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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

BIOCONTROL POTENTIAL OF *STREPTOMYCES* SPECIES AGAINST *FUSARIUM OXYSPORUM* F.SP. *ZINGIBERI* (CAUSAL AGENT OF RHIZOME ROT OF GINGER)

Manasa M, Yashoda Kambar, Pallavi S, Vivek M.N, Onkarappa R, Prashith Kekuda T.R*

P.G. Department of Studies and Research in Microbiology, Sahyadri Science College campus, Kuvempu University, Shivamogga-577203, Karnataka, India

**Corresponding author: p.kekuda@gmail.com*

ABSTRACT

The present study aimed at determining biocontrol potential of six *Streptomyces* species (SSC-MB-01 to SSC-MB-06) against *Fusarium oxysporum* f.sp. *zingiberi*, causal agent of rhizome rot of ginger. Primary screening for antifungal activity was tested by dual culture method. Agar well diffusion method was employed to screen inhibitory efficacy of ethyl acetate extracts obtained from fermentation broths of *Streptomyces* species. In both the assays, marked inhibitory activity was observed in case of *Streptomyces* species SSC-MB-02. In case of *Streptomyces* species SSC-MB-05, no inhibitory activity was observed in both the assays. The results obtained indicate the possible utilization of *Streptomyces* isolates for the protection of the ginger rhizomes from soft rot symptoms.

Keywords: Rhizome rot of ginger, *Fusarium oxysporum* f.sp. *zingiberi*, *Streptomyces*, Dual culture, Agar well diffusion

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belonging to the family Zingiberaceae is an important commercial crop being used as a spice and a medicine [1]. It earns a sizeable amount of foreign exchange for the country [2]. India is the largest producer of ginger accounting for about 1/3rd of total world output. In India, ginger is grown in various states such as Kerala, Karnataka, West Bengal, Andhra Pradesh, Orissa, Arunachal Pradesh and Sikkim [1,3]. The production of ginger is largely influenced by several diseases caused by bacteria, fungi, viruses, mycoplasma and nematodes. The crop suffers from diseases like bacterial wilt caused by *Ralstonia solanacearum*, rhizome rot caused by *Pythium* species, *Fusarium* species, *Sclerotium* species, *Pseudomonas* species and others [1, 4-7].The disease management involves cultural, biological and chemical approaches for pathogen suppression [8].

Actinomycetes are Gram-positive bacteria with high GC content in their genome and resemble fungi morphologically. Among actinobacteria, the largest genus is *Streptomyces* and it represents a large number of species. The members of *Streptomyces* dominate the actinomycete population of soil. The Streptomycetes possess a large linear chromosome, a rich repertoire of secondary metabolites. The members of *Streptomyces* have a complex life cycle that alternates between the filamentous vegetative mycelium and the spore-bearing aerial hyphae. Soil streptomycetes are saprophytic and are known to play significant roles in biotransformation and biodegradation. They are best known for production of secondary metabolites which accounts for more than half of the bioactive compounds in use *viz.*, antibiotics, anticancer agents, antiparasitic drugs, antifungals, antivirals, antiparasitic compounds, immunosuppressants, insecticides, antioxidants, enzyme inhibitors and herbicides [9-16].

Many microbial antagonists have been reported to possess antagonistic activities against plant fungal pathogens. Actinomycetes, in particular species of *Streptomyces*, have shown to be excellent biocontrol agents against phytopathogenic fungi [17-26]. In the present study, we report biocontrol potential of six *Streptomyces* species (SSC-MB-01 to SSC-MB-06) against *Fusarium oxysporum* f.sp. *zingiberi* recovered previously from rhizome rot specimen of ginger. The antagonistic *Streptomyces* species were isolated from a rhizosphere soil of Sahyadri Science College campus, Shivamogga, Karnataka, India and were shown to exhibit antibacterial activity [27].

2. MATERIAL AND METHODS

2.1. Antagonistic actinomycetes

In this study, we have used six *Streptomyces* species (SSC-MB-01 to SSC-MB-06) recovered previously from rhizosphere soil of Sahyadri Science College (Autonomous), Shivamogga-03, Karnataka which have shown antagonistic properties against bacteria and fungi [27].

2.2. Test fungus

The inhibitory efficacy of *Streptomyces* species was tested against *Fusarium oxysporum* f.sp. *zingiberi* isolated previously from a rhizome rot specimen of ginger.

2.3. Primary screening for inhibitory activity of *Streptomyces* species

Dual culture method was performed for screening the antifungal efficacy of *Streptomyces* isolates against *F. oxysporum*. Here, the *Streptomyces* isolates were inoculated at the centre of the Starch casein nitrate agar (soluble starch 10g; KH_2PO_4 2g; KNO_3 2g; NaCl 2g; casein 0.3g; $MgSO_4.7H_2O$ 0.05g; $CaCO_3$ 0.02g; $FeSO_4.7H_2O$ 0.01g; agar 15g; distilled water 1000ml) plates amended with dextrose and the plates were incubated at 30°C for up to 7 days. Afterwards, the test fungus was inoculated 3cm away from the growth of *Streptomyces* isolates by point inoculation method. The plates were further incubated at 30°C for 3 days and the extent of inhibition of test fungus was recorded [28].

