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BIO-DEGRADATIVE EFFICIENCY OF ASPERGILLUS *TERREUS* IN WASTE RUBBER MANAGEMENT

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ABSTRACT

Rubber products are widely used in our daily life which are mainly made up of Natural rubber (NR) obtained from the latex of tree *Hevea brasiliensis* commonly called Rubber tree. After the usage of these natural rubber products the disposal of these products is a worldwide solid waste problem. Microbial degradation of products can be a solution to this problem. In current work different fungal species were isolated from the soil where natural rubber samples were dumped. In all the isolated fungi *Aspergillus terreus* (22.6%) effectively degraded rubber. Degradation of rubber was further confirmed by SEM, FTIR and Schiff's staining methods. Further it was studied that laccase and manganese peroxidase enzyme produced by *Aspergillus terreus* were responsible for rubber degradation. Hence, it can be concluded that *Aspergillus terreus* can be effectively used for the degradation of rubber in an ecofriendly way.

KEYWORDS: *Aspergillus terreus*, *Hevea brasiliensis*, vulcanization, polyisoprene, laccase, manganese peroxidase.

INTRODUCTION

Rubber products are widely used in our daily life which are mainly made up of Natural rubber (NR) or cis-1,4 polyisoprene which is obtained from the latex of tree *Hevea brasiliensis* commonly called Rubber tree. Rubber trees grow throughout the world. Rubber trees are basically found in tropical & semitropical countries. Two main types of polyisoprenoids that differ according to their isomerism are synthesized by plants, the first one is the *cis* isomer natural rubber (NR) (poly(*cis*-1,4-isoprene)) and the second one is the *trans* isomer gutta-percha (GP) (poly(*trans*-1,4-isoprene)). The average composition of the natural rubber latex is 25-30% polyisoprene, 1-1.8% proteins, 1-2% carbohydrates, 0.4-1.1% neutral lipids, 0.5-0.6% polar lipids, 0.4-0.6 inorganic components, 0.4% amino acids etc., and other 50-70% water (Rose *et al.*, 2002).

For the manufacture of rubber products natural rubber latex should be subjected to vulcanization.

Approximately about 15.63 million tons of rubber is consumed globally in which 65% were used for tire production and other 35% is used for production of other rubber products such as rubber balloons, mats, rubber bands, pipes, gaskets, sheets etc.

After usage of these natural rubber products the disposal of these products are the worldwide solid waste problem as it cannot be easily re shaped due to vulcanization. So other alternatives such as microbial degradation of the product should be developed. (Lions *et al.*, 2000).

MATERIALS AND METHODS

For the isolation of fungi which were able to degrade natural rubber, the soil sample was collected from a local land fill of Shivamogga district and from rubber plantation, then it was brought to the laboratory, along with this natural rubber latex and natural rubber sheet samples were collected from rubber processing unit and

then it was brought to the laboratory and preserved in the refrigerator for further use.

Isolation of fungi by Serial dilution method

Serial dilution method was also followed for the isolation of fungi from the soil sample brought from the local land fill area and latex contaminated soil brought from the rubber plantation. In this method 1g of soil was weighed and it was added to the test tube containing 9ml of sterile saline and mixed properly with the help of clyclo mixer. From this test tube 1ml of suspension was pipetted out with the help of micropipette and transferred to another test tube containing 9ml of sterile saline and mixed well. The same procedure was repeated until 10^{-9} dilution was obtained and from the last test tube 1ml of suspension was discarded. Once the soil sample was serially diluted 10^{-4} and 10^{-5} diluted sample was plated on the petri plates containing Czapek Dox agar for the isolation of fungi to these plates antibiotic streptomycin was previously added to avoid the growth of bacteria. After plating Czapek Dox agar plates were incubated at 27±2°C for 7 days (Aneja, 2004). After incubation period the plates were observed for the growth of fungi and identified based on colony characteristics and microscopic appearance (Nagamani *et al.*, 2006).

