantly less virulent than isolate without dsRNAs.

Fusarium graminearum Hypovirus 2 (FgHV2/JS16) isolated from *F. graminearum* strain JS16 in China [27]. The genome is 6.4 kbp long, excluding poly (A) tail and belong to a newly proposed genus *Alphahypovirus* and family *Hypoviridae*. Li et al. [27] demonstrated that infection of FgHV2/JS16 activated the pathway of RNA interference in *F. graminearum* by relative quantitative real-time RT-PCR.

Two mitoviruses; *Fusarium coeruleum* mitovirus 1 (2.423 kbp) and *Fusarium globosum* mitovirus 1 (2.414 kbp), belongs to genus *Mitovirus* and family *Narnaviridae* isolated and characterized from *F. solani* variety *coeruleum* and *F. globosum*, respectively in Japan [41]. Osaki et al. [41] also characterized *Fusarium solani* partitivirus 2 (1.950 kbp) belong to genus *Alphapartitivirus* and family *Partitiviridae*, isolated from *F. solani* f. sp. *pisi*. Minor et al. [34] in Spain published the first report of characterization of the complete genome of *Fusarium oxysporum* f. sp. *dianthi* mycovirus 1 (FodV1) isolated from *F. oxysporum* f. sp. *dianthi* that infects Carnations (*Dianthus caryophyllus*). FodV1 is a new member of family *Chrysoviridae*, that contained 4 dsRNA segments, namely dsRNA 1, 2, 3 and 4 and having sizes of 3.555, 2.809, 2.794, and 2.646 kbp, respectively. The identity of the RNA segment was also obtained by means of DNase and S1 nuclease treatment as dsRNA is resistant to DNase and S1 nuclease treatment. dsRNA 1 and dsRNA 3 encoded a putative RNA dependent RNA polymerase and a putative coat protein, respectively. dsRNA 2 and dsRNA 4 encoded a hypothetical protein (P2 and P4) with unknown functions.

Two double-stranded RNA (dsRNA) mycoviruses, termed *Fusarium poae* dsRNA virus 2 (FpV2) and *Fusarium poae* dsRNA virus 3 (FpV3) were reported in China with respective genome sequences of 9.518 and 9.419 kbp, are both predicted to contain two discontinuous open reading frames (ORFs), ORF1 and ORF2. The two viruses were isolated from the plant pathogenic fungus, *Fusarium poae* strain SX63 from wheat and molecularly characterized [57].

Concluding remarks

In recent years a remarkably potent, economic and more importantly an eco-friendly bio-control methodology has been proposed to tackle diseases caused by the fungus. As the principle behind the bio-control methods is antagonistic interactions among pathogens, it proposes broad dimensions to be explored in the research of disease control. It includes usage of certain fungi, bacteria, yeasts, and mycoviruses as antagonistic pathogenic agents in biocontrol of other fungal pathogens. PGPR strains from different bacteria have exhibited potential antagonistic or antifungal activity both under in vitro and in vivo conditions against *Fusarium* species causing dry rot in potato. Mycovirus-associated hypovirulence has also been reported in several *Fusarium* species infecting different crops in several countries around the world. As most of the mycoviruses reported till date, contains double-stranded RNA (dsRNA) as their genome in form of isometric particles. The hypovirulence based biocontrol has already been successfully employed in control of chestnut blight caused by *Cryphonectria parasitica* in Europe. This has highly driven the mycologists and plant pathologists to explore various hypovirulent factors in several fungi on a worldwide scale. Mycoviral dsRNAs (of varying sizes ranging from 1 kb to 5 kb approx.) have also been reported to occur in *Fusarium* species infecting different crops, viz. in strains of *F. solani* from Japan, *F. graminearum* strains in China, *F. oxysporum* strains in Spain, etc. And further on, a number of mycoviruses have been successfully isolated, characterized and even classified into groups and families of viruses. But still, no studies have been able to identify the mycoviruses associated with hypovirulence in *Fusarium* species in India. Although research is underway at extensive scale in many parts of India to characterize the mycoviruses suspected to be the cause of hypovirulence in *Fusarium* species; for instance, presence of dsRNAs and geminivirus-related mycoviral sequences have been isolated from *F. solani* infecting apple plants in Himachal Pradesh district of India at Molecular Plant–Microbe Interaction (MPMI) Laboratory of Shoolini University, Solan, Himachal Pradesh, India.

Acknowledgements Authors are thankful to Hon'ble Vice Chancellor, Shoolini University of Biotechnology and Management Sciences for providing necessary facilities. We would also like to thank Department of Biotechnology (DBT), Govt. of India for providing financial support as research Grant No. BT/PR6464/GBD/27/430/2012. Authors would also like to acknowledge the help of Mr. Neeraj

Pizar, Assistant Professor (English), Shoolini University for thorough language editing in the manuscript.

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