ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Report on Rubber Degrading Bacterial Sps. from Vellore Soil Contaminated With Tyre Waste

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ABSTRACT

Rubber a natural polymer, which is widely used, is degraded at a very slow rate in the environment. This is leading to accumulation of rubber waste throughout the world and poses so many environmental problems. This experiment is an attempt to isolate and identify to increase the efficiency of rubber degradation in the nature. Various soils contaminated with tyre waste were collected along with the degraded form from various places of Vellore district of Tamilnadu. The samples were serially diluted and cultured in selective media in petriplates followed by Grams staining and biochemical tests identified that the isolate is *Bacillus species* is also a strong potent degrader of rubber and its products.

Keywords: serial dilution, selective media ,Grams staining, biochemical tests.

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ISSN: 0975-8585

INTRODUCTION

Rubber is a natural polymer of organic compound isoprene. The structure of rubber is given in Fig. 1.Rubber waste is getting accumulated and is posing a threat to environment by contaminating the soil. The main constituent of rubber is cis1,4-polyisoprene with an average molecular mass of 10^6 Da. Rubber is relatively resistant to degradation when compared to other natural polymers [1,3,4,7] . The rate and extent of the degradation depends on the rubber formulations i.e. Vulcanized rubber is less and more slowly degraded when compared to latex gloves or natural rubber [8-10,5]. It also depends on the bacteria species acting on the rubber and their interaction with the environment [11,2,12] . The two major problems today are wastage of rubber and disposal of waste tires which is leading to environment problems.

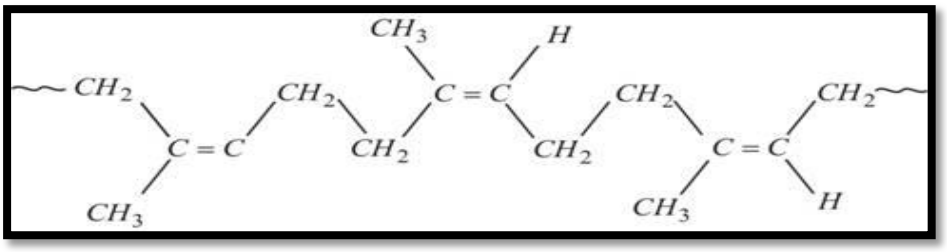

Fig.1: showing the structure of rubber

According to previous reports, natural rubber degrading bacteria mostly belongs to the group of Actinomycetes. Recently, certain thermophilic bacteria were also reported [13-16]to be rubber degrading. Degradation of natural rubber latex by two Gram negative bacteria viz. *Xanthomonas* sps. (and *Pseudomonas aeruginosa* were also reported. Gordonia polyisoprenivorans strain VH2 is also known to effective degrade natural and vulcanized rubber.

The main principle behind the selective media is to put the bacteria under stressed conditions and make it produce the enzyme to degrade rubber [17,18] . Carbon source is not added to the media and hence the bacteria utilizes the rubber as carbon source. Each bacterial species has unique biochemical pathway which is not yet determined clearly for all species but in actinomycetes species and most likely all the other bacterial species will have similar pathways the pathway [6].The pathway is given in figure [2].

If the tyres have undergone the process of vulcanization then the process of Desulphurisation, where in the sulphur is oxidized or used up is used . The mechanism is not yet clear (for further info please refer to [7].

Plasmids present in the most of the rubber degrading sps are responsible for the degradation of rubber for example*: Gordonia polyisopreivorans* strain VH2 , which is one of the most efficent degraders of rubber has a genome of circular chromosomes(5669,805 bp) and a circular plasmid (174,494 bp). It has 5,110 putative protien coding sequences including the genes responsible for rubber degradation activity. It is also suspected that most of the genes coding for rubber degrading protiens are from the plasmid [5].

