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RESEARCH ARTICLE

Natural rubber degradation by *Verticillium* sp. and *Chaetomium* sp.

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ABSTRACT

Rubber products are widely used in our daily life these products are mainly made up of Natural rubber (NR) which is obtained from the latex of tree *Hevea brasiliensis* commonly called Rubber tree. 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% aminoacids etc., and other 50-70% water. The different rubber products are manufactured by using vulcanized natural rubber. During manufacturing along with vulcanized natural rubber other chemical additives will be added. After usage of these natural rubber products the disposal of these products are the world wide solid waste problem. One of the solution to reduce this problem is to recycle the used waste rubber. But due to the chemical cross linking formed during vulcanization it is not possible to simply melt and reshape the products as in case of polythene. So other alternatives such as microbial degradation of the product should be developed. Microbial degradation is mainly carried out by various microorganisms such as bacteria and fungi. During the present study an attempt was made to isolate rubber degrading microorganisms. Rubber pieces were dumped in the soil for regular interval of time and then plated on the media to isolate the organism. In the isolated organism *Verticillium* species and *Chaetomium* species effectively degraded the rubber sample. The present study has showed that, it is possible to use these strains to degrade the rubber.

INTRODUCTION

Rubber products are widely used in our daily life these products are mainly made up of Natural rubber (NR) which is obtained from the latex of tree *Hevea brasiliensis* commonly called Rubber tree. 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% aminoacids etc., and other 50-70% water. Dry weight of the natural rubber latex contains more than 90% of cis-1,4-polyisoprene and less than 10% of non-rubber constituents like proteins, carbohydrates, lipids etc. (Rose *et al.* 2002) The natural rubber latex is sticky and viscous in nature and very sensitive to temperature therefore it can not be directly used for the manufacturing of rubber products. For the manufacture of rubber products this latex should be subjected to vulcanization. During vulcanization some percent of elemental sulfur is added to rubber latex and it is heated to 140-180°C under pressure so that original sticky viscous material is converted a non-sticky and elastic material. During vulcanization the polyisoprene molecules are covalently linked by bridges of elemental sulfur which makes the rubber rigid elastic and resistant to temperature. (Tsuchii *et al.* 2006)

The different rubber products are manufactured by using vulcanized natural rubber. During manufacturing along with vulcanized natural rubber other chemical additives will be added. The percentage of natural rubber content and the additives added mainly depends on the products manufactured. The global rubber consumption in 1998 was estimated to be 6.6 million tons of which 65% were used for tire production and other 35% is used for the 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 world wide solid waste problem. One of the solutions to reduce this problem is to recycle the used waste rubber. But due to the chemical cross linking formed during vulcanization it is not possible to simply melt and reshape the products as in case of polythene. So other alternatives such as microbial degradation of the product should be developed. Microbial degradation is mainly carried out by various microorganisms such as bacteria and fungi. The present study was taken to isolate the rubber degrading fungi from the soil so that it can be used to degrade the rubber waste. (Lions *et al.* 2000)

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MATERIALS AND METHODS

Natural rubber latex and rubber sheet samples were collected from Rubber processing unit and then it was brought to the laboratory. Rubber sheet was cut into small pieces and it was weighed and initial weight was recorded. Then these pieces were buried in the soil and left for a period of six months time interval. These rubber pieces were removed regularly at time interval of two, four and six months respectively. Then again rubber pieces were weighed and final weight was recorded.

Isolation of Rubber Degrading Microorganisms

For the isolation of rubber degrading fungi rubber piece, which were previously buried in the soil were collected and along with the rubber pieces even the soil from the area where the rubber was buried was collected and brought to the laboratory. Then these soil sample and rubber samples were plated on the Potato dextrose agar media and kept for incubation at room temperature or at 22±2ºC for 3 to 4 days for the isolation of fungi. After incubation period organisms were identified by staining and by comparing with standard manual. (Tsuchii *et al.* 1996)

