



Screening of Natural Rubber-Degrading Microorganisms from Rubber Processing Factory Waste in Vietnam

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Abstract — Natural rubber-degrading microorganisms were isolated from waste of rubber processing factory in Cam Thuy of Vietnam. Four of them belong to *Streptomyces* sp. that showed the higher abilities for natural rubber degradation than the others. They are able to use both deproteinised natural rubber (DPNR) and synthetic rubber *cis*-1,4-polyisoprene (SR) as a sole source of carbon. Gel permeation chromatography (GPC) analysis revealed that these strains degraded DPNR and SR to low-molecular-weight products. The growth of isolates occurs essentially on the latex glove pieces after one month of incubation in mineral salt medium. The total nitrogen contents of glove pieces were determined using Kjeldahl method, which were 10-20 times higher than that in un-inoculated sample. Moreover, the degradation was also confirmed by observing the growth of isolates on glove's surface using scanning electron microscopy (SEM).

Keywords — degradation, isolation, natural rubber, poly(*cis*-1,4-isoprene), *Streptomyces* sp.

I. INTRODUCTION

Natural rubber is a biopolymer containing poly(*cis*-1,4-isoprene). It is an important material for tires, latex gloves, catheters, and so on. The typical method for isolation degrading- microorganisms is using a clearing zone technique. Two distinct groups were found. Member of the first group form clearing zones around their colonies on latex overlay agar plates. Most of them belong to the *Actinomycetes* [3], [4]. Member of the second group do not produce clearing-zones on latex overlay agar plate like *Gordonia*, *Mycobacterium*, and *Nocardia*. They are supposed to grow adhesively on the surface and extensively disintegrate of rubber particles [5], [6]. In this study, we screened and isolated natural rubber-degrading microorganisms from waste of Cam Thuy rubber processing factory including *Streptomyces* sp. Characterization of these strains revealed transformation

of poly(*cis*-1,4-isoprene), DPNR and also the growth of them on the latex glove pieces.

II. MATERIALS AND METHODS

2.1 Isolation of Natural Rubber-Degrading Microorganism

Waste rubber and waste water were collected from Cam Thuy rubber processing factory in Thanh Hoa province in Viet Nam. Three grams of waste rubber or one milliliter with waste water was suspended in 9ml sterile distilled water, and 100 μ l of the supernatant after standing still was spread on a latex overlay agar plate, 0,4% (v/v) latex was included in minimal salt medium (MSM) [5] to prepare the overlay layer of each plate. Colonies with clearing-zones formed after incubation at 30°C for 3 days were transferred to a fresh latex overlay agar plates, and incubated at 30°C for 3 days.

2.2 Aldehyde Staining of Colonies on Overlay Agar

Aldehyde groups in latex overlay agar were stained for 30 minutes with Schiff's reagent. Afterwards, the reagent was removed, and the plates were washed with 10%(w/v) sodium hydrogen sulfite solution, following by distilled water.

2.3 Determination of 16S rRNA Gene Sequences

Total DNA of these strains were prepared as described by [1] with modifications were described by [6]. 16S rRNA gene amplification, and nucleotide sequencing analysis were performed as described in previous studies [4], [6]. Sequences were aligned with published sequence in Genbank database using the basic local alignment search tool (BLAST) at the National Center of Biotechnology Information.

2.4 Degradation of Poly(*cis*-1,4-isoprene) and DPNR in Liquid Culture

The method of this test was described by [4].

2.5 Degradation of Poly(*cis*-1,4-isoprene) of a Latex Glove Piece

The latex glove pieces were cut from latex glove and washed in distilled water after being dried to constant weights. Initial dry weight of each latex glove piece added to each test tube was 37 to 40 mg. Each test tube filled 5ml MSM was inoculated with 500 μ l of the cell suspension at 30°C. After 4 weeks incubation, latex glove pieces were taken to scanning electron microscopy (SEM). The rubber weight loss (%) was calculated according to the following equation, 100 x (initial weight of rubber - residual rubber after one month incubation)/initial weight. The cell suspended in the medium were collected by centrifugation and weighed after being dried. The nitrogen content of latex glove pieces and cell suspensions were determined following the Kjeldahl method.

2.6 Scanning Electron Microscopy

Latex glove pieces were fixed in a mixture of 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer and dehydrated by increasing the acetone concentration step wise up to 100%. After drying, the samples were coated with platine and examined with a scanning electron microscope S-4800 (Hitachi, Japan).

III. RESULTS AND DISCUSSION

3.1 Isolation of Natural Rubber-Degrading Microorganism

The bacterial colonies with clearing zone on latex overlay agar plates were picked up and transferred to fresh latex overlay agar plates. The reproduced colonies with clearing zone on latex overlay agar plates were subjected to repeat single-colony isolation on LB (Luria-Bertani Broth) medium three times. Four isolates with large clearing zone were selected for further investigations.