Screening of natural rubber degradation by using Mineral salt medium (MSM)

Natural rubber degrading ability of the fungi was checked in the laboratory conditions by growth experiment in mineral salt medium (MSM) (Pan *et al.*, 2009), where natural rubber was used as sole carbon source. Previously isolated fungi were inoculated into different conical flasks containing MSM and kept for incubation for 2 months on rotary shaker. Fungi were incubated at 27±2°C, triplicates were maintained. After the incubation period natural rubber discs were removed and observed for the growth of fungi. Then natural rubber discs were washed dried at 50°C in hot air oven for 24 hours and weight loss was checked (Tsuchii and Tokiwa, 2001).

Confirmation of natural rubber degradation by staining with Schiff's reagent

Evidence for degradation and mineralization of *cis*-1,4-polyisoprene rubber hydrocarbon chain was obtained by staining treated natural rubber discs with Schiff's reagent. In a tightly stopper bottle, 10 ml of fuchsin reagent was added to a sample and kept for incubation for 10-30 minutes at room temperature. After 10-30 minutes excess amount of the reagent was discarded and 10ml of the sulfite solution was added to suppress nonspecific reaction of untreated sample (Berekka *et al.*, 2000).

Confirmation of natural rubber degradation by Scanning Electron Microscopy (SEM)

Evidence for degradation and mineralization of *cis*-1,4-polyisoprene natural rubber hydrocarbon chain was obtained by observing the natural rubber discs under SEM. For the observation natural rubber discs buried in the soil and present in the MSM, which were subjected for degradation were observed under field emission-scanning electron microscopy (FEI-SIRION, Eindhoven, Netherland) (Lions *et al.*, 2000).

Confirmation of natural rubber degradation by Fourier Transform Infrared Spectroscopy (FTIR)

Chemical changes that arose directly on the natural rubber surface as result of the degradation process were determined using FTIR spectroscopy. NICOLET 380 FTIR spectrophotometer from Thermo Fisher Scientific, France was used which gives transmittance spectra in IR range 4000 to 400 nm. (Roy *et al.*, 2005).

Characterization of enzymes responsible for biodegradation of natural rubber

It was studied that laccase and manganese peroxidase enzymes were responsible for the natural rubber degradation.

Screening for Laccase and Manganese peroxidase enzyme production by fungi

Screening for laccase enzyme produced by *Aspergillus terreus* was done on plates containing following composition (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4, 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 Agar (pH-6) supplemented with 0.02% guaiacol. *Aspergillus terreus* was inoculated into this plate and the plate was incubated at 30°C for 7 days. Laccase activity was visualized on plates containing 0.02% guaiacol, since laccase catalyzes the oxidative polymerization of guaiacol to form reddish brown zones in the medium (Viswanath *et al.*, 2008).

For the screening of manganese peroxidase enzyme producing organisms H_2O_2 was added to the laccase screening media.

Mass production of enzymes by submerged fermentation

Pure cultures of *Aspergillus terreus* were inoculated to submerged state fermentation medium to produce extracellular enzymes by using MSM media and was maintained at the incubation temperature of 27±2ºC for 3 months (Shraddha *et al.*, 2011).

Determination of Laccase and Manganese peroxidase enzyme activity by using Spectrophotometer

Guaiacol (2mM) in sodium acetate buffer (10mM pH 5.0) was used as substrate. The reaction mixture contained 3ml 10mM acetate buffer of pH 5, 1ml guaiacol and 1ml enzyme source and enzyme blank contained 1ml of distilled water instead of enzyme source. The mixture was incubated at 30ºC for 15minutes, and absorbance was read at 450nm blank using UV spectrophotometer (Papinutti *et al.*, 2006). Manganese peroxidase enzyme activity was calculated by following laccase enzyme activity determination

procedure, for the reaction mixture 1 ml of H_2O_2 was added and incubated.

Protein estimation

Protein concentration was estimated to determine specific activity of enzyme. The protein concentration was determined by the Lowry's method, as described by Lowry's (1951) using Bovine Serum Albumin (BSA) as a standard, absorbance was read at 660 nm using JENWAY- 6305 UV-VIS Spectrophotometer.