Screening of Rubber Degrading Microorganisms

For the screening of rubber degrading microorganisms previously isolated microorganisms were inoculated directly on the previously weighed natural rubber sheets and then kept for incubation for 3

months. Organisms capable of degrading rubber utilized rubber as sole source of carbon and showed growth on the rubber sheets. After a time interval of 3 months rubber sample inoculated with organisms were washed thoroughly, dried and final weight was recorded. Along with this growth experiments was also carried out in Mineral Salt Medium to check rubber degradation. In this experiment mineral salt medium was and dispensed to the different conical flasks. To these conical flasks previously weighed rubber strips were added as a sole carbon source for the growth of microorganisms. To this flask Fungi which were previously isolated were inoculated and flasks were kept for incubation for 3 months. After incubation period rubber strips were checked for weight loss and also for the growth of the organisms. (Pan *et al.* 2009)

Confirmation of 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 natural rubber sheets which is containing actively growing colonies of microorganisms with Schiff's reagent. In a tightly stoppered bottle, 10 ml of fucshsin reagent was added to a sample and kept for incubation for 10- 30 minutes at room temperature. There will be development of purple color if there is the degradation of cis-1,4-polyoisoprene units by microorganisms which is a sole carbon source present in the rubber latex. During the degradation of cis-1,4-polyisoprene there will be release of aldehyde and ketone group. These aldehyde and ketone group reacts with Schiff's reagent and there will be development of purple color. After 10- 30 minutes excess amount of the reagent was discarded and 10ml of the sulfite solution was added in order to suppress nonspecific reaction of blank sample. (Terzi *et al.* 2009)

Confirmation of Rubber Degradation by Scanning Electron Microscopy

Evidence for degradation and mineralization of cis-1,4-polyisoprene rubber hydrocarbon chain was obtained by observing the rubber strip under scanning electron microscope. For the observation rubber strips buried in the soil and present in the Mineral salt medium which were subjected for degradation were taken out and washed with water and the sample was dried on a cleaned silicon wafer and electron conductivity was created externally to the sample by sputtering with gold nanoparticles using a gold sputter (Jeol JFC 1100E Ion sputtering device) and analyzed by Field emission-scanning electron microscopy (FEI-SIRION, Eindhoven, Netherland). (Tsuchii *et al.* 2001)

RESULTS

In the rubber sample, which were removed at the time interval of two month the initial weight of the rubber piece was 3 grams and after 2 months the final weight of the sample was 2.80 gram and there was a weight loss of 0.2 gram in the rubber sample. In the rubber sample which were removed at the time interval of 4th month the initial weight of the sample was 3 grams and the final weight of the rubber piece was 2.5 grams and there was weight loss of 0.5 grams of the rubber sample. In the rubber sample which were removed at the time interval of 6th month the initial weight of the sample was 3grams and the final weight of the sample was 2grams and there was decrease in 1 gram weight in the rubber sample.

Isolation of Rubber Degrading Microorganisms

When the rubber sample and the soil sample of 2, 4 and 6 months were plated on the potato dextrose agar medium different fungi were isolated. When the 2nd month rubber sample was plated organisms like *Verticillium* sp., *Chaetomeium* sp., *Aspergillus flavus,* were isolated. When 2nd month soil sample was plated organisms like *Chaetomeium* sp., *Verticillium* sp., and *Trichoderma* sp., were isolated (Fig. 1). When the 4th month rubber sample was plated organisms like *Verticillium* sp., *Chaetomeium* sp. and *Aspergillus flavus,* were isolated. When 4th month soil sample was plated organisms like *Verticillium* sp., *Chaetomeium* sp. were isolated (Fig. 2). When the 6th month rubber sample was plated organisms like *Verticillium* sp., and *Chaetomeium* sp., were isolated. When 6th month soil sample was plated organisms like *Aspergillus niger, Aspergillus flavus, Verticillium* sp., *Trichoderma* sp., were isolated (Fig. 3).

Figure 1: Growth of fungal colonies on when 2 months inoculated Rubber and Soil sample were plated.

Figure 2: Growth of fungal colonies on when 4 months inoculated Rubber and Soil sample were plated.

Figure 3: Growth of fungal colonies on when 6 months inoculated Rubber and Soil sample were plated.

