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Rubber gloves biodegradation by a consortium, mixed culture and pure culture isolated from soil samples

Chairat Nawong, Kamontam Umsakul, Natthawan Sermwittayawong*

Prince of Songkla University, Faculty of Science, Department of Microbiology, Songkhla, Thailand

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ABSTRACT

An increasing production of natural rubber (NR) products has led to major challenges in waste management. In this study, the degradation of rubber latex gloves in a mineral salt medium (MSM) using a bacterial consortium, a mixed culture of the selected bacteria and a pure culture were studied. The highest 18% weight loss of the rubber gloves were detected after incubated with the mixed culture. The increased viable cell counts over incubation time indicated that cells used rubber gloves as sole carbon source leading to the degradation of the polymer. The growth behavior of NR-degrading bacteria on the latex gloves surface was investigated using the scanning electron microscope (SEM). The occurrence of the aldehyde groups in the degradation products was observed by Fourier Transform Infrared Spectroscopy analysis. *Rhodococcus pyridinivorans* strain F5 gave the highest weight loss of rubber gloves among the isolated strain and posses latex clearing protein encoded by *lcp* gene. The mixed culture of the selected strains showed the potential in degrading rubber within 30 days and is considered to be used efficiently for rubber product degradation. This is the first report to demonstrate a strong ability to degrade rubber by *Rhodococcus pyridinivorans*.

Introduction

Natural Rubber (NR) is a biopolymer that is produced from some plant species. The key composition of natural rubber latex is *cis*-1,4-poly(isoprene) that consists of isoprene as a monomer. In nature, polyisoprene can be divided into two groups, e.g. *cis*-poly(isoprene) and *trans*-poly(isoprene). The major source of natural rubber was produced from the *Hevea brasiliensis*. However, not only *Hevea brasiliensis* but also many plants can produce latex such as *Ficus elastica*, *Ficus nitida* and *Euphorbia pulcherrima*.[1] The latex from the *Hevea brasiliensis* contains a polymer of *cis*-1,4-poly(isoprene) units and up to 90% of the dry weight of the latex is *cis*-1,4-poly(isoprene) and less than 10% are non-rubber constituents such as protein and carbohydrates.[2,3]

* *Corresponding author*.
E-mail: Kamontam.u@psu.ac.th (K. Umsakul).
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Global natural rubber production increased about 79.4% from 6.8 million metric tons in 2000 to 12.2 million metric tons in 2013.[4] Natural Rubber is utilized daily in numerous applications such as agriculture, transportation, and various farming equipment, in addition, it is the major component in rubber tires.[3] However, billions of discarded tires are currently stockpiled around the world and these are increasing exponentially and causing important environmental problems. At present, most rubber waste is eliminated by either burned or used as landfills but this process can cause serious pollution.[5] Biodegradation of rubber wastes has been much of interest. Roles of microbes in degradation process are releasing of extracellular enzymes to cleave polymer chains into small molecules and these products are absorbed into the cell for use as carbon and energy source. Final process, CO_2, H_2O and other metabolic products are released and they can be used by other living organisms. Therefore, the biodegradation is an alternative way to degrade the rubber waste and could overcome the environmental problems.[6–9]

However, microbial degradation of polyisoprene rubber is a very slow process that taking months or even years. Microbial degradation of *cis*-1,4-poly(isoprene) rubber is currently being intensively investigated. Rubber-degrading organisms can be 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*, *Micromonospora* and *Rhodococcus*.[10–12] In contrast, the second group of rubber-degrading organisms is the microorganisms, which do not produce any clear zone, therefore, they require the direct contact with the rubber substrates. The examples of this group are nocardioform, actinomycetes, i.e. *Gordonia* sp., *Mycobacterium* sp. and *Nocardia* sp.[10,13–15]

Lcp (latex-clearing protein) encoded by *lcp* gene has been considered as a key enzyme in NR degradation by clearing-zone-forming gram-positive bacteria. While RoxA (rubber oxygenase) is a key enzyme in NR degradation found in the gram-negative bacterium *Xanthomonas* sp. strain 35Y. RoxA controlled by *roxA* gene is an extracellular protein secreted by this strain during growth on NR. Purified RoxA degraded *cis*-1,4-poly(isoprene) by oxidative cleavage at the double bonds, yielding 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al as the main cleavage product; other minor cleavage products differed only in the number of repetitive isoprene units. In vitro experiments also revealed occurrence of two 18 O atoms in the reduced degradation product 12-hydroxy-4,8-dimethyltrideca-4,8-diene-1-ol, thereby disclosing a dioxygenase mechanism.[16–18]

Most efforts on the study of rubber biodegradation have been directed to using single bacterial strain which still gave poor results. It was hypothesized that the synergistic interaction among various microorganisms should accelerate the rubber degradation process. So in this study, the degradation of rubber latex gloves in a mineral salt medium (MSM) by a consortium, a mixed culture of the selected bacteria and a pure culture were compared. The weight loss, scanning electron microscopy (SEM) of the rubber glove substrate and viable cell counts were determined.

Materials and methods

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

The rubber-degrading microorganisms were isolated from soil samples collected from rubber contaminated ground in Songkhla province, Thailand. Soil samples were mixed with pieces of natural rubber (NR) glove (0.5×0.5 cm) incubated in sterile mineral salt medium (MSM) at 30 °C, 150 rpm. Mineral salt medium is composed of (g/L): Na_2HPO_4 9.0, KH_2PO_4 1.5, $(NH_4)_2NO_3$ 1.0, $MgSO_4$ 0.2, $CaCl_2$ 0.02, and Fe (III) NH_4 0.0012. The medium was adjusted to an initial pH of 7.0 before sterilization.[19] After a month of incubation, 0.5 mL of those culture broths were transferred into MSM with latex and further incubated under the same conditions as described above for another month to encourage the growth of rubber degrading bacteria. The developed culture broth was then assigned as the natural soil consortium. The rubber degrading bacteria were also screened and isolated from the natural soil consortium using NR latex agar plates.

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 to dry. All tested strains that grew well on the latex overlay agar were picked for further study.

Rubber glove degradation investigation of the individual tested bacteria, the mixed culture, and the natural soil consortium

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. Three experiments were designed to test the microbial activities for degrading rubber gloves. The first experiment was testing each individual isolated strain. The second experiment was testing the mixed cultures of high effective isolated strains (mixed cultures) and the third experiment tested the mixed cultures of all isolated strain (consortium). Each culture was incubated in MSM supplemented with dried latex glove pieces (0.6%, w/v) as a sole carbon source at 30 °C, 150 rpm for 4 weeks. Each experiment was performed in triplicate. The inoculated MSM without any latex glove pieces and the uninoculated MSM with latex glove pieces were used as biotic and abiotic control experiments, respectively. Viable cell counts of each culture were determined throughout the incubation time. The weight of latex gloves were measured before (W1) and after degradation (W2). Weight loss was calculated using equation: Weight loss = $[(W1 - W2)/W1] \times 100$.

Scanning electron microscope (SEM)

The surfaces of the NR glove film pieces were analyzed by scanning electron microscopy (SEM) to investigate the

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