

Comparative Studies on Rubber Biodegradation by Mixed Culture and Pure Culture Isolated From the Soil Samples

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Abstract— An increasing production of natural rubber (NR) compounds has led to major challenges in waste management. The degradation of NR is normally extremely slow. In this study, the degradation of rubber latex gloves in a mineral salt medium (MSM) using a mixed culture of the selected bacteria and a pure culture was investigated by monitoring weight loss, scanning electron microscopy (SEM) and viable cell counts. About 18% weight loss of the rubber gloves were detected after incubated with the mixed culture and the pure culture. The increased viable cell counts over incubation time indicated that cells used rubber gloves as a carbon source leading to the degradation of the polymer. The degradation of NR gloves was also determined by observing the growth of NR-degrading bacteria on the glove surface using SEM.

Keywords— Biodegradation, natural rubber, latex glove.

I. INTRODUCTION

Natural rubber is a polymer that is produced from several plant species. The main composition of natural rubber latex is poly-1, 4 isoprene that consists of isoprene as a monomer. In nature, poly isoprene can be divided into two groups, poly-cis isoprene and poly tran isoprene. Natural rubber is mainly produced from the *Hevea brasiliensis*. However, not only *Hevea brasiliensis* but also many plants can produce latex [16]. In addition, they are an alternative rubber such as *Ficus elastica*, *Ficus nitida* and *Euphorbia pulcherrima* [9]. The latex from the *Hevea brasiliensis* contains a polymer of cis-1, 4-polyisoprene units. Up to 90% of the dry weight of the latex is cis-1, 4-polyisoprene and less than 10% is non-rubber constituents such as protein, and carbohydrates [17].

Global natural rubber production increased to 12.2 million metric tons in 2013, a rise of 79.4 % from 6.8 million metric tons in 2000 [18]. These polymers can be used in thousands of ways i.e. in the fabrics, mining, power generation, agriculture, transportation, paper industries, insulation of cables, wires, and various equipments, in addition, it is the major component in rubber tires [16]. However, billions of discarded tires are currently stockpiled around the world and these are increasing exponentially and causing important environmental problems.

At present, much rubber waste is eliminated by either burned or used as landfills, but this process can cause serious pollution [6].

Biodegradation is the degrading process by microorganisms. Roles of microbes in degradation process are releasing of an extracellular enzyme to cleave polymer chain into small molecules and these products are absorbed into the cell for use as carbon and energy source. Final process, CO₂, H₂O and other metabolic products are released and they can be used for photosynthesis by the plants. Therefore, the biodegradation is an alternative way to degrade the rubber waste and could overcome these environmental problems [2], [3].

However, microbial degradation of polyisoprene rubber is a very slow process that is taking months or even years. Microbial degradation of poly (cis-1, 4-isoprene) rubber is currently being intensively investigated. So far rubber-degrading organisms have been classified into two different groups; the first group is rubber-degrading organisms producing clear zones on natural rubber latex agar plates. Members of these groups generally belong to the mycelium-forming actinomycetes such as *Actinoplanes*, *Streptomyces*, and *Micromonospora*. In contrast, the second group of rubber-degrading organisms is the organisms which cannot produce any clear zone, therefore they require the direct contact with the rubber substrates. The example of this group is nocardioform, actinomycetes i.e. *Gordonia*, *Mycobacterium* and *Nocardia*. *Xanthomonas* sp. strain 35Y is the only known gram-negative rubber degrading bacterium which does not belong to the actinomycetes [13].

In this study, the degradation of rubber latex gloves in a mineral salt medium (MSM) by a mixed culture of the selected bacteria and a pure culture was compared. The weight loss, scanning electron microscopy (SEM) of the rubber glove substrate and viable cell counts were determined.

II. METHODS

A. Isolation of rubber degrading microorganisms from soil samples by enrichment culture technique

The rubber-degrading microorganisms were isolated from soil samples collected from various contaminated rubber sites in Songkhla, Thailand. Approximate ten grams of the soils mixed with pieces of natural rubber (NR) glove were added to

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100 mL of sterile mineral salt medium (MSM) in each Erlenmeyer flask and were then incubated at 30°C at 150 rpm. Mineral salt medium is composed of (g/L): Na₂HPO₄ 9.0, KH₂PO₄ 1.5, (NH₄)₂NO₃ 1.0, MgSO₄ 0.2, CaCl₂ 0.02, and Fe(III)NH₄ 0.0012. The medium was adjusted to an initial pH of 7.0 before sterilization. After a month of incubation, 0.5 mL of each culture broth was transferred into a new flask containing fresh MSM with NR glove and further incubated under the same conditions as described above for another month to encourage the rubber degrading bacteria to grow. The developed culture broth was then assigned as the natural soil consortia and stored in 15% glycerol at -80°C. The rubber degrading bacteria were also screened and isolated from the culture broth using NR latex agar plates.