Screening of Rubber Degrading Microorganisms

For the screening of rubber degrading microorganisms, the organisms which were isolated from the soil and rubber pieces were used. In the isolated organism *Verticillium* sp. and *Chaetomeium* sp., were commonly isolated when the rubber and soil sample were plated. Thus these organisms were screened to test rubber degrading ability. For screening rubber degrading ability of these organisms they where plated on the previously weighed rubber sheets and kept for incubation for a month. After incubation period all 2 organisms showed growth on the rubber sheets, and there were weight loss in the rubber sheets. The initial weight of the *Chaetomeium* sp., inoculated sheet was 10gm and final weight was 7.64gm and there was a decrease in 2.36gm of weight (Fig. 4) and (Table 1). In case of *Verticllium* sp., initial weight of the rubber sheet was 10gm and final weight was 8.45gm and there was decrease in 1.55gm weight (Fig. 5) and (Table 2). In the time interval of 3 months *Chaetomeium* sp. 23.6% degradation and *Verticillium* sp. showed 15.5% degradation. 50% weight loss was observed in the rubber sample as the incubation period increased (Fig. 6).

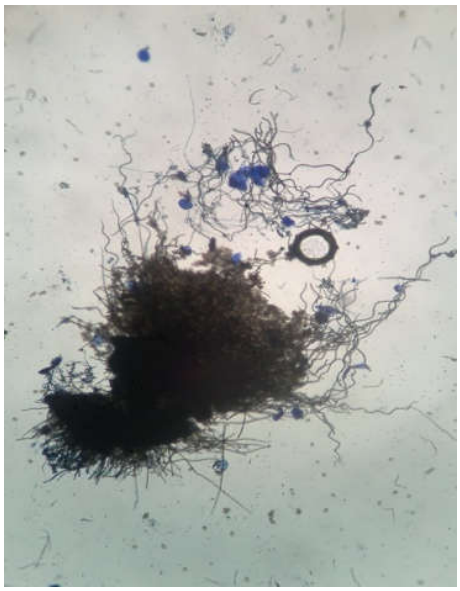
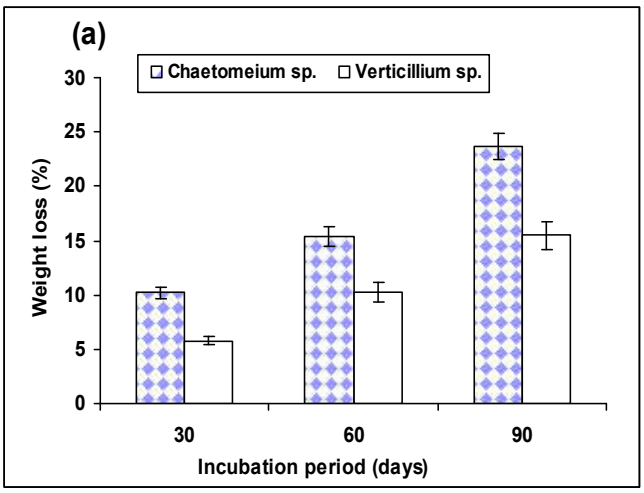


Figure 4: Stereo binocular microscopic observation of *Chaetomeium* sp. grown over rubber sheet.

Figure 5: Microscopic observation of *Verticillium* sp. grown over rubber sheet.



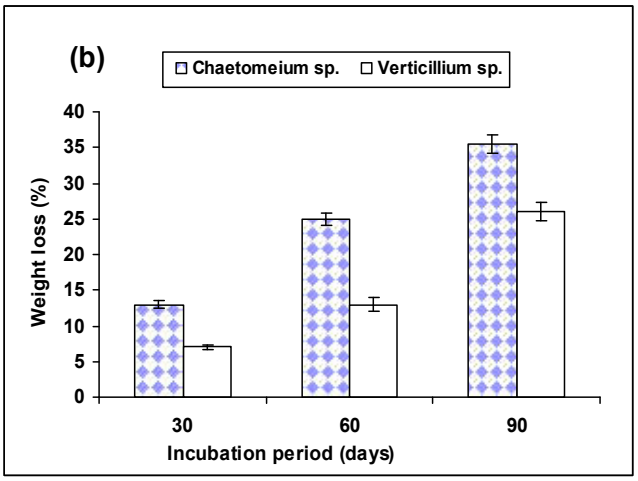


Figure 6: Percentage weight loss by *Chaetomeium* sp. and *Verticillium* sp. inoculated into the rubber sheet (a) and rubber containing mineral salt medium (b). Error bars are standard error of the mean.

