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EXPERIMENTAL ARTICLES

Degradation of Natural Rubber Latex by New *Streptomyces labedae* Strain ASU-03 Isolated from Egyptian Soil[1]

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Abstract—Natural rubber latex is one of the problems that raises the environmental concerns. In this study the degrading ability of *Ficus elastica* rubber latex by a bacterium strain ASU-03, isolated from Egyptian soil was assessed. The strain was able to produce clear zone around its colony on latex rubber containing medium and was identified by conventional methods as *Streptomyces* sp. Phylogenetic analysis of 16S rRNA (16S rRNA) and RNA polymerase β-subunit (*rpoB*) genes were applied. Results of the 16S rRNA gene analysis revealed that the strain was highly related to *Streptomyces* sp. (100% similarity), so the *rpoB* gene was partially sequenced to clarify the specific name of the isolate. Phylogenetic tree based on *rpoB* gene sequences indicated that strain ASU-03 was highly similar to the reference strain *Streptomyces labedae* and both were shared a one cluster. The current results demonstrated that the use of a *rpoB* gene-based method gives a better resolution in the species level identification. To our knowledge, this species has never been reported to be involved in natural rubber degradation. This was therefore the first report about the degradation of *Ficus elastic* by *S. labedae*. The degradation of *Ficus elastica* rubber latex was determined by measuring the increase in protein content of bacterium (mg/g dry wt), reduction in molecular weight (g/mol) and inherent viscosity (dL/g) of the latex. Moreover the degradation was also confirmed by formation of aldehyde or *keto* group by Schiff's reagent and by observing the growth of the *Streptomyces* strain using scanning electron microscopy.

Keywords: streptomyces, natural rubber latex, biodegradation, *rpoB* gene sequence, 16S rRNA gene, phylogenetic analysis

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Two types of polyisoprenoides, which differ according to their isomerism, are almost exclusively synthesized by many plants: poly (*cis*-1,4-isoprene) and poly (*trans*-1,4-isoprene). The *cis* isomer is the main constituent of Natural rubber latex (NR), a highly unsaturated hydrocarbon, with an average molecular weight about 10^6 Da [1, 2]. NR is produced by more than 2000 dicotyledonous plants. The *trans* isomer (gutta percha, GP) is synthesized by much fewer plants and occurs, for example, in South-east Asian trees *Palaquium gutta* and *Eucommia ulmoides*, the European shrub *Euonymus europaeus* and the South American tree *Couma macrocarpa*.

Degradation potential of the microorganism is dependent upon colonization on natural rubber and accompanied by a loss in the weight of the rubber hydrocarbon and a decline in the relative viscosity of the polymer solution. Many reports have been published on the biodegradation of natural rubber hydrocarbon as a sole carbon source by microorganisms and the biodegradation process have been found to be slow [1–8].

Investigations of bacterial degradation of natural rubber (NR) revealed two groups of NR-degrading bacteria according to their strategy for substrate utilization. Members of the first group form clear zones on latex overlay-agar plates, indicating an extracellular enzyme activity. Representatives belong to the genus *Streptomyces* other than *Streptomyces labedae* [2, 5, 7]. Members of the second group, which do not form clear zone, exhibit adhesive growth with direct contact of the cells with the NR material and extensive disintegration of the substrate. Representatives belong to the genera *Gordonia, Mycobacterium, Streptomyces, Nocardia* and *Bacillus* [9, 10].

Natural Rubber degrading bacteria can be useful for the disposal of discarded natural rubber products, the problems established as a result of wastage of valuable rubber and increase disposal of waste tires render our government to search for a mean to get rid of these two problems by biodegradation of natural rubber to eliminate the environmental pollution.

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Therefore, this work aimed to solve these problems by bacterial degradation of breaking down the rubber molecular weight through isolate and identify soil bacteria that have the ability to metabolize and degrade rubber latex of *Ficus elastica* as a sole carbon source. Phylogenetic analysis for the sequencing of the gene coding for 16S rRNA and the gene *rpoB* were applied to identify the most potent degrading strain to species level.

