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Research Article

ISOLATION AND CHARACTERIZATION OF PESTICIDE DEGRADING BACTERIA *ACINETOBACTER BAUMANNII* A85 FROM THE MICRO-NICHE OF *ORYZA SATIVA* L. AND ITS ANTIOXIDANT ACTIVITY

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A R T I C L E I N F O

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A B S T R A C T

Pesticide degradation is one of the major problems facing today which contaminate the environment. Microorganisms are most commonly used in the biodegradation of pesticides. The present study is focussed on the isolation and characterization of pesticide degrading bacterial strain from the micro niche of rice grains by 16srRNA gene sequencing, using colony PCR. The bacteria were isolated from the micro niche of rice grains using 50 ml of MSM medium, with 200mgl^{-1} of canistan and 200 mgl^{-1}of 20 % flubendiamide (diamide pesticide) and the cultures were maintained by suspending pure single colonies in nutrient agar broth. Morphological characterization of isolated strain was done by Gram staining and found that were pink coloured, aerobic and gram negative cocco-bacilli. The preliminary identification and characterization of isolated bacteria under study were done by using VITEK 2 machine, which read the isolated bacteria as *Acinetobacter baumannii*. Using colony PCR amplification of 16S rRNA gene sequence of *Acinetobacter baumannii* was done in order to confirm the bacterial strain with the help of BLAST search tool. The isolated bacterial strain, *Acinetobacter baumannii* was checked for the biodegradation of pesticides at different concentration, i.e., 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml. The maximum growth occurred in MSM medium with 2.0 mg/ml 20% of flubendiamide. Theisolated bacterial strain is also showed antioxidant property. It was observed that cell free extract of *Acinetobacter baumannii* A85 have highest inhibitory action at 72 hour culture.

INTRODUCTION

Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to other organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms [(1)]. Degradation of pesticides involves both biotic transformation processes mediated by microorganisms or plants and abiotic processes such as chemical and photochemical reactions. Biodegradation is generally recognized as the mass balance-wise most important route of pesticide degradation. Nowadays we are using various chemical agents to increase the productivity. In India the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion [(2)].

Plants, animals, and fungi (Eukaryota) typically transform pesticides by detoxification or through fortuitous metabolism by broad-spectrum enzymes; bacteria (Prokaryota) more commonly metabolize them for assimilation as essential nutrients and energy. In addition, facile horizontal transfer of biodegradation genes is known to occur within microbial populations, and this has been observed to spread newly evolved biodegradation pathways globally[(3)]. The microorganisms associated with the plants have many other uses. They may show antagonistic activity against other organisms invading the plants. So they can be used as bio-control agents to control the infection and spreading of pathogen [(4)]. Patented Biological Control Agents (BCAs) are made of bacteria[(5)]. The present study is focussed on the biodegradation of pesticide flubendiamide using bacterial strains isolated from rice grains.

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MATERIALS AND METHODS

The grains of rice (*Oryzasativa* L.) were collected from common farmers of AdatGramaPanchayat, Thrissur and stored at 4°c.

Isolation of pesticide degrading bacteria from rice grains

The rice grains were surface sterilized with 70% ethanol for 1-2 minutes, 2.5% sodium hypochlorite for 2 minutes, followed

by three rinses with sterile distilled water. 0.1 g surface sterilized rice grain was grounded into fine powder using mortar and pestle and collected in Erlenmeyer flask containing 50 ml of MSM medium, with 200mgl^{-1} of canistanand 200 mgl^{-1}of 20 % flubendiamide (diamide pesticide). Cultures were incubated at 30^{0}C and shaken at 100 rpm for 7 days. Two millilitres of the culture were then transferred to a fresh MSM medium containing 200 mgl^{-1} of 20% flubendiamide. Cultures from the fifth transfer were plated on nutrient agar and incubated for 24 hr at 30^{0}C[6].