When growth experiment was conducted by using mineral salt medium weight loss was observed and growth of fungi was observed on the rubber strips. Initial weight of the *Chaetomeium* sp., inoculated rubber strip was 2g and the final weight was 1.29g and there was a weight loss of 0.71g. Initial weight of *Verticillium* sp., inoculated sample was 2g and final weight was 1.48g and there was a weight loss of 0.52g (Table 2). In the time interval of 3 months *Chaetomeium* sp. showed 35.5% degradation and *Verticillium* sp. Showed 26% degradation. 50% weight loss was observed in the rubber sample as the incubation period increased (Fig. 7).

Figure 7: *Chaetomeium* sp. showing positive result to Sciff's base test.

Table 1: Showing percentage of weight loss of rubber treated with fungal organisms

Sl. No.	Name of the organism	Initial weight (g)	Final Weight (g)	Weight Loss(g)	Weight loss In percentage
1.	*Chaetomeium* sp.	10±0.51	7.64±0.14	2.36	23.6%
2.	*Verticillium* sp.	10±0.66	8.45±0.18	1.55	15.5%

All results are expressed as Mean ± Standard Deviation of Mean; n=6

Table 2: Showing percentage of weight loss of rubber.

Sl. No.	Name of the organism	Initial Weight (g)	Final Weight (g)	Weight Loss(g)	Weight loss In percentage
1.	*Chaetomeium* sp.	2± 0.10	1.29±0.02	0.71	35.5%
2.	*Verticillium* sp.	2±0.11	1.48±0.02	0.52	26%

All results are expressed as Mean ± Standard Deviation of Mean; n=6

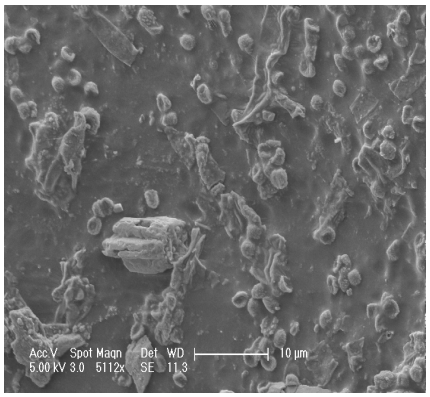
Confirmation of Rubber Degradation by Staining with Schiff's Reagent

For the confirmation of rubber degradation, rubber sheets on which there was growth of the organism were stained with Schiff's reagent. Along with this treated sample uninoculated rubber sheet sample was maintained as blank. Rubber sheets which were inoculated with microorganism turned to purple color and there was no color formation in the blank. Formation of purple color in the treated sample is due to the presence of aldehyde and ketone group which is produced as a result of degradation of cis-1,4-polyisoprene units (Fig. 6 and 7).

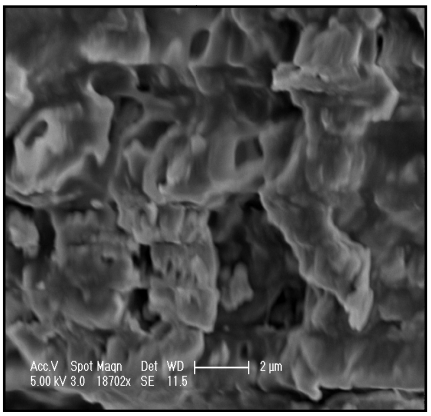
Confirmation of Rubber Degradation by Scanning Electron Microscopy

When rubber strips were observed under scanning electron microscope biofilm formation was observed and complete disintegration of the rubber strip material was also observed. The structural changes in the form of pits and crosions observed under scanning electron microscopy indicated surface damage of rubber incubated with *Chaetomeium* sp. and *Verticillium* sp. This study suggested that the fungal strains were able to adhere to the surface of rubber and cause surface damage by brake down of cis-1, 4-polyisoprene chain (Fig. 8).