MATERIALS AND METHODS

Sampling and fractionation of crude latex. Latex from *Ficus elastica* grown in both farms of Faculties of Science and Agriculture, Assiut University was collected using tapping method [11]. It was obtained by injuring the trunk of *F. elastica* and withdrawing by sterile disposable syringe under sterile conditions into sterile eppendorf tubes. The fresh latex was centrifuged at 17,000 rpm for 20 min at 4°C in SR4000 Prolabo centrifuge (France). It was separated into a sticky top layer containing Natural Rubber (NR) as reported in our previous study [10]. The natural rubber fractions was separated and washed three times by deionized water to remove impurities and dried at 30°C for 24 h [12]. A known weight (20 mg) was dissolved in 2 mL of tetrahydrofuran (THF), and its molecular weight was then determined by gel permeation chromatography (GPC) [10].

Isolation and growth assessment. Microorganisms were isolated on Mineral Salts Medium (MSM) (8.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.1 g $Ca(NO_3)_2$, 20 mg $CaCl_2 \cdot 2H_2O$, 20 mg $FeSO_4 \cdot 7H_2O$, 0.5 mg $Na_2MoO_4 \cdot H_2O$, and 0.5 mg $MnSO_4$ per liter of deionized water) containing 30 mg yeast extract and 20 g agar per liter [13]. Pure NR was prepared and dissolved in tetrahydrofuran (200 mg/20 mL THF), then spotted on MSM agar medium (each 50 μL/colony) then left in an oven at 30°C until THF evaporated and the NR became white in appearance. The isolates were inoculated with tooth pick on the top of pure NR spot then incubated for 15 days at 36°C. The colony diameter was measured at the end of incubation period.

Assessment of biodegradation ability. Natural rubber (dried) was cut into pieces (20 mg each) and washed three times with ethyl alcohol, then left to evaporate at 30°C for 15 min. The NR-bacterial degrading strain was cultivated in sterile 100 mL Erlenmeyer flask containing 20 mg sterile piece of natural rubber/25 mL sterile MSM broth. The inoculated flasks were incubated in a shaking incubator at 150 rpm for 15 and 30 days at 36°C. After each incubation period the rubber pieces and the microorganism were separated by centrifuging at 10000 rpm for 15 min. The microorganism (pellet) was subjected for protein determination while natural rubber pieces (top layer) were collected, washed with deionized water, and dried at 30°C for 24 h and then subjected for analytical methods (molecular weight, viscosity and Schiff's reagent) as well as SEM photography as follow:

Protein determination. The total protein was determined according to the method adopted by Lowery et al. [14].

Viscosity determination. A known weight of NR was dissolved in 15 mL THF and the inherent viscosity of NR solutions at 29°C was determined using an Ubbelohde suspended level viscometer. The inherent viscosity of NR was calculated according to the method of Aly et al. [15] as follow:

$$\eta_{rel} = \frac{\eta}{\eta o} \quad \text{Relative Viscosity,}$$

$$\eta_{sp} = \frac{\eta - \eta o}{\eta o} = \eta_{rel} - 1 \quad \text{Specific Viscosity,}$$

$$\eta_{inh} = \frac{In\eta_{rel}}{c} \quad \text{Inherent Viscosity.}$$

Molecular weight determination by gel permeation chromatography (GPC). As described by Hesham et al. [10], a known weight of standard and the reset of NR after growing of organism were washed, dried, dissolved in 2 mL THF and passed through a 0.45-μm-pore size filter. GPC of the model Agilent technologies 1100, Germany (present in National Research Center, Doki, Cairo) was used. THF was used as the eluent with flow rate 1 mL/min. Polymethylmethacrylate and polystyrene standards were used to calibrate the columns using the refractive index detector 10^4 Å, Colum PL gel Micrometer 100; 10,000; 100,000. The GPC apparatus was run under the following conditions: flow rate = 2 mL/min, injection volume = 100 μL, sample concentration = 1 g/L. The values of molecular weight were computed by means of a computer program.

