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Processed exudate of pathogen can kill wild pathogens: Successful in vitro application of newly synthesized novel antimicrobial therapeutic against multiple human pathogens & plant pathogens

[1]Arghya Chakravorty, [2] Aishwarya Das, [3] Arabinda Samanta, [4] Nalluri Abhishek*

[1]SANCHARAN, 4289/2003, Agartala, India – 799001
[2]Department of Microbiology, Vidyasagar University, Midnapore, India – 721102
[3]Department of Botany, Jhargram Raj College, Jhargram, India - 721507
[4]Center for Fuel Cell Innovation, Huazhong University of Science and Technology, Hubei-430074, China

Corresponding Author: nalluri_abhi@hust.edu.cn

Abstract: *Rhizoctonia solani* is a plant pathogenic fungus. In this study synthesis of an antimicrobial therapeutic agent is carried out using cell free culture supernatant of *Rhizoctonia solani*. The synthesized therapeutic or biomedicine was analyzed using UV-VIS spectrophotometry which showed the appearance of SPR centered near about 520nm. The therapeutic agent can be cadmium sulphide nanoparticles (CdSNPs). Antimicrobial assay of this newly synthesized therapeutic agent was performed against four prokaryotic pathogens which are usually human pathogen & seven eukaryotic pathogens which are mainly plant pathogen. The newly synthesized therapeutic agent revealed their effective antibacterial activity against *Bacillus subtilis* MTCC430 by forming 0.8 mm clear zone, against *Bacillus cereus* MTCC441 by forming 1 mm clear zone, against *Vibrio parahaemolyticus* by forming 0.9 mm clear zone. MIC has been recorded as 111.12 µg/ml for *B. subtilis* MTCC430, 107.11 µg/ml for *B. cereus* MTCC441 & 79.38 mg/ml for *Vibrio parahaemolyticus*. The newly synthesized therapeutic is also revealed positive activity against *Sclerotinia sclerotinum* & *Alternaria alternata*.

***Key Word*– Antimicrobial therapeutic agent, Anti-fungal activity, Anti-bacterial activity, *Rhizoctonia solani*, CdS nanoparticles.**

I. INTRODUCTION

Microorganism has gained immense importance in recent trends of biotechnology. Fungi is one such diversified microorganism in the field of medicine, agriculture etc. These are of significant interest because of their easy scale up procedure and economic feasibility. Medicines based on application of the principles of natural sciences, especially biology and biochemistry is termed as biomedicine. Biomedicine are now of great importance due the problem of multiple drug resistance of the pathogens against traditional drugs. The therapeutics prepared by using microorganism and their exudates are eco-friendly and also cost effective.

In this study, efforts have been made to synthesize an antimicrobial from the exudate of the pathogenic fungi Rhizoctonia solani. Antimicrobial assay of this synthesized therapeutic was conducted against four pathogenic bacteria (*Bcillus subtilis* MTCC430, *Bacillus cereus* MTCC441, *Vibrio parahaemolyticus*, *Shigella flexneri* MTCC7061) & seven pathogenic fungi (*Sclerotinia sclerotinum*, *Fusarium spp. Geotrichum spp. Cercospora spp. Colleotrichum lagenarium*, *Alternaria alternata*, *Rhizoctonia solani*).

The bacteria are human pathogens, among which *B. subtilis* is considered as a positive model organism. The seven fungi are all plant pathogens.

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II. MATERIALS AND METHODS

Microorganisms:

All the microorganisms; i.e. four pathogenic bacteria - *Bcillus subtilis, Bacillus cereus, Vibrio parahaemolyticus, Shigella flexneri*& seven pathogenic fungi - *Sclerotinia sclerotinum, Fusarium spp. Geotrichum spp. Cercospora spp. Colleotrichum lagenarium, Alternaria alternata, Rhizoctonia solani* were collected from laboratory.