Testing of Natural Rubber degrading ability of enzymes

Degrading ability of the enzyme which were purified was tested by adding enzymes to the flasks containing acetate buffer and previously weighed natural rubber sheets and kept for incubation on rotary shaker for a period of 15 days at room temperature. After incubation period rubber discs weighed to check weight loss and subjected for staining to confirm natural rubber degradation (Fujisawa *et al.*, 2001).

RESULTS

Isolation of microorganisms by Serial dilution method

In isolation of rubber degrading fungi by serial dilution method different fungi were isolated and recorded. In the isolated organism *Aspergillus terreus* was pre-dominant and commonly isolated. Thus, it was screened to test natural rubber degrading ability (Fig.1).

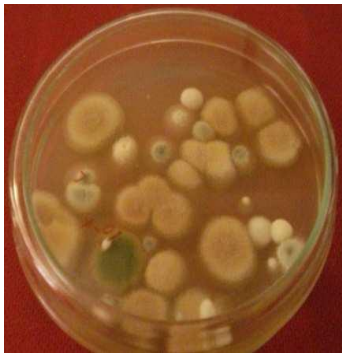


Fig. 1: Isolation of microorganisms by serial dilution method.

Screening of natural rubber degradation by using Mineral salt medium (MSM)

The growth experiment was conducted by using mineral salt medium weight loss was observed and growth of fungi was observed on the natural rubber discs. Initial weight of *Aspergillus terreus* inoculated sample was 3g and final weight was 2.32g and there was a weight loss of 0.68±0.026g and the percentage of weight loss 22.6% degradation.

Confirmation of Natural rubber Degradation by Staining with Schiff's Reagent

Rubber sheets which were inoculated with *Aspergillus terreus* turned purple and there was no colour formation in the blank. Formation of purple colour in the treated sample is due to the presence of aldehyde and ketone group which is produced because of degradation of cis-1,4-polyisoprene units.

Confirmation of natural rubber degradation by Scanning Electron Microscopy (SEM)

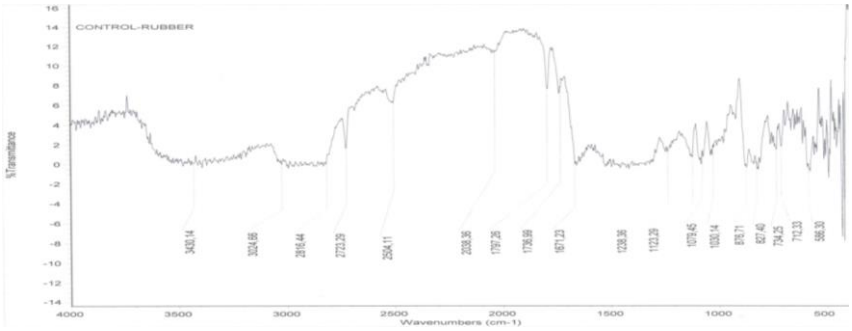
Natural rubber discs were observed under SEM, bio-film formation, complete disintegration and formation of cavities on the natural rubber discs was observed (Fig 2).

***A. terreus* treated** **Buried in soil** **Control**

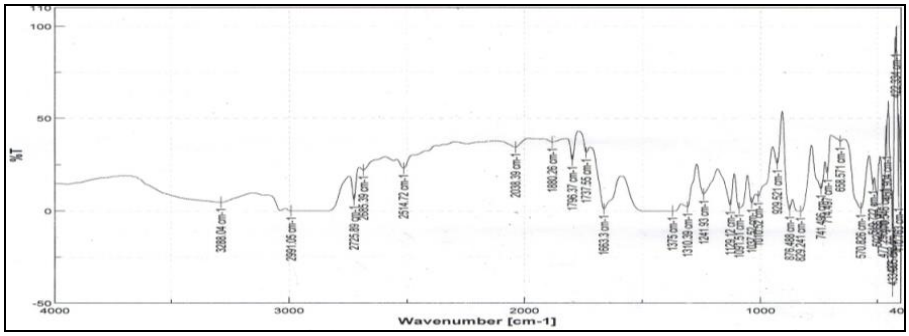
Fig. 2: SEM images of natural rubber showing degradation.

