STUDY ON ANTI-HELMINTHOSPORIUM COMPOUND PRODUCING BACTERIA BACILLUS SUBTILIS

[1]Maitry Shah,[2] Krishna Bhavsar, [3]Naresh Butani,
[4]Nafisa Patel, [5]Ravin Rawal

[1,2,3,4,5] Naran Lala College of Professional and Applied Sciences, Navsari, Gujarat, (India)

ABSTRACT

Phyto pathogenic fungi are a serious problem for crop and vegetable due to its damaging effects. In indirect way it also causes serious problem in human and animals by producing mycotoxins. Helminthosporium spp. is one of phyto pathogenic fungi responsible for causing blight in very important food crop like, wheat and oats. It also causes leaf spots in many plants and responsible for root rots in cereals. Spot blotch caused by Bipolaris sorokiniana is one of the most important foliar diseases limiting wheat production in warmer, nontraditional growing areas. Due to infection of such fungi up to 15% crop loss has been reported. So, it is very important to manage this pathogen. Infection caused by such fungi can be controlled by application of antifungal compounds or by applying biocontrol agents. Major limiting factor for antifungal compound application is its low solubility and toxicity. In order to find new safer antifungal compound or biocontrol agent, present research work has been undertaken. From the agricultural soil samples, various microorganisms had been isolated and screened for its antifungal compound production. Anti-Helminthosporium activity was studied by agar cup method. Screened bacterium was identified as Bacillus subtilis, having capacity of production of antifungal compound. Bacillus subtilis can be applied as biocontrol agent.

Keywords: Antifungal activity, Antifungal compound, Helminthosporium spp., Bacillus subtilis, Phyopathogen

I. INTRODUCTION

Fungal pathogens pose serious problems worldwide and cause a number of plants and animal diseases such as ringworm, athlete's foot, and several more serious diseases. Plant diseases caused by fungi include rusts, smuts, rots, and may cause severe damage to crop[1]. Fungal pathogens cause devastating losses of crops and postharvest fruits throughout the world [2], and are a major threat to crop. Post-harvest food spoilage also represents a potential health hazard for humans due to the production by phytopathogens of toxic metabolites [3]. *Helminthosporium* is a generic name, for a group of fungal species, well known for its phytopahogenicity. These species are commonly associated with leaf spots or blights, foot rots, and other disease syndromes on cultivated and wild *Poaceae*. Their ability to cause devastating disease has occasionally resulted in famine and loss of human life [4] or in great economic loss [5]. Other plant groups are also affected: palms [6], cacti [7], *Leucospermum spp*., beans, forage legumes[8], rubber, Musa, and coffee [9] are reported as hosts. In addition, mycoses in domestic animals and man increasingly are being attributed to these fungi. They have been

implicated in mycotoxicoses in grazing animals, and mycoparasitic activity by them has been reported [10]. Spot blotch caused by *Bipolaris sorokiniana* is one of the most important foliar diseases limiting wheat production in warmer, nontraditional growing areas. Due to infection of such fungi up to 15% crop loss has been reported [11].

Antifungal antibiotics, effective against fungal disorders, are relatively few mainly because of their solubility and toxicity problems [12,13]. Many artificial chemical fungicides have been used to prevent and kill fungi in various environments. However, because of their huge populations and high frequency of mutation, a large amount of pathogenic fungi may easily acquire resistance to frequently used fungicides. Several important chemical fungicides such as anilinopyrimidine, benzimidazoles, demethylation inhibitors (DMI), dicarboximide, phenylpyrrole, Qo respiration inhibitors, and strobilurin, have lost their efficacy against pathogenic fungi in the field [2]. Excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide [1]. To reduce the risk of crop disease and enhance the safety of food and the environment, new, safer fungicides should be discovered and developed [14].

In order to screen new antifungal compound, we had isolated many microorganism and screened for its antifungal activity. Morphological and metabolic characteristic of isolated strain was studied in detail. Antifungal activity against *Helminthosporium spp*. was studied using agar cup method.

II. MATERIAL AND METHODS

2.1 Sample Collection

Several soil samples were collected from the vicinity of Navsari City, Gujarat, India. Soil samples were collected in sterile polyethylene bag, close tightly and immediately transferred to Microbiology Laboratory of NLCPAS, Navsari. Samples were stored in refrigerator till next use.

2.2 Isolation of Microorganism

Serial dilution spread plating method is used for the isolation and enumeration of microorganisms. Isolation was carried out on sterile nutrient agar plate (composition : 0.5% peptone; 0.3% beef extract; 1.5 % agar; 0.5% NaCl; pH was adjusted to 7.2). Aliquots were spread on the plates. Plates were incubated at 37 C for 24 h. Next day, various bacterial colonies were observed and subjected for screening of anti-Helminthosporium activity.