Chemicals:

These chemicals were used for this research purpose: potato dextrose broth (HiMedia Laboratories), potato dextrose agar (HiMedia Laboratories), nutrient broth (HiMedia Laboratories), thioacetamide (MERCK), cadmium nitrate (MERCK), agar agar (HiMedia Laboratories), deionized water etc.

Czapek-Dox media (modified):

Starch 3g, KCl 0.05g, $NaNo_3$ 0.3g, $MgSo_4$ 0.05g, K_2HPO_4 0.1g, $FeSO_4$ 0.001g, agar 2g were dissolved in 100 ml of distilled water [p^H 7.0(±0.2)] and autoclaved 10 psi for 30 minutes.

Synthesis of Therapeutic Agent:

2 mm^2 mycelia inoculum of pathogenic fungus Rhizoctonia solani was inoculated separately in 250 ml Erlenmeyer flasks containing 50 ml of sterilized Czepak-Dox media and incubated at 28 ºC with gentle shaking at 150 rpm. After incubation for 7 days cells were harvested aseptically by zero mess shives and then washed several times with sterilized deionized water (mili Q).Washed mycelium was then suspended in 250 ml Erlenmeyer flask containing 50 ml sterilised deionized water and incubated for 3 days at 28 ºC with gentle shaking at 150 rpm. After that, biomass was precipitated by centrifugation at 5000 rpm for 20 min. and cell free supernatant was collected for further experiment.

0.001M $Cd(NO_3)_2$ solution and 0.005M thioacetamide was prepared priorly. Then 6ml of prepared 0.001M of $Cd(NO_3)_2$ was taken in a beaker and heated at 80 ºC for 5 min. 4ml of 0.005M thioacetamide was added followed by the cell free supernatant of *Rhizoctonia solani*. The solution was heated up to 20 min at 80 ºC. The color of the solution changed to bright yellow. The color change indicated that there was formation of the therapeutic agent.

The yellow color solution was analyzed using UV-Vis spectrophotometer. The appearance of SPR centered near 520 nm indicated that the synthesized therapeutic agent can be a cadmium sulphide nanoparticle.

Antimicrobial Activity Study:

Antimicrobial effectiveness of the newly synthesized therapeutic agent was determined by standard agar well diffusion method. The sensitivity of newly synthesized therapeutic agent against test organisms was calculated by measuring the clear zone produced due to the diffusion of the solution in the agar. It has been observed that the diameter of zone of inhibition is directly proportional to the concentration of antimicrobial agents, upon a limit (after which due to decrease in diffusion capacity remain or less same).

Bactericidal Activity Analysis:

Evaluation of bactericidal activity of the newly synthesized therapeutic agent was conducted against four human pathogenic bacteria .At first the solidified nutrient agar plates were swabbed with the four pathogenic bacterias namely (two gram positive *B. cereus* MTCC441 and *B. subtilis* MTCC430; two gram negative *S. flexneri* MTCC7061 and *V. parahaemolyticus*. The wells were prepared on the agar plate with the help of a cork borer (0.6 cm diameter). As a standard, streptomycin solution (0.2 mg/ml) was prepared and poured in the well of each plate.

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The newly synthesized therapeutic agent (100 mg/ml) was loaded onto the wells of each plate. The plates were then incubated for 24 h at 37 °C and the antibacterial activity was determined by measuring the diameter of the zone of inhibition using a ruler and expressed in mm.

Fungicidal Activity Analysis:

Actively growing fungal plant pathogens (*Sclerotinia sclerotinum*, *Fusarium* sp., *Geotrichum* sp., *Cercospora* sp., *Colleotrichum lagenarium*, *Aternaria alternata* and *Rhizoctonia solani)*were used for the preparation of fungal plates. A small piece of the mycelium was transferred to the middle of sterile standard potato dextrose agar (PDA) plates & incubated for 2 days at 25 °C. After 2 days, three wells (6 mm) were prepared by using a sterile cork borer at equal distance around the mycelia growth of pathogenic fungi. The newly synthesized therapeutic agent at different concentrations (4 µg/ml, 2 µg/ml, 1 µg/ml) were loaded onto each well and allowed to grow for 3 days. The inhibition of growth of plant pathogenic fungi around the well refers to antifungal activity of the synthesized therapeutic agent.