ISSN: 0975-8585

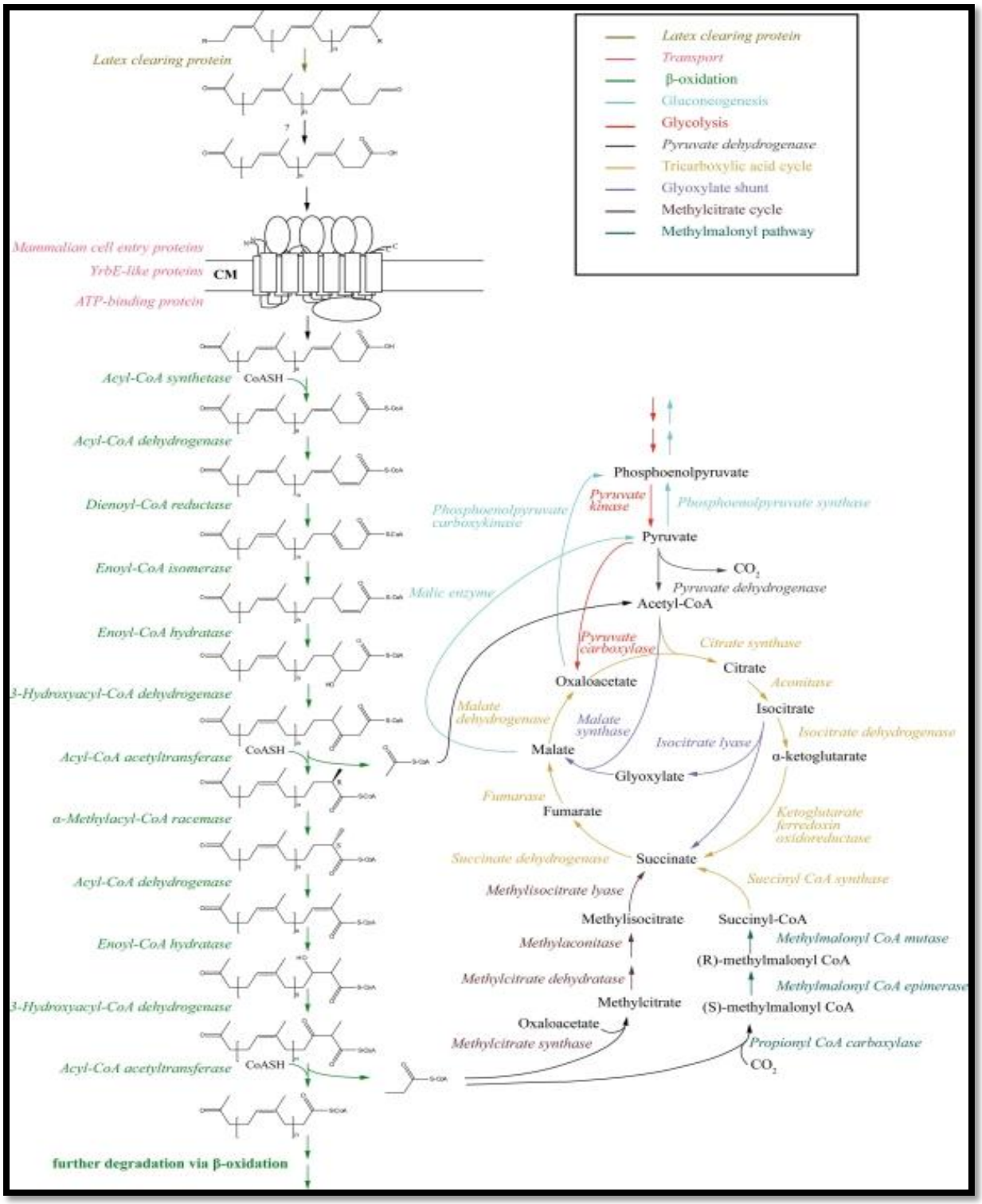

Fig.2 :Showing the pathway of rubber degradation in Gordonia and related sps.

There are two diffrent types of rubber degrading bactria. The first one *is Corynebacterium, Mycobacterium, Nocardia* and the other one is *streptomycetes* and related genera. *Streptomyces sp. K30* is one example. The first group needs direct physical contact to rubber material and shows adhesive growth, these strains show good rubber degradation activities in submerged cultures but do not grow very well on pertiplate [19-21] it is suspected that

ISSN: 0975-8585

Bacillus species also belong to this group . Members of the second group grow and degrade the rubber very well on pertiplate and show lesser growth in submerged cultures.