Detection of aldehyde or keto group by Schiff's reagent. Reagent was applied to detect aldehyde or ketone groups in polyisoprene degradation products. 10 mL of fuchsin reagent was added to the samples (50 μL of the NR dissolved in *n*-hexane) as described by [16]. The composition of the fuchsin reagent was prepared as follow: 2 g of fuchsin dissolved in 50 mL of glacial acetic acid plus 10 g of $Na_2S_2O_5$ plus 100 ml of 0.1 N HCl + 50 mL of H_2O [17]. Positive results are indicated by purple color at room temperature after 30 min.

Scanning electron microscopy (SEM). The morphological change during growth of the bacteria on NR was also assessed by SEM. The inoculated rubber samples as well as the control were fixed overnight in 5% glutaraldehyde, and then dried at 50°C. The samples were mounted on metal stubs and coated with gold and palladium (Jeol JFC1100E Iosputtering Device). Micrographs were taken by means of a Joel JSM-4500 LV electron microscope operating at 15 kV in Electron Microscopy Unit, Assiut University.

Phenotypic and Molecular Identification of the Bacterial Strain

Phenotypic identification. The isolate was identified based on its morphological and biochemical properties using Bergey's Manual of Systematic Bacteriology [18].

DNA isolation, amplification conditions with temperature-time profiles for different genes, and construction of phylogenetic trees. The extraction of total bacterial genomic DNA was performed according to the procedures described by Hesham [19]. Molecular genetics identification of the isolate was done by the analysis of partial sequences of the two genes (16S rRNA gene and *RNA polymerase β-subunit gene (rpoB gene)*. Amplification of 16S rRNA gene was performed with bacterial universal primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-CGGCTACCTTGTTACGACTT-3) [20] generating a PCR product corresponding to nucleotide positions 27 to 1492 of the *Escherichia coli* 16S rDNA sequence. A fragment containing 352 bp of the *rpoB* gene from *Streptomyces* strain was also amplified, using forward primer (SRPOF1, 5'-TCGACCACTTCGGCAAC-CGC-3') and reverse primer (SRPOR1, 5'-TCGATCGGGCACATGCGGCC-3'), as described previously by Kim et al. [21]. PCR reactions for the two genes were carried out in a final volume of 50 μL containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at a concentration of 0.2 mM, 1.25 IU of *Taq* polymerase, each primer at a concentration of 0.2 mM, and 1 μL of the DNA template. PCR was performed for the 16S rRNA gene with the following program: 5 min denaturation at 95°C, followed by 36 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1.5 min extension at 72°C, and a final extension step of 7 min at 72°C. On the other hand, the reaction mixture of the *rpoB* gene was subjected to 30 cycles of amplification (30 s at 95°C, 30 s at 60°C, and 45 s at 72°C) followed by a 5-min extension at 72°C. To verify the presence of appropriate sized of each amplicons, 5 μL from each PCR product was subjected to electrophoresis in 1% agarose gel according to standard methods. Product of the correct size was purified with a TakaRa garose Gel DNA Purification Kit Ver.2.0 and sequenced in both directions using an ABI 3730 automated sequencer. The bacterial 16S rRNA and *rpoB* genes sequences obtained were then aligned with known 16S rRNA gene sequences as well as *rpoB* gene sequences in GenBank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/), and percent homology scores were generated to identify bacteria. Phylogenetic trees were constructed with MEGA version 4.0 using a neighbor-joining algorithm, plus the Jukes-Cantor distance estimation method with bootstrap analyses for 1,00 replicates was performed.

Nucleotide sequence accession number. The sequences of the gene coding for 16S rRNA and the *rpoB* gene of *Streptomyces* strain ASU-03 reported in this study have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under Accession nos. KC420524 and KC420525 respectively.