Confirmation of natural rubber degradation by Fourier Transform Infrared Spectroscopy (FTIR)

Natural rubber disc, which was treated by *Aspergillus terreus* was subjected to FTIR, peaks at the wave 2725.89cm^{-1} and 1663.3cm^{-1} were found, which indicates the release of aldehyde and ketone group because of degradation. Presence of alkanes (2991.05cm-1), carboxylic acid (2514.72cm^{-1}), carbonyl (1796.37cm^{-1}) and alcohol (1091.51cm^{-1}) groups were observed, which confirms rubber degradation (Fig. 3).



Control



***A. Terreus* treated**

Fig. 3: Confirmation of natural rubber degradation by FTIR.

Enzymatic studies of natural rubber degradation

It was studied that laccase and manganese peroxidase enzymes were responsible for the rubber degradation.

Screening of Laccase and Manganese peroxidae enzyme producing organisms

Aspergillus terreus was inoculated on the laccase and manganese peroxidase medium there was a formation of reddish brown colour around the colonies since laccase and manganese peroxidase catalyzes the oxidative polymerization of guaiacol to form reddish brown zone. *Aspergillus terreus* which showed positive result for rubber degradation showed positive result for laccase and manganese peroxidase enzyme screening.

Spectrophotometrical analysis of laccase and manganese peroxidase enzyme activity

Aspergillus terreus showed maximum activity of both laccase and manganese peroxidase enzyme activity was maximum in 10th week.

Table 1: Showing laccase and manganese peroxidase activity.

A. Terreus	1st week	2nd week	3rd week	4th week	5th week	6th week	7th Week	8th week	9th week	10th week	11th week	12th week
Laccase	0	0	0.0011 ±0.0004	0.0019 ±0.0004	0.0029±0 .0003	0.0044 ±0.0001	0.0062 ±0.0002	0.0085± 0.0001	0.0096±0 .0003	±0.0002 0.0115	0.0118± 0.0002	0.0086± 0.0002
Mangenese peroxidase	0	0	0.0015 ±0.0003	0.0032 ±0.0001	0.0041 ±0.0001	0.0061 ±0.0003	0.0079 ±0.0004	0.0101 ±0.0003	0.0115 ±0.0002	0.0129 ±0.0003	0.0118 ±0.0002	0.0086 ±0.0001

DISCUSSION

Present study was carried out to isolate natural rubber degrading fungi. It was studied that *Aspergillus terreus* is capable of degrading natural rubber. Degradation of natural rubber was studied by performing growth experiments in MSM, and degradation was confirmed by staining, SEM and FTIR studies. Further enzyme responsible for degradation was studied. Laccase and manganese peroxidase were the enzymes responsible for degradation.

Similar attempts were made by several other scientists to degrade rubber by using microorganisms.

Roy *et al.*, (2005) tried to study natural rubber (NR) biodegradation through solid-state fermentation (SSF) and submerged fermentation (SMF) has been carried out for both bacterial as well as fungal species. There was a change in the organic carbon content along with the average molecular weight of the treated rubber samples indicated rubber hydrocarbon utilization and its degradation.

Berekaa *et al.*, (2000) conducted similar work and tested the biodegrading ability of different bacteria belonging to the genera *Gordonia* (strains Kb2, Kd2 and VH2), *Mycobacterium*, *Micromonospora* and *Pseudomonas*. All strains were able to use natural rubber (NR) as well as NR latex gloves as sole carbon source.

Similar study was carried out by Tokiwa *et al.*, (1999) in his study he showed that forty-seven percent of a tire tread strip with a natural rubber content of 100 phr (parts per hundred of rubber) was completely mineralized by a mutant strain, Rc, of the rubber-degrading organism, *Nocardia* sp. Strain 835A.

