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ACTINOBACTERIA IN BIODEGRADATION AS A PROMISING SOLUTION FOR RUBBER DEGRADATION

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ABSTRACT

The study involves the use of Natural Rubber Degrading Actinobacteria isolated from rubber plantation soil for the degradation of the artificial rubber. Plate assay method by mineral salt medium was followed for the screening of bacteria for knowing its capacity to mineralize the rubber. Degradation of the rubber and the ability of the Isolates able to utilize the artificial rubber which was confirmed by Schiffs test and FTIR studies. In the current investigation it was concluded that the Actinobacteria have the capacity to mineralize artificial rubber. Hence such isolated cultures can be used in the utilities.

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INTRODUCTION

Recent years, waste disposal is a problem and the interest in the biodegradability of polymers, especially when the public is having greater concern about protecting human health and preserving the quality of our environment. Rubber became an integral part of contemporary life, and already formed a significant part of wastes in municipal landfills. Considering the environmental impact of solid wastes, recycling and composting options are expected to increase as the landfills capacity decreases. Managing the waste is a challenge facing the global community. Bioplastics can be composed of starches, cellulose, biopolymers and a variety of other materials (Aamer Alsihah *et al* 2008). As plastics and rubbers are not biodegradable, dumping of these causes a grave threat to human health and environmental pollution, it's a need of the hour to work on the degradation aspects of these polymers. Hence the present study focuses on the mineralization of artificial rubber by Actinomycetes strain isolated from contaminated soil of rubber plantation area.

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The growth Conditions were optimized for maximum mineralization which was evaluated by FTIR studies. Further the isolated strains were also applied in degradation of Rubber. The major users of natural rubber are tire and footwear industries. Natural rubber processing sector consumes large volumes of water and energy and uses large amount of chemicals procedures are used for preparing products from latex, there will always be an aqueous liquid as a byproduct (Rungruang and babel,2008).

MATERIALS AND METHODS

Rubbers: The Natural Rubber sheet was obtained from rubber rubber processing unit and Latex gloves was purchased from Medical store.

Microorganisms: Strains were isolated as described previously Linos *et al* 1998.The isolates were isolated from Rubber plantation soils of different areas in Kerala.

Medium and Growth of the Microorganism: Soil samples were collected from the different sites of rubber plantation areas and transported to the lab, the normal dilution technique was followed for the isolation of microbes.

Plating in mineral salt media with latex as carbon source is the media used for the isolation process. Mineral salt medium of Na_2HPO_412 $H_2O(9.0)$, $KH_2PO_4(1.5)$, $NH_4NO_3(1.0)$, $MgSO_4.7H_2O(0.2)$, $CaCl_2.2H_2O(0.02)$, $Fe(III)NH_4$Citrate (0.0012) and 0.1ml of the trace element solution 1000 x modified from Jendrossek *et al* 1997.The added carbon source for initial isolation was natural rubber latex 0.6% added to both liquid and solid MSM prior to autoclaving. The plating process with latex as carbon source was followed by Ibrahim *et al* 2006.

Screening of Rubber Degrading Organism

Soil samples were processed by the enrichment of cultures with mineral salt medium,0.1ml of each serial dilution was spread on MSM agar plates with an NR latex as the sole carbon source and incubated at 30ºC and incubated for 5days and the Colonies producing translucent halos are considered as positive and were purified by alternate transfer to MSM/NR media. The rubber degrading organisms previously isolated were inoculated directly on capable of degrading rubber utilized and rubber used as a sole source of carbon and showed growth on the latex agar. Growth experiments was carried out in mineral salt medium .In this experiment the mineral salt medium was dispensed to the conical flasks, to these conical flasks previously weighed rubber strips / latex gloves pieces were added. Rubber sample inoculated with organisms in mineral salt broth were kept in the shaker at 150 rpm at room temperature. To these flasks the Actinomycetes which are previously isolated were inoculated and the rubber sheets were checked for weight loss and also for the growth of the organisms after a time interval of 3 months. After the time period it was washed thoroughly, dried and final weight was recorded.

Identification of the Organisms able to degrade NR

Identification Method include morphology and basic biochemical tests Imvic ,TSI, and Urease was done, 16sRNA gene sequence analysis was also performed to identify and classify the isolates. Genomic DNA was extracted by standard methods (Warneke *et al* 2007). Nucleotide sequence of isolates were compared for identification using the data available of the gene bank databases at the National Center for Biotechnology Information.