B. Screening of NR-degrading bacteria

The degradation of the rubber gloves by the selected strains was investigated through plate assay. Latex overlay plates were prepared by spreading 5 mL of solubilized latex concentrate as a thin film on MSM agar plates and then left it to evaporate until dried. All tested strains that grew well on the latex overlay agar were picked for further study.

C. Rubber glove degradation of the individual tested bacteria and the mixed culture

Latex glove pieces were washed once with distilled water prior to drying in an oven at 60 °C until obtained a constant weight before being used as a carbon source. Two experiments were designed to test the microbial activities for degrading rubber gloves. The first experiment was testing each individual isolated strain and the second experiment was the randomly mixed cultures of isolated strain. Each culture was incubated in MSM supplemented with dried latex glove piece (0.6% w/v) as a sole carbon source and were incubated at 30 °C, 150 rpm for 4 weeks. This experiment was performed in triplicate. The inoculated MSM without any latex glove pieces and the non-inoculated MSM with latex glove pieces were used as control experiments. Viable cell counts of each culture were determined throughout the incubation time. Weight loss of incubated latex gloves at the end of incubation period was measured and calculated in gram unit.

D. Scanning Electron Microscope (SEM)

The surface of the latex glove film pieces from the inoculated samples and the control were analyzed by scanning electron microscopy (SEM) to investigate the surface changes after the degradation. The samples were washed with sterilized distilled water and then were mounted on aluminum stubs. Gold coating was carried out in vacuum by evaporation to make the samples conducting. SEM images of the tested and control samples were compared.

III. RESULT AND DISCUSSION

A. Isolation of rubber-degrading microorganisms from soil samples

Ten bacterial isolates, F1 to F10, obtained from soil samples were capable of growing on MSM agar containing NR. Isolate F5 and F7 were identified as Gram-positive and others were

Gram-negative bacteria. All ten isolates did not produce clearing zone around the colonies indicated that they could degrade the latex concentrate by a production of an intracellular enzyme [16].

B. Weight loss of degraded latex glove

The weight loss of latex glove pieces after one month incubation was around 0.5-18%. The highest weight loss of 18.82% received from the mixed cultures (Fig. 1) and 9.36% from the pure culture of isolate F5 compared to only 0.2% of the control samples (see Table I). Moreover, NR glove had been broken down into little pieces (Fig. 2) indicated that the bacteria had the ability to degrade natural rubber

TABLE I
WEIGHT LOSS IN LATEX GLOVE AND VIABLE CELL COUNTS OF PURE CULTURE

Isolate	Weight loss of latex glove	Viable cell counts (CFU/mL)	
		Day 0	Day 30
F1	4.31%	1.22×10 ⁸	3.20×10 ⁸
F2	4.45%	1.15×10 ⁸	4.30×10 ⁸
F3	3.92%	1.21×10 ⁸	3.00×10 ⁸
F4	0.88%	1.20×10 ⁸	1.40×10 ⁸
F5	9.36%	1.21×10 ⁸	1.50×10 ⁹
F6	4.52%	1.20×10 ⁸	4.50×10 ⁸
F7	4.31%	1.26×10 ⁸	3.40×10 ⁸
F8	1.54%	1.23×10 ⁸	2.40×10 ⁸
F9	0.53%	1.25×10 ⁸	1.31×10 ⁸
F10	1.22%	1.19×10 ⁸	2.10×10 ⁸
Control	0.20%	0.00	0.00

C. Viable cell counts

Mixed culture grew well with the highest viable cell count of 6.20×10⁹ CFU/mL (see Table II). The best growth related to the highest weight loss of latex glove pieces among selected strains. The increased viable cell counts through the end of the incubation time indicated that the bacteria had the ability to degrade natural rubber and used rubber gloves as the sole carbon source.

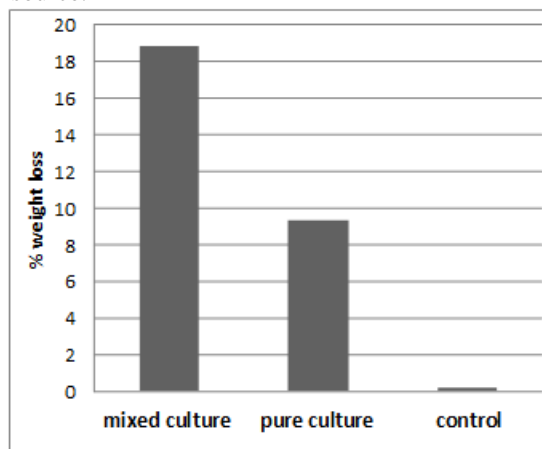


Fig. 1 Percentage weight loss of rubber gloves after incubation with various inocula.