Mechanism of the rubber degradation is not yet well explored but the general mechanism goes like this, there is an oxidative cleavage of the double bond in the polymer backbone for initiation has been explained by Tsuchii and Takeda. Recently two different enzymes which degrade rubber were discovered one is RoxA which was isolated from Xanthomonas sp., and other one is Lcp was isolated from *streptomyces sp.*

MATERIALS AND METHODS

Sample Collection

Ten Contaminated soil and tyres samples were collected from different places in Vellore and stored. The tyres are shown in Fig. 3.

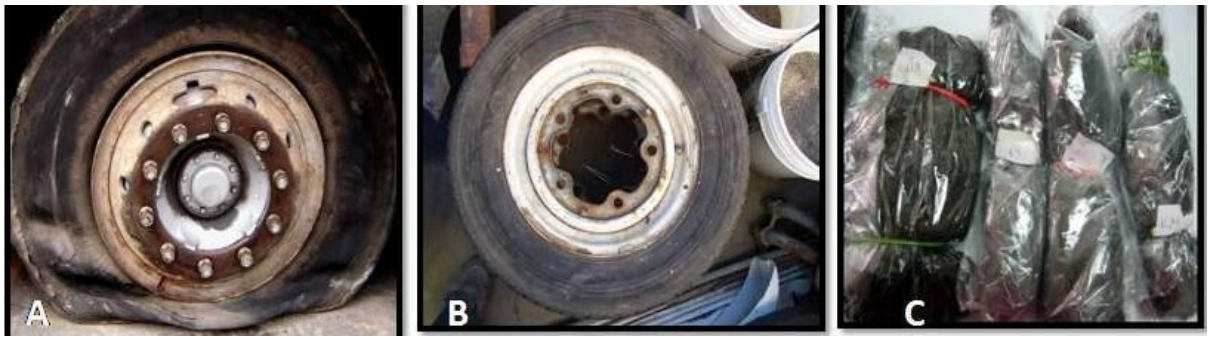

Fig 3: Sample collection from the from the soil contaminated with degraded tyre wastes

Media used

One gram of soil was added to 25 ml solution (g/L)(solution 1): K_2HPO_4. $3H_2O$ 9.17; KH_2PO_4 2.68; MgSO4 0.1; NH_4Cl 1 in an Erlenmeyer flask. Latex glove pieces, 2-5cm in diameter were added to each flask. This was placed on a rotating shaker (70rpm) and incubated at 35^{o}C. Sub-culturing (transfer of the latex glove piece to fresh media) was done after 10 days. Thereafter, above mentioned solution was routinely added to the cultures [1, 3].

Pre treatment of rubber gloves

The gloves were soaked for 2 hrs in 70% ethanol + acetone solution for the rubber to be broken down for easier degradation [1, 3]. The media was pour plated and streak plated in a petriplate that had (g/L) NH_4Cl- 1g, agar 15, X10 solution(1) 100 ml, with pieces(0.5-1 cm) of treated rubber embedded in the gar. The plates were incubated at 30^{0} C for about 20 days. Staining and Biochemical tests has also been used to identify the isolated bacterial species.

RESULTS AND DISCUSSION

Five potential strains of Bacterial growth(turbidity) was observed and the rubber was started degrading from 23 hrs onwards . The degraded rubber was distinguished from

ISSN: 0975-8585

the non degraded one by its porous surface and cuts and cracks on it . Based on the characteristics of rubber degrading bacteria the degradation would be due to biofilm formation. The respective 0th day and 10th day turbidities are shown in Fig.4 .The activity in petri plate is shown in Fig. 5. The results obtained from Biochemical tests has also been used to identify the isolated bacterial species which has been shown in Fig. 6. The result of Gram's stain was a "+ ve" and the shape was rod shaped,he catalase test was also performed and the result was a positive Fig. 7. hence the isolated sps was suspected for Bacillus sps. It was then inferred that Bacillus sps. also degrades rubber, a 16sRNA sequencing will help in determining the strain and amino acid sequencing helps in determining the protein responsible for rubber degradation activity.