The 16S rRNA gene sequences of four isolates were determined, and showed the highest similarity to those of *Streptomyces griseorubens* [AY999870] (99% identity), *S. albogriseolus* [AB184780] (99% identity), and *Nocardio*

alba [FJ4811638] (99% identity) (Fig. 1). However, *Nocardio* *alba* belongs to *Streptomyces* group, therefore these isolates were designated as *Streptomyces* sp. strain NMD1, strain M4D1b, strain M4T1c, and strain T37. To analyze the phylogenetic relationship among them and the other known rubber-degrading microorganisms, a phylogenetic tree was constructed by the neighbor-joining method (Fig. 1).

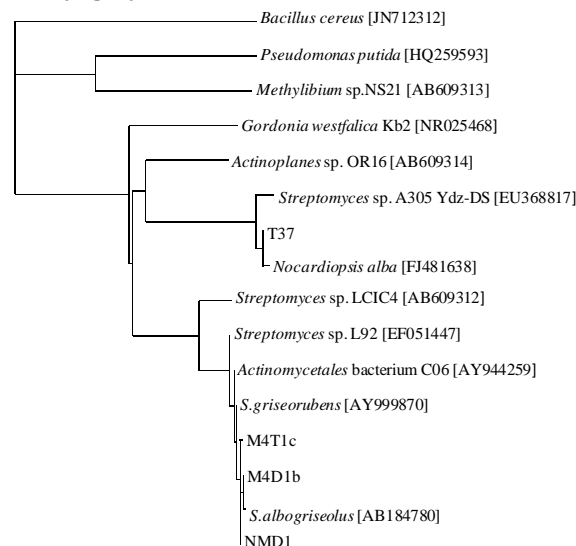


Fig. 1 Phylogenetic tree of 16S rRNA gene sequences of isolates (NMD1, M4D1b, M4T1c, and T37) with the other rubber-degrading microorganisms. The tree was constructed by the neighbor-joining method, and the scale corresponds to a genetic distance of 0.1 nucleotide substitutions per position (10% difference).

In order to determine whether the rubber-degradation products contain aldehyde group, formation of aldehydes on latex overlay agar plates was examined by staining with Schiff reagent. When Schiff's reagent was poured into latex overlay agar plates after inoculation for 3 days at 30°C, purple color developed around the colony of NMD1, M4D1b, and M4T1c. In contrast, no color development was detected around the colony of T37.

3.2 GPC Analysis of Poly(*cis*-1,4-isoprene) and DPNR Degradation

To examine the rubber-degradation ability of isolates, these strains were incubated in mineral salt medium (MSM) containing 0.25% (w/v) poly(*cis*-1,4-isoprene) or 0.5% (v/v) DPNR in flask. The whole culture broth after 30 days incubation was extracted with the equal volume of n-pentane at low temperature. Along with the poly(*cis*-1,4-isoprene) incubation at 30°C, SR-GPC profiles of isolates show a large peak area at about 27th minute of retention time deriving from rubber degradation product about 10kDa (Fig. 2). Interestingly, in the case of strain M4D1b, this peak lightly shifts to the higher retention time demonstrating for the lower degree of polymerization. This data clearly indicated that poly(*cis*-1,4-isoprene) was degraded by our isolates except T37. Among them, M4D1b is most potential strain for synthetic rubber degradation. In the case of DPNR-GPC profile (Fig. 3), also a significant peak was observed at 26th minute of retention time by M4D1 but not other strains. Hence, the DPNR degradation

by isolates is confirmed by further GPC analysis considering more longer incubation time.

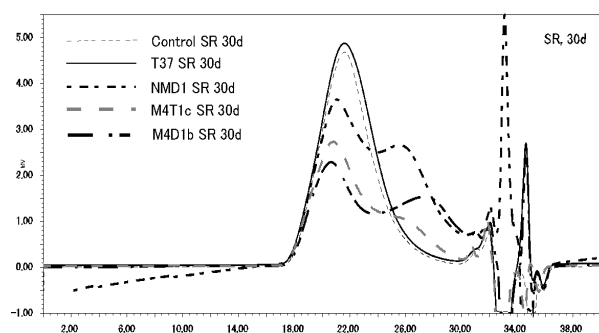


Fig. 2 GPC elution profiles for the residual polymers after incubation of isolates (NMD1; M4D1b; M4T1c; and T37) for 30 days (solid line) and no inoculum (dashed line) as a control; SR, Poly(cis-1,4-isoprene).