TABLE II
WEIGHT LOSS IN LATEX GLOVE AND VIABLE CELL COUNTS OF VARIOUS INOCULA

Isolate	Weight loss of latex glove	Viable cell counts (CFU/mL)	
		Day 0	Day 30
mixed culture	18.82%	1.15×10^8	6.20×10^9
pure culture (F5)	9.36%	1.21×10^8	1.50×10^9
pure culture (other strains)	>5.00%	1.20×10^8	$>1.00 \times 10^9$
control	0.20%	0.00	0.00

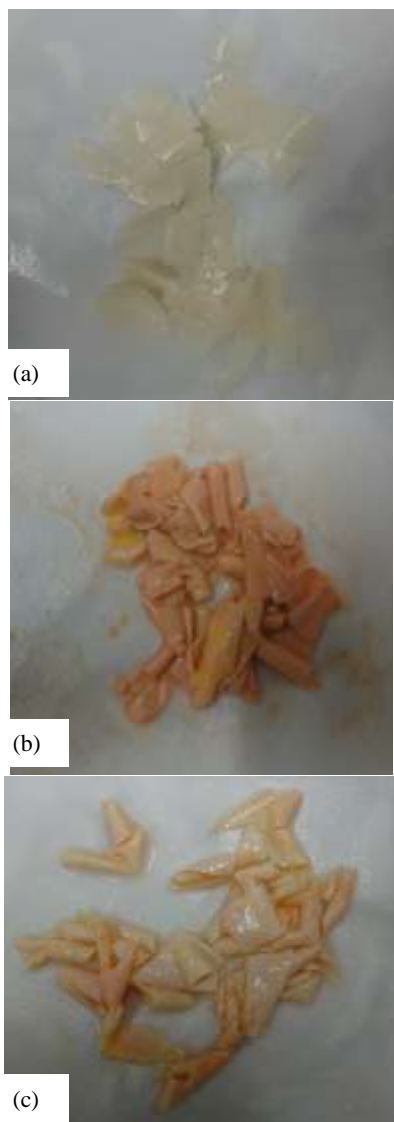


Fig. 2 Image of the latex glove pieces after incubation for 30 days: (a) control, (b) mixed culture and (c) pure culture (isolate F5).

D. SEM

Fig. 3a shows the surface of the control piece of NR glove (uninoculated). They were smooth without any pits whereas the glove pieces that were exposed to mixed culture for 30 days showed a rough appearance i.e. there were numerous irregular cracks, surface erosions, and pits in various sizes on

the surface of polymer films (Fig. 3b). This is a similar situation to some other species e.g. *Bacillus* sp. AF-666 [13] and *Streptomyces* strain La 7 [7]. Interestingly, isolate F5 colonized not only on the surface but also deeply inside the polymer holes (Fig. 3c). The colonization and growth on the NR glove surface were confirmed by SEM and showed that there were numerous small holes on latex glove degraded by F5 than in mixed culture.

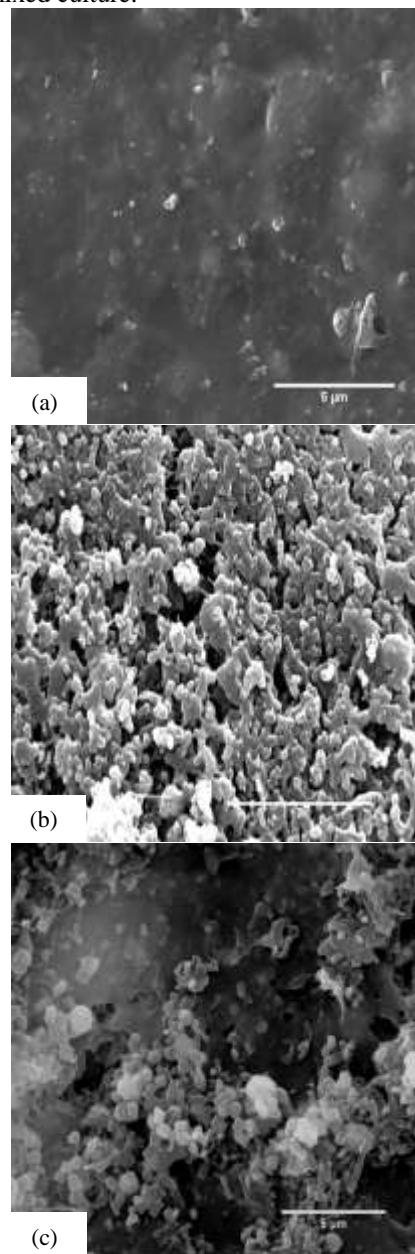


Fig. 3 Scanning electron micrographs of the latex glove pieces after incubation for 30 days: (a) control, (b) mixed culture and (c) pure culture (isolate F5).

IV. CONCLUSIONS

In the present study, a mixed culture and pure culture were able to degrade poly (cis-1, 4-isoprene) which is the monomer compositions of rubber. The weight loss of rubber, the increase in viable cell counts and the appearance changes and the microbial growth of the NR glove surface after one-month

incubation confirmed the biodegradation of natural rubber by these isolates. Mixed culture of the selected strains reached the highest rubber degradation with the percentage of weight loss of 18.82 % after one-month incubation (Fig. 1). This mixed culture has shown the potential to be used efficiently for rubber degradation.

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Isolation of rubber degradation bacteria from soils for complete natural rubber degradation