Staining with Schiff Reagent

Staining of Natural rubber sheets and latex gloves with Schiff reagent was done followed by (Tsuchii, *et al.,* 1996).In a tightly stoppered bottle, 10ml of the fuschin reagent added to the sample and the purple color was developed for 10-30 min at room temperature. An amount of excess reagent was then discarded, and 10 ml of the sulfite solution was added in order to suppress the non specific color reaction of the blank sample. The composition of the fuchsin reagent (Ehrlich *et al*., 1948) was the following : 2g of fucshin dissolved in 50ml, glacial acetic acid 10 g , $Na_2S_2O_5$ 100ml, 0.1NHCl 50 ml of H_2O.The composition of the sulfite solution was 5g of $N_2S_2O_5$,5ml of concentrated HCl (37-38%)in a 100ml aqueous solution.

Mineralization of artificial rubber by the Actinomycetes Strain

To study the mineralization of rubber, rubber sheet as well as latex gloves was used as a substrate. To the 150ml of Mineral salt medium prepared and sterilized 2mm of artificial rubber strips were added and the culture were inoculated and incubated at 37°C in orbital shaker at 100rpm for three months. The strips were examined for mineralization by SEM after 90 days .Further results were confirmed by analyzing the compounds released due to the mineralization of artificial rubber by performing FTIR Spectroscopy. The Actinomycetes which are capable of Degrading natural rubber latex were only used for the degradation of artificial rubber (Manasa. M, Veena Gayathri 2016).

Confirmation using Schiff reagent Tests

Evidence for degradation and Mineralization of Cis-1-4,Polyisoprene rubber hydrocarbon chain was obtained by staining treated artificial rubber strips with Schiff reagent. In a tightly stopper bottle,10 ml of fuchsin reagent was added to the sample and kept for incubation for 10-30 minutes at room temperature .After 10-30 minutes excess amount of the reagent was discarded and 10 ml of the sulfite solution was added in order to suppress non specific reaction(Nayanashree *et al*., 2014).

Evaluation of the products produced by Mineralization of (Poly-1-4,cis isoprene)

Chemical changes that were found directly on the artificial rubber surface as a result of the mineralization were determined using FTIR Spectroscopy. FTIR Analysis was done by the standard procedure; preliminary results from Fourier transform infrared spectroscopy (FTIR) strongly confirmed the degradation of natural rubber. It is assumed that degradation of the polymer backbone is initiated by the oxidative cleavage of double bonds in the polymer chain (Braaz *et al*. 2004). FTIR analysis was done by STIC Kochi.

Application of the Actionmycetes Strain for the degrading rubber

The efficiency of the Actinomycetes Strain to degrade the rubber samples were studied.MSM broth were prepared in a conical flasks, sterilized and cooled. In this the Actinomycetes isolated inoculated and then 2mm strips of rubber were added in each conical flask including the control as well. Degradation of the samples was studied and the results obtained using FTIR was compared and confirmed.

RESULTS

Isolation, Identification and Screening of Actinobacteria: Actinobacteria were isolated from different soil samples collected from different sites of rubber plantation areas in Kerala. Identification of the organisms include culture 1,2&5 is positive for MR, VP, Indole, Urease, Citrate, TSI ,Culture 3&4MR negative all the other positive and the culture no:6 urease positive all the other negative. All the six are Gram positive.

Medium and Growth of Organism:

The *Actinobacteria* colony from MSM media was selected based on powdery , earthy smell and colony which produce zone around was identified as the rubber degrading organism according to the colony character. Pure, isolated colonies were streaked on MSM medium. sixisolate able to grow well on media.

On by routine sub culturing other isolates lost their activity and the 6 isolate which were active were selected for further research work and were designated as 1, 2, 3, 4, 5, 6.The 6 selected isolates showed difference in their culture characteristics. The 6 isolates on MSM media showed the difference in the *mycelial* character, Colony morphology of the Isolates.1. Fast growing with dull white colored spore's powdery colonies.2.Brown colored, powdery, substrate mycelium, well isolated colonies were found active.3.Light brown colored colony, 4.yellowish white colored, 5.pinkish colored, 6.Dark pinkish colored colony subsequently.

Mineralization of the artificial rubber by the Actinomycetes strain:

Artificial Rubber sheet and latex gloves was used as the substrate as the breakdown compounds and was studied in a period of 90 day duration. During these 90 days mineralization rate was estimated every 5 days. Mineralization was maximum on 20th day. This was further confirmed by performing Schiff's reagent test and FTIR studies.

Schiff's reagent Test

Rubber sheets and latex gloves which were inoculated with Actinomycetes strains formed purple color and there were no color formation in the control sample. The formation of the purple color in the mineralized sample is due to the presence of aldehyde and ketone group which is produced as a result of the degradation of cis-1-4, poly isoprene units. The reaction is shown (Fig.1& Fig.2, Fig.3 and Fig.4)) which include both samples.