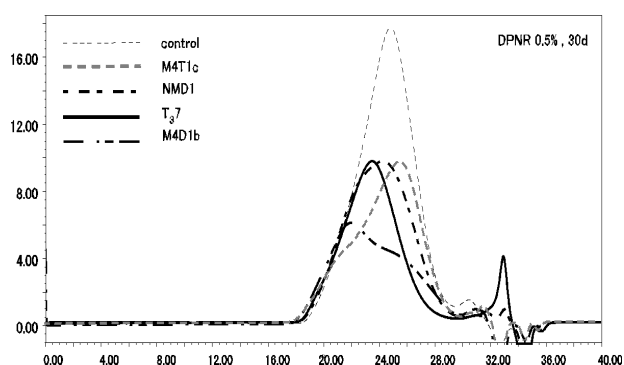


Fig. 3 GPC elution profiles for the residual polymers after incubation of isolates (NMD1; M4D1b; M4T1c; and T37) for 30 days (solid line) and no inoculum (dashed line) as a control; DPNR, Deproteinised natural rubber.

3.3 Degradation of Latex Glove Piece

The weight loss of latex glove pieces as well as cell mass and nitrogen content during incubation with isolates is shown in Table 1.

After one month incubation, the degradation of latex glove piece seems weakly, it was around 2-4%, however in control sample without inoculation the weight loss of the glove piece was 0.26%. The nitrogen contents of glove pieces were 10-20 times higher than that in control sample. During incubation, the isolates cells were growing on the surface of the latex pieces and in the culture liquid. The nitrogen contents in cell suspensions were higher than that in latex glove pieces, indicating soluble latex degradation products that may serve as carbon sources for the isolates. In case of strain NMD1, its weight loss and nitrogen total were the highest than other strains, suggesting this strain was the strongest degrader than other isolates. Nevertheless, the degradation of latex glove by strain NMD1 was lower than those of *Streptomyces* sp. strain La7 [2]. After 32 days incubation without shaking at 30°C, the weight loss of latex glove was 13.5%. In another study Tsuchii et al. 1996 showed the degradation of natural rubber by a strain of *Nocardia* after 21 days of incubation, 60-70 mg of latex glove strips were almost completely degraded [5].

Table 1. Degrdaton of latex glove piece by isolates through determination weight loss, cell mass and nitrogen content after 30 days incubation at 30°C

Sample	Latex glove piece		Suspended cells	
	Weight loss (%)	Nitrogen content (%)	Cell mass (mg)	Nitrogen content (%)
Control	0.26 ± 0.02	0.01 ± 0.01	0	0
NMD1	4.28 ± 0.32	0.22 ± 0.02	0.5 ± 0.3	1.84 ± 0.26
M4D1b	3.11 ± 0.5	0.16 ± 0.05	1.6 ± 0.4	1.33 ± 0.07
M4T1c	2.05 ± 0.76	0.12 ± 0.03	0.7 ± 0.3	1.50 ± 0.10
T37	2.43 ± 0.34	0.11 ± 0.02	1.3 ± 0.7	1.23 ± 0.17

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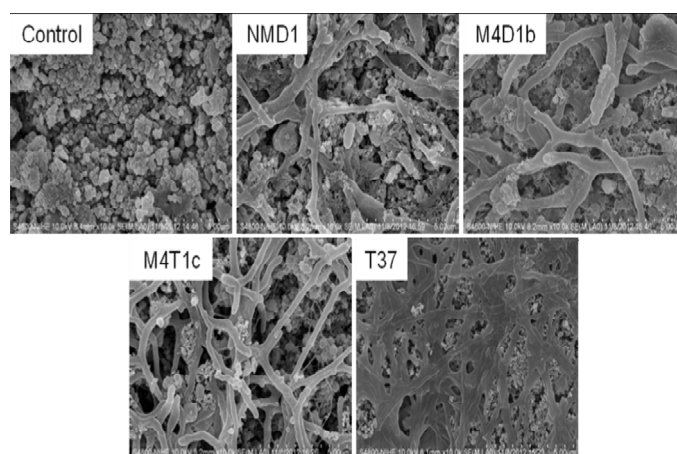


Fig. 4 SEM of isolates (NMD1; M4D1b; M4T1c; and T37) growing on latex gloves and surface of latex glove as a control sample after one month incubation in MSM medium

Obtained SEM clearly indicated the growth as well as the colonization of each isolate on the glove piece. In case of strain NMD1 and M4D1b, it seems these strains colonized not only on the surface but also deeply inside. The colonization and/or growth on the rubber surface shown by SEM micrographs have been observed for *Streptomyces* sp. [2].

IV. CONCLUSIONS

In the present study, four rubber-degrading bacteria, *Streptomyces* sp. strain NMD1, strain M4D1b, strain M4T1c, and strain T37, which produced clearing-zones around their colonies on latex overlay agar plates, were isolated. These strains degraded poly(*cis*-1,4-isoprene) to low-molecular-weight products. The results of the weight loss, the increase in nitrogen contents, the detection of growth by SEM confirmed the degradation of natural rubber by these strains.

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