RESULTS AND DISCUSSION

Isolation and conventional identification of rubber-degrading bacteria. Several bacterial strains were isolated from soil samples on latex agar plates. One isolate, ASU-03 could grow rapidly on both latex liquid and agar media. This isolate produced also clear zone during growth on opaque mineral salts agar with latex as a carbon source. It was selected as the most efficient strain to assess its natural rubber-degrading ability. The isolate was later identified based on its morphological and biochemical properties using *Bergey's Manual of Systematic Bacteriology* [18] as *Streptomyces* sp. (data not shown). The identity was confirmed by 16s rRNA and *rpoB* gene sequencing and phylogenetic analysis as *S. labedae*.

Rubber viscosity and molecular weight and bacterial protein of *S. labedae* ASU-03. The *Streptomyces* strain gave the higher mycelial protein (2.66 mg/g dry weight) and lower rubber viscosity values (6.98 dL/g) and was selected for further study to trace the biodegradation process after 15 and 30 days of incubation at 36°C.

The protein content for *S. labedae* grown on natural rubber increased significantly from 2.22 mg/g cell dry wt after 15 days to 4.07 mg/g cell dry wt after 30 days. On the other hand, rubber viscosity decreased significantly from 6.9 dL/g after 15 days of incubation with *S. labedae* to 4.3 dL/g after 30 days. Regarding molecular weight of separated natural rubber, it decreased from 6.7×10^5 after 15 days to 5.1×10^5 g/mol after 30 days (degradation rate of *S. labedae* after 30 days comparable with the control 41.4%) (table).

These results are in agreement with those of Tsuchii et al. [22] who found biodegradation of natural rubber after treatment for 6 weeks by strains of *Streptomyces coelicolor* and *Pseudomonas citronellolis*. A significant shift in the molecular weight to lower values was observed from the results of gel permeation chromatographic (GPC) analysis of recovered natural rubber. The shift was moderate after 2 weeks (Mw, 4×10^5 to $5 \times 10^5 = 20\%$) and was more evident after 6 weeks (Mw, 1×10^5 to $2 \times 10^5 = 50\%$). Also, a similar change in molecular weight was noted for *Pseudomonas* sp. after 20 weeks (Mw 2.7×10^5 to $1.9 \times 10^4 = 70.4\%$) [23]. Bode et al. [24] found that *Streptomyces lividans, S. coelicolor*, and *Pseudomanas citronellolis* could grow on opaque latex agar (purified natural latex of *Hevea brasiliensis*) at 30°C for 1 week. They found that only *S. coelicolor* 1A showed clearing zone formation which indicated utilization of the natural rubber. While growth of *S. lividans* 1326 and *P. citronellolis* were

Degradation of *Ficus elastic* rubber fraction by the mesophilic *Streptomyces labedae* strain AUS-003 through determination of protein content (mg/g dry wt); viscosity (dL/g) and molecular weight (g/mol) after shaking incubation for 15 and 30 days at 36°C

Analysis parameters	Protein content mg/g dry wt		Viscosity measurements dL/g		Molecular weight g/mol × 10^5	
Time (days)	15	30	15	30	15	30
Control (rubber only)			7.1 ± 0		8.7	
Rubber + *Streptomyces* strain	2.22[a] ± 0	4.07 [a] ± 0	6.9 [a] ± 010	4.31[a] ± 0.18 (39.44)	6.7	5.1 (41.38)

Figures in the table are mean of three replicates ± standard deviation. Values in brackets represent % percentage of degrading natural rubber by microorganisms in case of viscosity and molecular weight after 30 days. a—Values Significant at PC 0.05 level.

poor, and the formation of clearing zones could not be observed even after prolonged incubation, and apparently, *S. lividans* 1326 and *P. citronellolis* are unable to utilize natural rubber latex as a carbon source. Also Braaz et al. [25] reported that the *Xanthomonas* sp. produced clearing zones during growth on opaque mineral salts agar with latex as a carbon source. Spence and Van Niel [26] referred to pure cultures of four rubber-degrading actinomycetes forming clear zones on latex overlay plates. Nette et al. [27] described also three natural rubber-degrading actinomycetes namely *Proactinomyces ruber, Actinomyces elasticus,* and *A. candidus*. They also stated that decrease in the molecular weight indicates that these organisms are able to cleave the carbon backbone of the natural rubber.