CONCLUSION

Rubber products are widely used in our daily life. These products are made up of natural vulcanized rubber and other chemical additives. Due to vulcanization of the natural rubber these rubbers are very resistant to high temperature and persist in environment for very long time. Rubber materials have been increasingly used now a days in different areas after usage its disposal is a very big solid waste problem. It cannot be easily recycled due to the sulphur cross linking formed during vulcanization. If they are burnt, they release enormous amounts of carbon-di-oxide and some other gases which causes environmental pollution and contribute to the global warming. Rubber products such as balloons which are disposed of in the natural environment are dangerous to wild animals if they are consumed by animals.

So, one of the alternative ways to solve these problems is to subject these products to biodegradation. During the present study, rubber discs were dumped in the soil removed at regular intervals of time and then plated on the media to isolate the organism. In the isolated organism and *Aspergillus terreus* effectively degraded the rubber sample. The present study has showed that, it is possible to use *Aspergillus terreus* to degrade natural rubber effectively. Along with this, enzymes responsible for natural rubber degradation were also characterized.

REFERENCE

1. Aneja, K.R. 2004. Experiments on Microbiology, Plant Pathology and Biotechnology. 4th ed. New Age International Pvt. Ltd. New Delhi, 1-607.
2. Berekaa, M.M., Lions, A., Reichelt, R., Keller, U. and Steinbuchel, A. 2000. Effect of pretreatment of rubber material on its Biodegradability by various rubber degrading Bacteria, *FEMS Microbiology Letters*, 184: 199-206.
3. Fujisawa, H., Hirai, H., and Nishida, T., 2001. Degradation of Polyethylene and Nylon-66 by laccase mediator system, *Journal of Polymer and Environment*, 9: 103-108.
4. Lions, A., Rudolf Reichelt, R., Keller, U. and Steinbuchel, A. 2000. A Gram-negative bacterium identified as *Pseudomonas aeruginosa*, AL98, is a potent degrader of natural rubber and synthetic cis-1,4-polyisoprene, *FEMS Microbiology Letters*, 182: 155-161.
5. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951. Protein measurement with the folin phenol reagent, *Journal of general Microbiology*, 31: 3017-3027.
6. Nagamani, A., Kunwar, I.K. and Manoharachary, C. 2006 *Handbook of Soil Fungi.* I. K. International Pvt. Ltd. New Delhi, 1-477.
7. Pan, L., Gung, G.J., Yin, B. and Cheng, P.S. 2009. Contribution to deterioration of polymeric materials by slow growing Bacteria *Nocardia corynebacterioides*, *International Biodegradation and Biodeterioration*, 63: 24-29.
8. Papinutti, L., and Martinez, J. M., 2006. Production and Characterization of Laccase and Manganese peroxidase from the ligninolytic fungus *Fomes sclerodermeus*, *Journal of technology and biotechnology*, 81: 1064-1070.
9. Rose, K., and Steinbüchel, A., 2005. Biodegradation of Natural Rubber and Related Compounds: Recent Insights into a Hardly Understood Catabolic Capability of Microorganisms, *Applied Environmental Microbiology*, 71: 2803–2812.
10. Roy, V.R., Das, M., Banrjee, R. and Bhowmick, A. 2005. Comparative studies on rubber biodegradation through Solid state and Submerged fermentation, *Process Biochemistry*, 42: 181-186.
11. Shraddha, Shekher, R., Sehgal, S., Kamtania, M., and Kumar, A., 2011. Laccase microbial source Production, Purification, Potential biotechnological application, *Enzyme Research*, 1-11.
12. Viswanath, B., Chandra, M. S., Pallavi, H., Reddy, 2008. Screening and Assessment of Laccase Producing Fungi isolated from different environmental samples, *African Journal of Biotechnology*, 7: 1129-1133.
13. Tsuchii, A. and Tokiwa, Y. 1999. Colonization and disintegration of Tire Rubber by a colonial Mutant of *Nocardia*, *Journal of Bioscience and Bioengineering*, 87: 542-544.
14. Tsuchii, A. and Tokiwa, Y. 2001. Microbial degradation of Tire rubber particles, *Biotechnology Letters*, 23: 963-969.