2.3 Screening of Microorganism

Screening was carried out by spreading *Helminthosporium spp.* on the sabouraud dextrose agar plate (Composition: Table 1) . Each isolated microorganism was streaked in circular form in the center of plate. Inoculated plates were incubated at 30 C for 24- 48 hr. Screening was done on the basis of its size of zone of inhibition produced by microorganism.

2.4 Identification of isolate

Morphological study of isolate was carried out by studying Gram staining characteristics. Various biochemical characteristic analyses were done on the BD Pheonix^{TM} [15].

2.5 Production and Extraction of Antifungal compound

Stage 1: Fermentation

Nutrient broth (pH 7.2,100 ml) was prepared and then under aseptic conditions a loop full of purified growth added. This broth was incubated at 28°C in shaking incubator at 150 rpm for 5 days. After 5 days the inoculums for fermentation process was ready for use.

Stage 2: Extraction

After fermentation, the medium was harvested and centrifuged to remove cells and debris. Supernatant is collected in a sterilized screw cap bottle. Filter the fermented broth. The filtrate was mixed with ethyl acetate in the ratio of 1:1 (v/v) and shaken vigorously for 1 h in a solvent extraction funnel.

The solvent phase that contains antibacterial compound was separated from the aqueous phase. Solvent phase was evaporated to dryness in water bath at 80 - 90°C and the residue is used to check antibacterial activity [16].

2.6 Study of Antifungal activity

Determination of antifungal activities of pure bacterial culture was performed by using modified agar cup method. To determine the effect of bacteria on *Helminthosporium spp*., bacteria was spread on the surface of sabouraud dextrose agar.

One mycelial disk (6 mm in diameter), from the edge of 3 to 5- day-old-colony of *Helminthosporium* spp. grown on SDA, was put onto the center of each plate. Inoculated sabouraud dextrose agar plates were incubated at 28°C for 4 days. Control plates were prepared in the same way without spreading bacteria. After 4 days, diameter of fungal colony was measured.

III.RESULT AND DISCUSSION

3.1 Sample Collection

Soil is considered as a rich source of the microorganisms. Soil samples were collected from the agricultural land from the vicinity of the Navsari District, Gujarat. Soil-borne bacteria that are antagonistic to plant pathogens could make a substantial contribution to prevention of plant diseases[17].

3.2 Isolation of Microorganism

Isolation was done on Nutrient Agar plate. Total 30 different types of bacteria were isolated from the soil samples on the basis of its colony characteristics (Fig. 1).

3.3 Screening of Microorganism

Screening of the organism was done its ability to form zone of inhibition. As a result of screening one bacterial strain, designated as UKB2, was selected for further study (Fig. 2). UKB2 was Gram positive, motile, rods in chain. Colony was irregular shaped, flat and become creamy after 48 hours.

Table 1 Composition of Sabouraud Dextrose Agar

Sr. No.	Component	Amount
1	Peptone	10 gm
2	Glucose	40 gm
3	NaCl	05 gm
4	Agar	20 gm
5	Distilled Water	1000 ml
	pH	5.2

Figure 1 Colony Characteristics of UKB2 **Figure 2 Screening of anti-*Helminthosporium* microorganism**

3.3 Identification of Isolate

On the basis of its Biochemical characteristics, studied with the help of Pheonix^{TM} , UKB2 was identified as *Bacillus subtilis*. Pheonix^{TM} is one of the reliable automated identification systems [18].

3.4 Study of Antifungal activity

Antifungal activity was studied by the agar cup method. Results showed that the diameter of the fungal colony on the test plate was 3 mm while on control the diameter of fungal colony was 70 mm (Fig. 3).

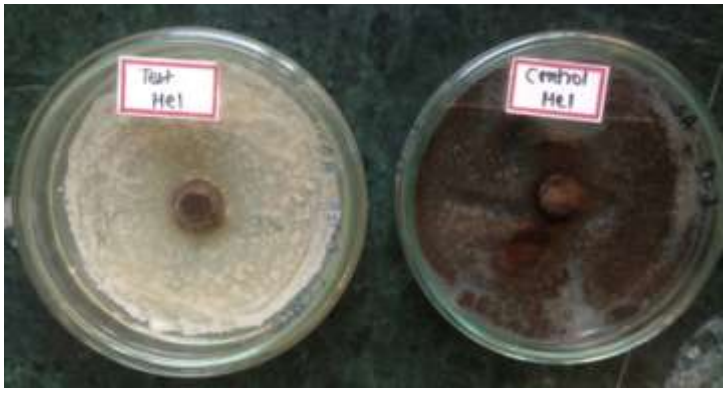

Figure 1 Anti-Helminthosporium Activity of *Bacillus Subtilis*

There are various reports available that variety of antifungal compound is produced by *Bacillus subtilis*. It had been already reported that an antifungal compound, known as Bacillomycin, is produced by *Bacillus subtilis*. It also produced some volatile compounds, having antifungal activity[19].