2.4. Secondary screening for inhibitory activity of *Streptomyces* species

Ethyl acetate extracts of *Streptomyces* species were obtained as described in our previous study [27]. The efficacy of ethyl acetate extracts to inhibit the growth of *F. oxysporum* was assessed by Agar well diffusion assay. Here, the spore suspension from well grown culture of *F. oxysporum* was swabbed uniformly on sterile Potato dextrose agar (peeled potato 200g; dextrose 20g; agar 15g; distilled water 1000ml; pH 5.6) plates. Later, using a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. The wells were filled with 100µl of ethyl acetate extracts (5mg/ml of dimethyl sulfoxide [DMSO]) of *Streptomyces* species and the plates were incubated at 30°C for 3 days. The zones of inhibition (ZOI) formed around the wells were measured [27].

3. RESULTS

The inhibitory activity of six *Streptomyces* isolates against the mycelial growth of *F. oxysporum* in dual culture method is shown in Table 1. All isolates except isolate SSC-MB-05 displayed inhibitory activity against *F. oxysporum*. Among isolates, the isolate SSC-MB-02 exhibited strong inhibition of *F. oxysporum* followed by isolate SSC-MB-04 and others.

Table 2 shows inhibitory activity of ethyl acetate extracts of fermentation broths of six *Streptomyces* species against *F. oxysporum*. Extracts of all isolates except SSC-MB-05 showed inhibition of test fungus with zone of inhibition ranging between 1.0-1.9cm. Like in primary screening, the isolate SSC-MB-02 displayed stronger inhibitory activity followed by SSC-MB-04 and others.

Table 1: Inhibition of F. oxysporum by Streptomyces species in dual culture

Streptomyces species	Extent of inhibition
SSC-MB-01	+
SSC-MB-02	+ + +
SSC-MB-03	+
SSC-MB-04	+ +
SSC-MB-05	-
SSC-MB-06	+

'-' No inhibition; '+' Inhibition

Table 2: Inhibition of F. oxysporum by solvent extract of Streptomyces species

Extract/standard	ZOI in cm
SSC-MB-01	1.2±0.09
SSC-MB-02	1.9±0.05
SSC-MB-03	1.0±0.01
SSC-MB-04	1.5±0.10
SSC-MB-05	0.0±0.0
SSC-MB-06	1.2±0.15
Clotrimazole	2.8±0.20
DMSO	0.0±0.0

4. DISCUSSION

A large number of fungi are able to cause diseases in a variety of plants including commercial crops. Fungicides have been extensively used for controlling plant diseases. However, the extensive use of these fungicides poses several problems such as the fungicide residues in food commodities, environmental pollution and possible development of resistance among pathogenic fungi. Due to increasing public concern about the ill effects of synthetic agents, availability of a sustainable and environmentally friendly method for disease control is highly desirable. The use of microbial antagonists is one of the best and alternate strategies for the control of plant pathogens. During recent years, the interest in biological control of plant pathogens by beneficial microorganisms has increased consistently and it forms a possible substitute for various chemical control methods. The use of antagonistic microorganisms has been advantageous as they are able to inhibit the growth and proliferation of phytopathogens with little or no side effects [29, 30].

In the present study, the bioactive *Streptomyces* species exhibited inhibitory activity against *F. oxysporum* f.sp. *zingiberi* isolated from rhizome rot disease of ginger. Streptomycetes have shown to be promising biocontrol agents capable of inhibiting fungal pathogens of several plant diseases. Getha and Vikineswary [17] found inhibitory activity of *S. violaceusniger* strain G10 against *F. oxysporum* f.sp. *cubense* the causal pathogen of wilt disease of banana. *S. olivaceus* isolated from soil showed

inhibitory activity against *F. oxysporum* f.sp. *melonis*, the causal agent of root rot disease of greenhouse cucurbits [18]. *Streptomyces* sp. Strain 3 was found to be a potential biological agent for control of Fusarium head blight (FHB) caused by *F. graminearum* [19]. Streptomycetes from rhizosphere of *Araucaria* were shown to have the ability to inhibit the growth of *Fusarium* and *Armillaria* causing pine rot [20]. The treatment with the Talc-based formulation of *S. griseus* of seeds and seedlings of tomato showed a significant reduction in the disease severity caused by *F. oxysporum* f. sp. *lycopersici* [21]. *S. alni* isolated from rhizosphere soil of grapevine was found to exhibit antagonistic activity against *F. oxysporum* the causative agent of root rot of grapevine [22]. Treatment of tomato seeds with *S. miharaensis* strain KPE62302H was found to induce a significant reduction in the incidence of Fusarium wilt in tomato plants compared with untreated controls [23]. *Streptomyces* sp. C-11 and C-26 isolated from the soil sample showed antagonistic activities against *F. subglutinans* in dual culture test [24]. *S. rochei* ACTA1551 strongly suppressed the growth of *F. oxysporum* f.sp. *lycopersici in vitro*. The strain was able to protect tomato seeds from *F. oxysporum* infection *in vivo* [25]. Kanini *et al*. [26] found inhibitory efficacy of indigenous *Streptomyces* isolates against the soil-borne fungal plant pathogen *Rhizoctonia solani*.

5. CONCLUSION

In our study, the six soil *Streptomyces* species and their ethyl acetate extracts exhibited inhibition of *F. oxysporum* f.sp. *zingiberi* in both dual culture method and agar well diffusion assay. The species of *Streptomyces* could be exploited as biocontrol agents for rhizome rot disease of ginger. Further, *in vivo* studies are to be carried out.

6. ACKNOWLEDGEMENTS

Authors are thankful to Dr. N. Mallikarjun, Associate Professor and Chairman, P.G Department of Studies and Research in Microbiology and Principal, Sahyadri Science College (Autonomous) for providing all facilities and moral support to conduct work. Authors are also thankful to Dr. Shobha K.S, Lecturer, P.G Department of Studies and Research in Microbiology for providing the fungal culture.

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