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CONTROL OF PHYTOPATHOGENIC BACTERIA USING PLASTIC DEGRADING NITROGEN FIXING MICROBES

Packialakshmi. N*[1] and Badhru Nisha. R[1]

*[1]PG and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Trichirappalli - 620020, Tamil Nadu, India.

ABSTRACT

The soil samples were collected from the polythene and plastic waste dumped site. The collected samples were subjected to serial dilution and plating technique. The isolated microbial strains were identified based on their cultural morphological and biochemical study. It was concluded that *B.subtilis*(I), *B.subtilis*(II), and *B.megaterium* degrade the polythene and plastic materials effectively. The infected leaves of *Abelmoshus esculentus*, *Solanum melongena* and *Solanum lycopersicum* were collected and the bacterial phytopathogens *Pseudomonas aeruginosa*, *Pseudomonas solanacearum* and *Xanthomonus vesicatoria* were isolated and identified through biochemical tests. The antagonistic effect of isolated plastic degrading microbes against bacterial phytopathogens was analyzed by zone of inhibition method. The functional groups were identified through IR. From this present study we concluded that the plastic degrading microbes can control the bacterial phytopathogens without any effects in plant.

KEYWORDS

Antimicrobial activity, Plastic degrading microbes and Phytopathogens.

Author of correspondence:
Packialakshmi. N,
PG and Research Department of Microbiology,
Jamal Mohamed College (Autonomous),
Trichirappalli - 620 020,
Tamil Nadu, India.

Email: packia_lakshmi_1977@yahoo.com

INTRODUCTION

Microorganism are frequently present in soil, manure and decaying plant tissues which are able to degrade wastes that are correlated with the substrate organic matter. Agriculture soil is a dynamic medium in which a large number of pathogenic and non-pathogenic bacterial and fungal flora live in close association. Microbes in the soil are the key to carbon and nitrogen recycling. Microorganisms produce some useful compounds that are beneficial to soil health, plant growth and play an important

role in nutritional chains that are important part of the biological balance in the life in our planet[1].

Biodegradation or biotic degradation or biotic decomposition is the chemical dissolution of materials by bacteria or other biological means. The term is often used in relation to ecology , waste management, biomedicine and the natural environment (bioremediation) and is now commonly associated with environmental eco-friendly products that are capable of decomposing back into natural elements. Organic material can be degraded by degraded aerobically with oxygen or anaerobically without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process[2, 3].

Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases, Beyond good agronomic and horticultural practices, growers often very heavily on chemical fertilizers and pesticide. Such inputs to agricultural have contribute significantly to the spectacular improvements in crop productivity and quality over the past 100 years. However, the environmental pollution caused by excessive use and misuse of agrochemicals as well as fear mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture.

A variety of biological controls are available for use, but further development and effective adoption will require greater understanding of the complex interaction among plants, people, and the environment. The present study concentrates to use plastic degrading microbes to control plant bacterial pathogens.

MATERIALS AND METHODS

Collection of soil sample

Soil samples were collected from polythene and plastic dumped site of Sankarapuram, Villupuram District, Tamil Nadu, India.

Serial dilution Technique (Aneja, 1996)

Serial dilution was performed by using the collected soil sample to isolate the sample bacteria. 1 gram of soil sample were diluted in the test tube containing 9 ml of sterile distilled water and mixed thoroughly to make a 1:10 dilution (10^{-1}). The 1 ml of diluted sample was transferred to the next test tube and serially diluted into the serious of test tubes, having 9 ml of sterile distilled water with sterile pipettes up to 10^{-6} dilutions[4].

Isolation of Bacteria from soil sample and Phytopathogens from infected leaves.

Identification of the microorganism

The collected sample microorganisms were grown in nutrient medium. The medium is defined as the substrate in which microorganisms could grow and multiply. For the present study, synthetic medium was used for culturing, conducting biochemical test and antibiotic sensitivity purpose. Synthetic media was one in which all the constituents are chemically defined. They are used to study the specific nutritional requirements of the microbes and following tests were conducted to identify the organisms.

Collection of Plant Pathogens

The infected leaves of *Abelmoschus esculentus, solanum melongena,* and *solanum lycopersicum* were from the Agricultural field of Sankarapuram, Villupuram District, Tamil Nadu, India.