Bode et al. [28] found that the percentage of biodegradation of natural rubber after treatment for 10 weeks by strains of *Streptomyces griseus* 1D 18%, and *Streptomyces coelicolor* 1A 10–18% after 6 weeks. Also Rose et al. [4] found that the percentage of biodegradation of natural rubber after 12 weeks of incubation by *Streptomyce* spp. K30 was 13.4%. Heisey and Papadatos [13] reported that the percentage of biodegradation by *Streptomyces* spp. 10% after 6 weeks. From the results of these authors we confirmed that the degradation rate by our strain *Streptomyce labedae* after 4 weeks (41.4%) was the best.

Detection of aldehyde or keto group resulted from degradation of natural rubber by Schiff's reagent. The 30-day-old-treated natural rubber sample with *S. labedae* strain was tested using Schiff's reagent for the releasing aldehyde or keto group compounds. These samples gave positive results with Schiff's reagent indicated by formation of purple color on releasing aldehyde or keto group compound due to NR degradation. These results are in agreement with those of Tsuchii et al. [22], Linos et al. [16] and Rose et al. [4] who pointed out that the purple color produced by the Schiff's reagent was evidence that isoprene oligomers containing aldehyde groups have been produced and accumulated during the microbial degradation.

Scanning Electron Microscopy (SEM). The 15- and 30-day-old-treated natural rubber samples with the mesophilic *Streptomyces* strain was photographed using SEM. These SEM microphotographs clearly indicate the growth as well as the colonization of the *Streptomyces* strain on the NR surface (Fig. 1). The growth after 30 days was more pronounced than after 15 days of incubation, however, the strain was not able to grow in rubber latex-free mineral medium. In this respect, the colonization and/or growth on the rubber surface shown by SEM micrographs have been observed for *Streptomyces* sp. other than *Streptomyces labedae* [4]. Tsuchii and Tokiwa [29] explained the degradation of rubber as a two step reaction. They found that the crude enzyme isolated from *Xanthomonas* sp. could degrade the isoprene chain mainly into two fractions. In the first step, the original polymer with very high molecular weight was degraded into polymers with medium molecular weights. In the second step, the polymers with medium molecular weight were again degraded to form polymers with low molecular weight. Therefore, the wide molecular weight distribution of the degraded fraction in the MS suggested the random scissions of the original polymer in endwise form [22]. The mechanism involved in the degradation of NR may be the oxidative cleavage which is very well reflected in the reduction of double bond character and in the presence of aldehydes. Previous studies on NR biodegradation with various microorganisms indicated that during rubber degradation, oxidative cleavage of the double bond in the poly *cis*-1,4 isoprene backbone occurred as the first step [6]. Presence of aldehydes and ketones have been reported among the degradation products in several studies [10, 16, 28].

Molecular identification and phylogenetic analysis using 16S rRNA gene sequence. The genomic DNA was extracted from the isolated bacterial strain ASU-003 and universal primers 27F and 1492R were used for the amplification and sequencing of the 16S rRNA gene