Fig. 4 : Showing the turbidness of solution on the day of inocoulation and 10 days after that respectively (A,B) .

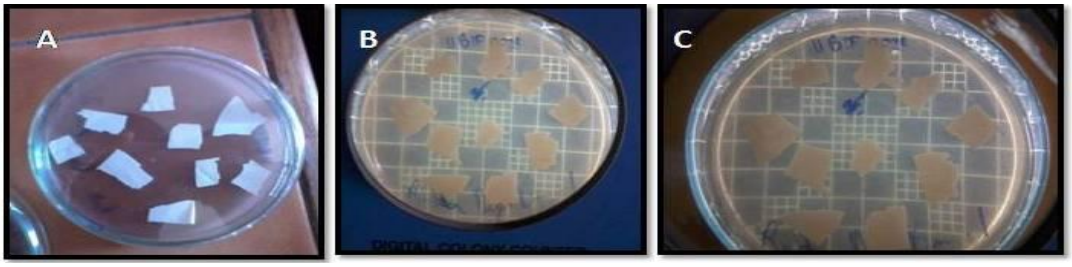

Fig. 5 : Showing the developments of organism growth on days:0,6,10 respectively as shown in A, B and C respectively.

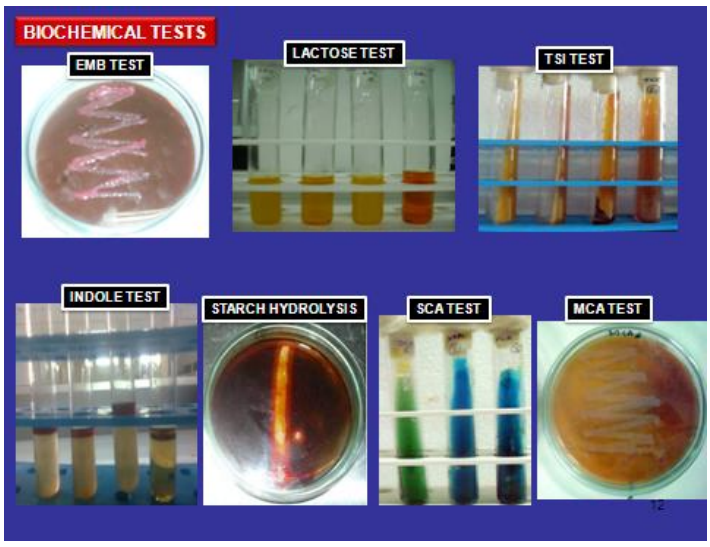

Fig.6: Different Biochemical Test performed for the isolated microorganism

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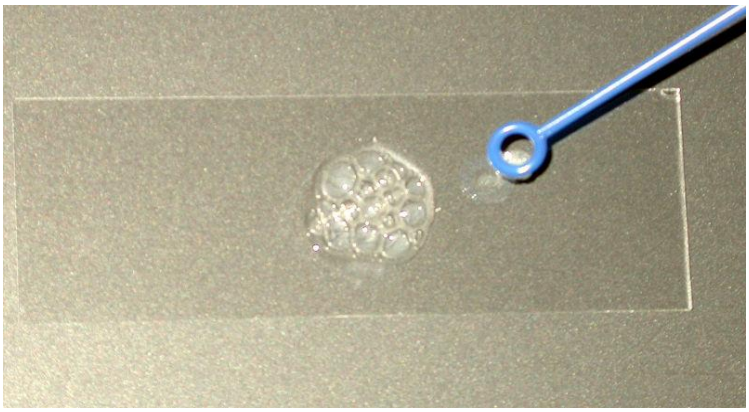

Fig.7: Slide showing catalase positve.

ACKNOWLEDGEMENT

We want to express our sincere gratitude and heartfelt thanks to our beloved and honorable Chancellor Dr. G. Viswanathan , VIT University for constant support , encouragement and for providing infrastructure to carry out this research work and DST for financial assistance.

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