Serial dilution Technique (Aneja, 1996)

Serial dilution was performed by using the collected sample to isolate the sample bacteria. The infected leaves were washed in the test tube containing 9 ml of sterile distilled water and mixed thoroughly to make a 1:10 dilution (10^{-1}). The 1 ml of diluted sample was transferred to the next test tube and serially diluted into the serious of test tubes, having 9 ml of sterile distilled water with sterile pipettes up to 10^{-6} dilutions.

Method

Spread plate technique was employed using Muller Hinton Agar medium (MHA).

Culture examination

Once the growth was found primary plate, then the identification was carried out by the following

systematic method, for examining the types of colony, colour change in the medium, morphology of the cells under stained and unstained conditions and biochemical tests were done by using Bergey's manual.

Disc Diffusion method

The disc diffusion method provided a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of substance. The disc is placed. The inhibition zone produced by the diffusion of the substance from the disc into surrounding medium.

Preparation of disc and medium preparation

Disc usually consist of absorbent paper impregnated with isolated microbes it was most convenient to use Whattman No.1 filter paper for preparing the disc. Dry discs of 6mm diameter were prepared and sterilized in autoclave.

For rapidly growing aerobic and facultative anaerobic bacteria, Muller Hinton agar was recommended, although this medium supports the growth of the most bacterial pathogens.

Procedure

Circular disc of 6mm diameter were prepared from Whatmann No.1 filter paper and sterilized in autoclave. These paper discs were impregnated with the sample of different concentration in the respective solutions and placed on Muller Hinton agar seeded with test bacterium. The plates were incubated at 37^{o}C for 24 hours the zone of inhibition around each disc was measured and the diameter was recorded.

Chi-square Test

The chi-square test was applied (George and Cochran, 1994). The purpose of Chi square test was decided to whether the set of observed date (anti-biogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (Ronald and Jones, 1933).

IR Spectrum Analysis

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600 cm^{-1}.

FTIR spectrum of the compound obtain from chemical precipitation was done using Shimadzu IR Affinity 1 instrument.

RESULTS AND DISCUSSION

The Present study deals with the isolation of Plastic degrading microbes by using enrichment medium. All the isolates were subjected to various tests to confirm identify.

In this study, plastic dumped site soil samples from sankarapuram, Villupuram district, Tamil Nadu, were used for the isolation of bacteria species using serial dilution and plating methods. Serially diluted sample were poured into the nutrient agar plates (10^{-3} to 10^{-6}) dilution (Figure No.1).

Gram positive and rod shaped motile organism showed positive results for MR, Catalase, Urease, oxidase and citrate test, and negative results for Indole, VP and catalase. For Triple Sugar Iron test, it showed the production of acid butt and the organism were identified as *Bacillus subtilis*. Whereas *Bacillus megaterium* negative results for Indole, MR, Citrate and Positive results were obtain for VP. With all the above results obtained, these organisms were confirmed according to Bergey's manual of systematic Bacteriology.

In earlier study also found that plastic degrading microbes isolated from forest soil to determine the plastics degrading of *Micrococcus luteus Masoniella* sp.[5,6,7].

Based on the colony morphology and microscopic observation the phytopathogenic bacteria's were isolated from infected leaves of *A.esculanthus, S.melongena* and *S.lycopersicum* and identified as *Pseudomonas aeruginosa Pseudomonas solanacearum* and *Xanthomonas vesicatoria* (Figure No.2) with all the above results obtained, these organisms were confirmed according to Bergey's manual of systematic Bacteriology.

Earlier study reported that accumulating of polythene wastes in the environment poses an ever increasing ecological threat. This study examined

the impacts of soil composting and poultry manure on biodegradation of polythene[8-11].

The *Bacillus subtilis I, Bacillus subtilis II* and *Bacillus megaterium* strain at 64 µg concentration under investigation revealed a wide spectrum of antagonistic activity against phytopathogenic bacteria such as *Pseudomonas aeruginosa Pseudomonas solanacearum* and *Xanthomonas vesicatoria* (Table No.1) (Figure No.3).