Identification of Minimum Inhibitory Concentration (MIC):

Broth dilution method was carried out for determining MIC of the newly synthesized therapeutic agent, against three bacteria *B. subtilis* MTCC441, *B. cereus* MTCC430 and *V. parahaemolyticus* separately. Various concentrations of the synthesized therapeutic agent was taken in a series of test tubes containing 3.0 ml of sterile nutrient broth were inoculated with 100 µl (10^8 CFU/ml) bacterial culture. Control tubes were maintained without the synthesized therapeutic agent. After that tubes were incubated for 24 hours at 37 °C. The MIC was determined by observing the visible turbidity and by measuring optical density (O.D.) at 600 nm of the culture broths respectively.

III. RESULT AND DISCUSSION

Ultraviolet-visible spectroscopic study:

The newly synthesized therapeutic agent was analyzed using UV-Vis spectrophotometer. UV-vistra showed the appearance of single and SPR centered around 520 nm which indicated that the therapeutic agent synthesized from exudate of Rhizoctonia solani can be a cadmium sulphide nanopartical. (CdSNPs).

Antibacterial activity observation:

Antibacterial potency of the synthesized therapeutic agent was significant against Gram positive bacteria. Maximum inhibitory zone was observed against *Bacillus subtilis* MTCC441 of 1.8 mm followed by *Bacillus cereus* MTCC430 of 1.5 mm. The synthesized therapeutic did not show any antimicrobial effect on Gram negative bacteria. It can be very useful against Gram positive as it shows appreciable clear zone in respect to streptomycin.

Antifungal activity observation:

Among the seven tested plant pathogenic fungi, the synthesized therapeutic agent only inhibit the growth of three pathogenic fungi. After performing the test with different concentration of the synthesized therapeutic agent, it was found that 4 µg/ml concentrations inhibit the growth of *Sclerotinia* sclerotinum and produces a hollow zone around the well loaded with the synthesized therapeutic, but can't inhibit in lower concentration. It also inhibits the growth of *Alternaria alternata and Geotrichum* sp. at the same concentration.

Minimum inhibitory concentration (MIC):

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MIC of the newly synthesized CdSNPs was determined against *B. subtilis* MTCC430, *B. cereus* 441 and *V. parahahaemolyticus* with the help of standard broth dilution method. It was observed that the growth of the tested two Gram positive bacteria *B. subtilis* MTCC430, *B. cereus* MTCC441 and Gram negative *V. parahaemolyticus* was inhibited by all the concentration of the synthesized therapeutic agent (μg/ml) used and MIC has been recorded as 79.38 μg/ml for *B. subtilis* MTCC430, 111.13 μg/ml for *B.cereus* MTCC441 and 23.33 mg/ml for *V. parahaemolyticus*. Culture without the synthesized therapeutic agent did not show any growth inhibition.

IV. Conclusion

In recent trends of biotechnology, microorganisms are serving as bio machinery for synthesis of many therapeutic agents. The increasing resistance of pathogens against traditional antimicrobials is giving rise to the need of developing new antimicrobial therapeutics. The processed exudate of the plant pathogenic fungus, Rhizoctonia solani can serve as a new antimicrobial. As stated above the newly synthesized therapeutic is a biomedicine and can be a CdSNPs as well. Further research is needed to analyse the synthesized therapeutic as a nanopartical. The synthesized therapeutic has application in the field of health and agriculture; as the study reveals its potency as an antimicrobial against plant and human pathogens, namely Sclerotinia sclerotinum , Alternaria alternata, Geotrichum sp. and B.subtilis MTCC441, B.cereus MTCC430. The process is cost effective and easy to synthesize. Further toxicological study is required to check if its application is hampering human health as well as the environment.

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