Fig. 1. Latex Gloves Control

Fig. 2. Latex Gloves

Fig. 3. Inoculated Rubber sheet

Fig.4.Rubber sheet Control

FTIR Analysis

Rubber which was utilized by the Actinomycetes strain was studied for degradation products with FTIR analysis. FTIR spectrum of Natural Rubber on 90 days (Fig 1-4) artificial rubber was subjected to FTIR studies. Peaks were observed for 90 days at the wave length. FTIR results onto the particular wavelength shows the degradation rate. The broad absorption band at 3388.44/cm showed the polymeric association in control. But it was narrowed and shifted to 3483.74/cm in the treated sample. treated sample of rubber sheet, and 3417.89 as well as 3441.60 for latex gloves respectively.

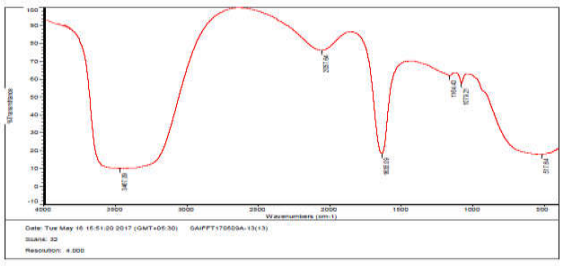

Fig.1. Showing FTIR Results of Control Rubber Sheet

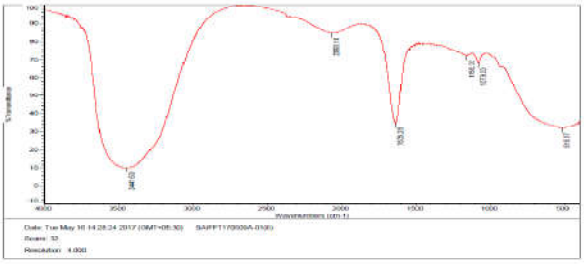

Fig2. Showing FTIR results of inoculated Rubber sheet

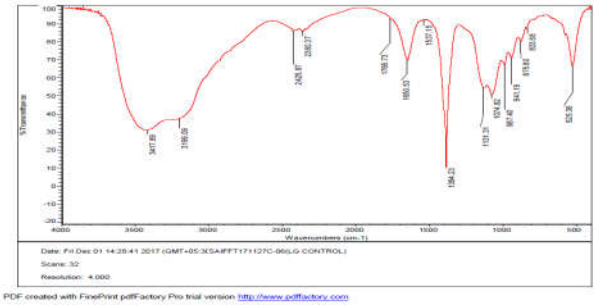

Fig 3.Showing FTIR results of Control Latex Gloves

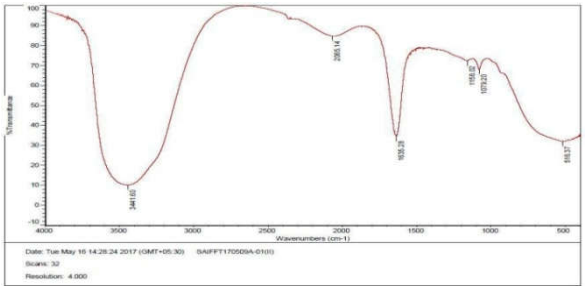

Fig.4.Showing results of Inoculated Latex Gloves

Conclusion

In the present work efforts has been done in the screening and characterization of the rubber degrading Actinobacteria from soil and study its activity. As a preliminary step *Actinobacteria* were isolated from rubber plantation rhizosphere soil. 20 isolates were screened for further work on minimal salt media, rubber latex was used as sole carbon sources. Six isolates showed as positive and designated as RCA 1,2,3,4,5,6. These isolates were screened for their activity. Colony morphology of the Isolates.1. Fast growing with dull white colored spore's powdery colonies.2.Brown colored, powdery, substrate mycelium, well isolated colonies were found active.3.

Light brown colored colony, 4.yellowish white colored, 5.pinkish colored, 6.Dark pinkish colored colony. The rubber degradation of non filamentous bacteria is usually limited compared to filamentous bacteria and it has not been so extensively studied. In an attempt to study the degradation, performed with the rubber latex and all the isolates showed positive result by showing Schiff's test with the rubber sheet of 30 days of incubation. The aim of the work was to screen biodegrading actinobacteria to minimize pollution. The genus level identification was carried out by biochemical methods and comparing the result with Bergeys manual of determinative bacteriology. The DNA sequence was performed and the nucleotide sequence was submitted in the NCBI. The isolates which confirm that it belongs to Streptomyces as gene data bank. By the 30 days after incubation period in the mineral salt broth and with rubber sheet inoculated with the isolate was confirmed by Schiff's test. Schiff's test showed purple color on the rubber sheet inoculated with the organisms. FTIR results onto the particular wavelength shows the degradation rate. The broad absorption band at 3388.44/cm showed the polymeric association in control. But it was narrowed and shifted to 3483.74/cm in the treated sample of rubber sheet, and 3417.89 as well as 3441.60 For latex gloves respectively.

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