3.5 Study of Antifungal activity of extracted compound

Ethyl acetate extracted metabolites also showed antifungal activity.

IV. CONCLUSION

As *Helminthosporium spp*. is causing very serious problems in our food crops like wheat, new infection management strategies must be devised. As *Bacillus subtilis* is showing promising anti-Helminthosporium activity, it can be applied as biocontrol agent. Extracted compound is also showing this activity, so, purified compound can be also applied after toxicity study of the compound. In future, purified compound isolation and its toxicity should be studied.

REFERENCES

[1] H. Sharma, and L. Parihar, Antifungal activity of extracts obtained from actinomycetes, *Journal of Yeast and Fungal Research*, *1(10)*, 2010, 197-200.

[2] H. Chen, L. Wang, C. Su, G. Gong, P. Wang, and Z. Yu, Isolation and characterization of lipopeptide antibiotics produced by Bacillus subtilis, *Letters in applied microbiology*, *47(3)*, 2008, 180-186.

[3] Z. Zhang, S. Zhang, and M. Wua, Isolation, Purification and Structure Determination of a Novel Antifungal Compound.

[4] S. Padmanabhan, The great Bengal famine, *Annual Review of Phytopathology*, *11*, 1973, 11-24.

[5] A. Ullstrup, The impacts of the southern corn leaf blight epidemics of 1970-1971, *Annual Review of Phytopathology*, *10(1)*, 1972, 37-50.

[6] L. Forsberg, Foliar diseases of nursery-grown ornamental palms in Queensland, *Australasian Plant Pathology*, *14(4)*, 1985, 67-71.

[7] A. Chase, Stem rot and shattering of Easter cactus caused by Drechslera cactivora [Rhipsalidopsis gaertneri, Ornamental], *Plant Diseases (USA)*, 1982.

[8] M.B. Ellis, Dematiaceous hyphomycetes, *Dematiaceous hyphomycetes.*, 1971.

[9] V. Smedegård-Petersen, Pathogenesis and genetics of net-spot blotch and leaf stripe of barley caused by Pyrenophora teres and Pyrenophora graminea, 1976.

[10] J. Alcorn, The taxonomy of" Helminthosporium" species, *Annual Review of Phytopathology*, *26(1)*, 1988, 37-56.

[11] E. Duveiller, and I. Garcia Altamirano, Pathogenicity of Bipolaris sorokiniana isolates from wheat roots, leaves and grains in Mexico, *Plant pathology*, *49(2)*, 2000, 235-242.

[12] S. Raytapadar, and A. Paul, Isolation of actinomycetes from different soils of West Bengal and variations in their antimicrobial spectrum, *Environment and ecology. Kalyani*, *13(4)*, 1995, 884-888.

[13] J. Berdy, The discovery of new bioactive microbial metabolites: screening and identification, *Progress in industrial microbiology*, *27*, 1989, 3-27.

[14] F. Coloretti, S. Carri, E. Armaforte, C. Chiavari, L. Grazia, and C. Zambonelli, Antifungal activity of lactobacilli isolated from salami, *FEMS microbiology letters*, *271(2)*, 2007, 245-250.

[15] E. Stefaniuk, A. Baraniak, M. Gniadkowski, and W. Hryniewicz, Evaluation of the BD Phoenix automated identification and susceptibility testing system in clinical microbiology laboratory practice, *European Journal of Clinical Microbiology and Infectious Diseases*, *22(8)*, 2003, 479-485.

[16] H.M. Atta, An antifungal agent produced by Streptomyces olivaceiscleroticus AZ-SH514, *World Applied Sci. J*, *6(11)*, 2009, 1495-1505.

[17] M. Islam, Y. Jeong, Y. Lee, and C. Song, Isolation and Identification of Antifungal Compounds from *Bacillus subtilis* C9 Inhibiting the Growth of Plant Pathogenic Fungi, *Mycobiology*, *40(1)*, 2012, 59-66.

[18] J. Snyder, G. Munier, and C. Johnson, Direct Comparison of the BD Phoenix System with the MicroScan WalkAway System for Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae* and Nonfermentative Gram-Negative Organisms, *Journal of Clinical Microbiology*, *46(7)*, 2008, 2327–2333.

[19] L. Yongfeng, C. Zhiyi, T. Ng, Z. Jie, M. Zhou, S. Fuping, L. Fan, and L. Youzhou, Bacisubin, an antifungal protein with ribonuclease and hemagglutinating activities from *Bacillus subtilis* strain B-916, *Peptides*, *28*, 2008.