Biodegradation studies of isolated bacterial strain:

Biodegradation assays were performed in 250 ml conical flasks containing 100 ml of MSM medium amended with series of 20 % flubendiamide concentrations, i.e., 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml. 0.1 ml of bacterial culture kept overnight was inoculated into each flasks. The flasks were incubated at 35±2^{0}C on rotary shaker at 100 rpm. Each sample is streaked on sterile petri plates with nutrient agar to check the growth of bacteria. From each sample 2 ml is centrifuged at 5000 rpm for 20 min. To remove residual nutrients, cells were washed twice by centrifugation (5000 rpm, 20 min) using 0.85% NaCl. The obtained residue used to measure the dry biomass of bacteria [7].

Molecular identification of bacterial strain using 16S rDNA

Pure cultured bacterial colony was grown in liquid nutrient medium for 20-24hrs. Cultures were then centrifuged at 16,000 rpm for 1 minute and discard supernatant. Pellets were collected in eppendorf tube was resuspended in lysis buffer (CTAB). Remove the supernatant and add mercaptoethanol into it and incubate at 70°c for 60 mins. Add equal volume of phenol: chloroform: isoamylalcohol (25:24:1) into the supernatant and centrifuge for 10 mins at 4°c. Add chloroform and take upper aqueous layer. Later add 1/10th volume of sodium acetate and 7/10th volume ice-cold isopropanol. Mix the tube gently and incubate at room temperature for 15 minsand then centrifuge (12,000 rpm) and collect the pellets as the sample DNA. Then sample was added with 50 ml TE buffer PH 8.0 and stored at -20°c.For molecular identification of bacteria, add 25 µl of PCR Master mix in 0.2 ml eppendorf tube. The universal primer used – 8F 5' AGAGTTTGATCCTGGCTCAG 3' and 1522R 5' AAGGAGGTGATCCAGCCGCA 3', to ampilify 1500 bp of 16srDNA gene. Amplified product was then visualized by gel electrophoresis.

Sequencing

Sequencing of the 16s rRNA gene of the bacterial isolates was done in Sci. genome, Kakkanadu and the sequence obtained were submitted to NCBI-Gen Bank and the percentage of sequence matching was also analysed to know the strain of isolated bacteria with the accession number using BLAST search tool.

Determination of antioxidant activity of isolated bacterial strain

Free radical scavenging activity of the cell free extract was measured using spectrophotometer. To 500µl of the cell free extract, 3.0 ml of freshly prepared solution of 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) at a concentration of 0.05 mg/ml (methanol) was added. Control was prepared using 500µl of methanol added to 3mL DPPH solution, mixed in dark and incubated for 30 min. Absorbance was recorded at 517nm after 30min of incubation in the dark with un-inoculated nutrient broth serving as blank. The readings were recorded in triplicates and the average absorbance value was calculated. Ascorbic acid (100µg/ml) was used as reference standards [8]. The percentage of radical scavenging activity was calculated according to the equation as follows,

$$\text{Percentage of radical scavenging} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

RESULTS

Isolation and characterization of pesticide degrading bacteria

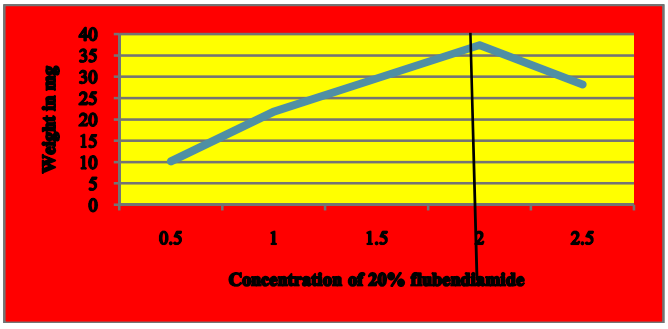
One bacterial strain was isolated which have the ability to grow in MSM medium amended with 20% flubendiamide. The sequence obtained was 995 kb length (AAACA----GGTCCT) was subjected to BLAST. The percentage of sequence matching analysed and the sequence was submitted to NCBI – Genbank. From the Genbank results, it was observed that the isolated bacterium shows more similarity towards the first bacteria of NCBI check list. The bacteria under study shows maximum score 1700, total score 10189, 97% query cover and 0.0 E-value. And it was concluded that the isolated bacterium was *Acinetobacter baumannii* A85.