In the present study the antibacterial activity of phytopathogens were analysed by *invitro* disc diffusion method (I, II, IIIrd day). The result conclude that compare with the I and II and IIIrd day report the III rd day most of the pathogen were inhibited. Above result lead to the conclusion the data was consistent with the hypothesis, the diameter of zone of inhibition obtained from the observed data showed the similarities with the experimental data. In the present study Table No.2, 3 and 4 showed the analysis of infrared spectrum of *Bacillus subtilis I, Bacillus subtilis II* and *Bacillus megaterium.* The band in the IR spectra indicates the presence of Aldehydes, Alkanes, Alcohol, and Aromatic compounds, Compared with all the three tables, the Alkanes and Aromatic compounds commonly present in all samples.

Table No.1: Zone of inhibition formed by isolated microbes against phytopathogenic bacteria

S.No	Antibacterial agent	Name of the Bacteria	µg	Zone of inhibition in Diameter (mm)		$X^2=\Sigma[0-E)^2]/E$
				Standard Value	Observed value (Aqueous)	
1	*Bacillus subtilis 1* (Pellet)	*Pseudomonas aeruginosa*	64	22	13	3.68
		Pseudomonas solanacearum	64	22	15	2.22
		Xanthomonas vesicatoria	64	22	21	0.04
2	*Bacillus Subtilis 2* (Pellet)	*Pseudomonas aeruginosa*	64	22	11	5.5
		Pseudomonas solanacearum	64	22	11	5.5
		Xanthomonas vesicatoria	64	22	11	5.5
3	*Bacillus megaterium* (Pellet)	*Pseudomonas aeruginosa*	64	22	9	7.68
		Pseudomonas solanacearum	64	22	12	4.5
		Xanthomonas vesicatoria	64	22	21	0.04

Total value $X^2(0.05) = 3.81$

Table No.2: Infra-red spectrum analysis *Bacillus subtilis* 1 (3rd day)

S.No	Peak value	Stretching	Interpretation
1	3466.88	C-H stretching	Alkanes
2	3429.63	C-H stretching	Alkanes
3	2367.79	C-H stretching	Alkanes
4	2337.68	C-H stretching	Alkanes
5	2082.68	C-H stretching	Alkanes
6	1638.42	C=O stretching	Aldehyde and ketone
7	1438.12	C-O stretching	Alcohols
8	1359.37	C-O stretching	Alcohols
9	1114.06	C-O stretching	Alcohols
10	1022.47	C-O stretching	Alcohols
11	671.62	C-H def.stretching	Aromatic compounds

Table No.3: Infra-red spectrum analysis *Bacillus subtilis* 1(3rd day)

S.No	Peak value	Stretching	Interpretation
1	3908.73	C-H stretching	Alkanes
2	3755.87	C-H stretching	Alkanes
3	3433.47	C-H stretching	Alkanes
4	2372.32	C-H stretching	Alkanes
5	2339.38	C-H stretching	Alkanes
6	2080.24	C-H stretching	Alkanes
7	1854.11	C=0 stretching	Alcohol
8	1637.89	C-C stretching	Alkanes
9	1441.84	C-Hdef stretching	Aromatic compounds and ketone
10	1406.51	C-Hdef stretching	Aromatic compounds and ketone
11	1358.01	C-Hdef stretching	Aromatic compounds and ketone
12	1089.80	C-O stretching	Alcohols
13	680.61	C-Hdef stretching	Aromatic compounds and ketone

Table No.4: Infra-red spectrum analysis *Bacillus megaterium* (3rd day)

S.No	Peak value	Stretching	Interpretation
1	3918.23	C-H stretching	Alkanes
2	3441.08	C-H stretching	Alkanes
3	2392.01	C-H stretching	Alkanes
4	2081.76	C-H stretching	Alkanes
5	1638.41	C=C stretching	Alkanes
6	1414.63	C-Hdef stretching	Aromatic compounds and ketone
7	1436.26	C-Hdef stretching	Aromatic compounds and ketone
8	1358.95	C-Hdef stretching	Aromatic compound and ketone
9	1109.81	C-O stretching	Alcohols
10	676.60	C-Hdef stretching	Aromatic compounds and ketone