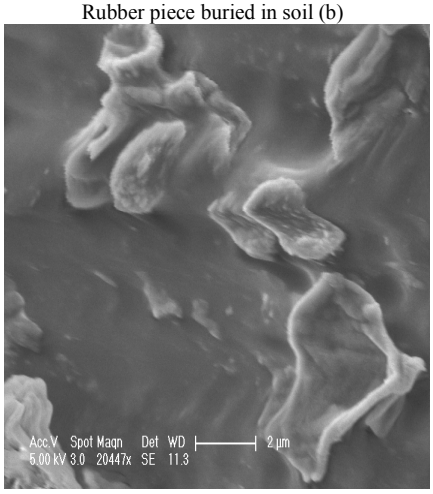
Figure 8: *Verticillium* sp. showing positive result to Sciff's base test.



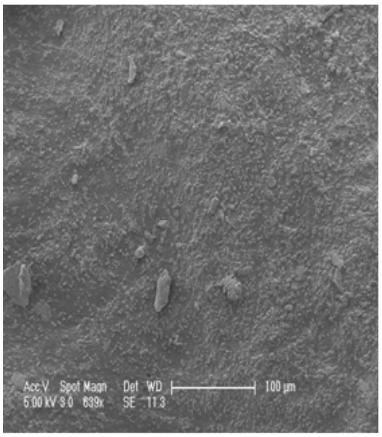
Control (a)



Rubber piece buried in soil (b)



Rubber treated with *Chaetomeium* sp. (c)



Rubber treated with *Verticillium* sp. (d)

Figure 9: SEM photograph of control (a), Rubber piece buried in soil (b) Rubber treated with *Chaetomeium* sp. (c) and Rubber treated with *Verticillium* sp.(d).

DISCUSSION

Rubber products are widely used in our daily life these products are mainly made up of Natural rubber (NR) which is obtained from the latex of tree *Hevea brasiliensis* commonly called Rubber tree. 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% aminoacids etc., and other 50-70% water. The different rubber products are manufactured by using vulcanized natural rubber. During manufacturing along with vulcanized natural rubber other chemical additives will be added. The percentage of natural rubber content and the additives added mainly depends on the products manufactured. After usage of these natural rubber products the disposal of these products are the world wide solid waste problem. One of the solutions to reduce this problem is to recycle the used waste rubber. But due to the chemical cross linking formed during vulcanization it is not possible to simply melt and reshape the products as in case of polythene. So other alternatives such as microbial degradation of the product should be developed. Microbial degradation is mainly carried out by various microorganisms such as bacteria and fungi. During the present study an attempt was made to isolate rubber degrading microorganism. Rubber pieces were dumped in the soil for regular interval of time and then plated on the media to isolate the organism. In the isolated organism *Chaetomeium* sp. and *Verticillium* species effectively degraded the rubber sample. (Breberg *et al.* 2002)

In the time interval of 2 months *Chetomeium* sp., showed 23.6% degradation and *Verticillium* sp. Showed 15.5% degradation. For the confirmation of rubber degradation, rubber sheets on which there was growth of the organism were stained with Schiff's reagent. Along with this treated sample uninoculated rubber sheet sample was maintained as blank. Rubber sheets which were inoculated with microorganism turned to purple colour 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 as a result of degradation of cis-1,4-polyisoprene units. The present study has showed that, it is possible to use these three strains to degrade the rubber. Similar attempts were made by several other scientists to degrade rubber by using microorganisms. Roy *et al.* (2005) made an attempt to study on 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 Tsuchii *et al.*(1996) 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 rubber are very resistant to high temperature and persist in environment for very long time. Rubber materials have been increasingly used now days in different area after usage its disposal is a very big solid waste problem. It can not be easily recycled due to the sulphur cross linking formed during vulcanization. If they are burnt they release enormous amount of carbon-di-oxide and some other gases which cause environmental pollution and contribute to the global warming. Rubber products such as balloon which are disposed in the natural environment are considered to be dangerous to wild animals if they are consumed by animals. One of the alternative ways to solve this problem is to subject these products for biodegradation. During the present study rubber pieces were dumped in the soil for regular interval of time and then plated on the media to isolate the organism. In the isolated organism *Chaetomeium* sp. and *Verticillium* species effectively degraded the rubber sample. The present study has showed that, it is possible to use these three strains to degrade the rubber.

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