Pesticide degradation activity of Acinetobacter baumannii

The isolated bacterial strain, *Acinetobacter baumannii* was checked for the biodegradation of pesticides at different concentration, i.e., 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml. The maximum growth occurred in MSM medium with 2.0 mg/ml 20% of flubendiamide. Dry biomass was calculated. Maximum biomass was obtained in 2.0 mg/ml concentration as shown in table no.1.

Table no 1 The dry biomass of isolated bacteria grown in different concentration of flubendiamide

Sl. No	Concentration of flubendiamide in medium (mg/ml)	Dry biomass of bacteria (mg/ml)
1	0.5	10.2
2	1.0	21.8
3	1.5	29.6
4	2.0	37.4
5	2.5	28.2



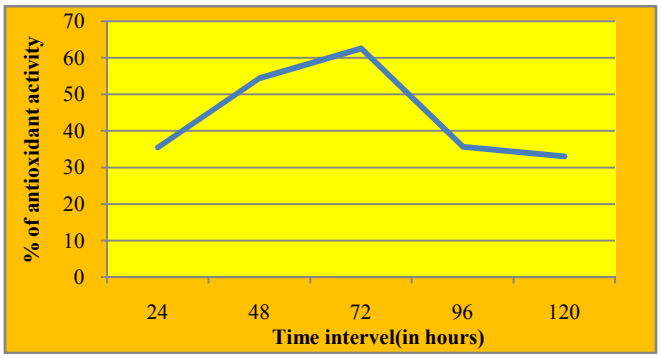
Graph 1 Dry biomass of bacteria in different concentration of pesticide flubendiamide

Antioxidant activity of isolated bacteria Acinetobacter baumannii A85

DPPH, a relatively stable organic radical has been widely used in the determination of antioxidant activities of cell free extracts of bacteria. The scavenging ability of the cell free extracts of the *Acinetobacter baumannii* A85 was compared with the standard antioxidant ascorbic acid. The cell free extract of the bacteria at different incubation period was used to check the antioxidant property. The 72 hour culture reported the highest percentage inhibition of DPPH. The rate of inhibition decreases from this incubation period.

Table no 2 Antioxidant activity of *Acinetobacter baumannii* A85

Sl. No.	Incubation period (in hours)	Percentage inhibition of DPPH
1	24	35.48 %
2	48	54.38 %
3	72	62.5 %
4	96	35.6 %
5	120	33.0 %



Graph 2 Antioxidant activity of *Acinetobacter baumannii* A85

DISCUSSION

Rice (*oryza sativa*, L) is economically important cereal which plays an important role in Indian economy. The present study is based on the isolation and characterization of bacterial strain from the micro niche of rice grains. The isolated bacterial strain was studied for their pesticide degrading activity. Also the isolated bacteria were checked for the antioxidant activity. Pesticide degradation study of freshly incubated strain was studied under different concentration of pesticides to find out the rate of bacterial degradation of pesticides. It was found that the maximum degradation of pesticide is at 2.0 mg/ml pesticide. Theisolated bacterial strain is also checked for antioxidant property. It was observed that cell free extract of *Acinetobacter baumannii* A85 have highest inhibitory action at 72 hour culture.

CONCLUSION

The isolation and molecular identification of pesticide degrading bacterial strain *Acinetobacter baumannii* A85 paved way for the bio-degradation of pesticides. Since this bacterial strain possesses this activity, it can act as a good biodegradation agent against pesticide like flubendiamide which is not easily degraded in natural condition. Also bacteria show good antioxidant activity and thus in future studies bioactive compounds can be isolated.

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