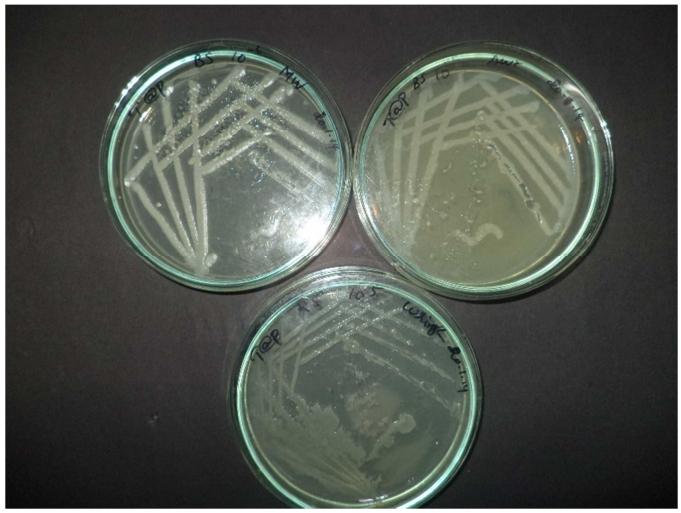


Figure No.1: Isolation of plastic degrading microbes from soil sample

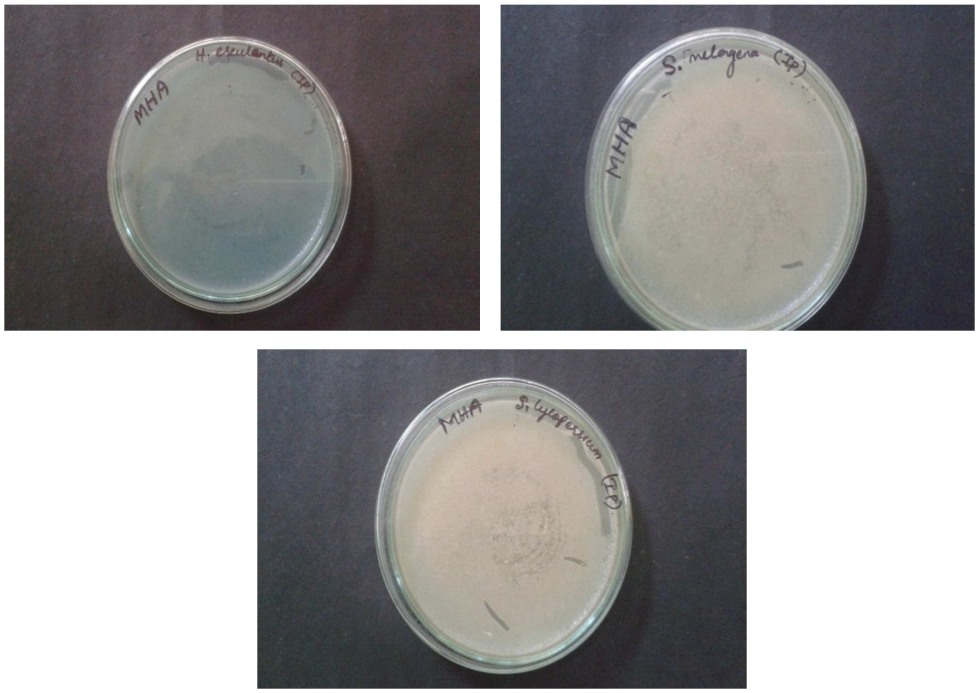
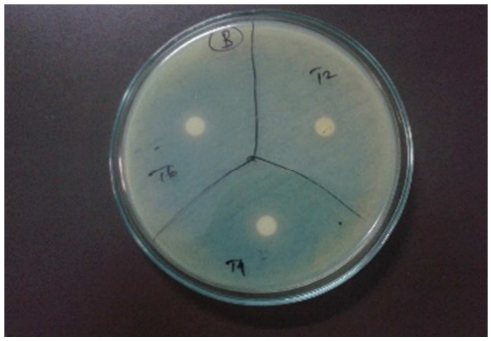


Figure No.2: Isolation of Bacterial pathogen from infected leaves

Pseudomonas aeruginosa *Pseudomonas solanacearum*

Xanthomonas vesicatoria



1. Bacillus subtilis 1(T2) 2. Bacillus Subtilis II (T4) 3. Bacillus megaterium (T6)

Figure No.3: Zone of inhibition formed by isolated microbes against phytopathogenic bacteria

CONCLUSION

The present study we concluded that the plastic degrading microbes can control the bacterial phytopathogens without any effects in plant and fix the nitrogen to the plant growth.

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CONFLICT OF INTEREST

None declared.

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