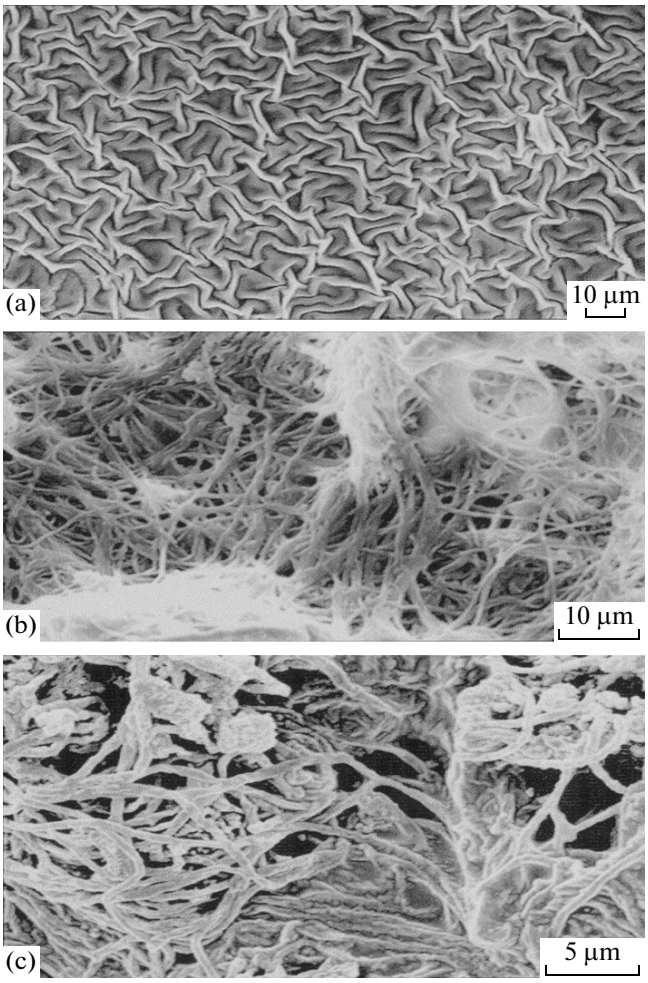

Fig. 1. Scanning Electron Microscope (SEMs) of the uninoculated rubber (a): and Degradation of rubber fig latex (as revealed by the inoculation of 25 mL mineral salts medium amended with 20 mg rubber with *Streptomyces labedae* ASU-03 and grown under 150 rpm by shaking at 36°C conditions for 15 days (b) and 30 days (c).

fragment. An almost complete sequence (~1.5 kb) of 16S rRNA gene was obtained for the *Streptomyces* strain ASU-03 and compared with the sequences of 16S rRNA regions in GenBank database by BLAST search. Results show that the 16S rRNA sequence of the isolated strain was highly homologous to *Streptomyces* sp. RJA2969, with 100% sequence similarity. To confirm the position of the strain ASU-03 in phylogeny, a number of sequences representative some *Streptomyces* species were selected from GenBank database for construction of a phylogenetic tree. As shown in Fig. 2, the phylogenetic tree indicated that strain ASU-03 and *Streptomyces* sp. shared a one cluster. Therefore, the strain ASU-03 was identified as *Streptomyces* sp. Recently, identification based on 16S rRNA gene sequencing and phylogenetic analysis was used for the family *Streptomycetaceae* [30–32].

Phylogenetic analysis using rpoB gene sequence. To get the full identification for the *Streptomyces* strain ASU-03, the *rpoB* gene analysis was performed using the *rpoB* primers (SRPOF1 and SRPOR2), the size of the amplified region was 352 bp for the strain ASU-03, which is the expected size of the *rpoB* fragment for the *Streptomyces* strains [21]. The *rpoB* DNA of the purified PCR products (352 bp) were sequenced and the obtained sequence (321 bp) data were compared with those of known *Streptomyces* strains, available at the GenBank database.

The alignment and comparison of the *rpoB* DNA sequence of the strain ASU-03 to the published *rpoB* DNA sequence belonging to eight reference *Streptomyces* strains (*S. labedae* JF424010, *S. erythrogriseus* JF424008, *S. speibonae* JF424004, *S. scabiei* EF198099, *S. albogriseolus* AY280729, *S. xanthocidicus* JF424024, *S. coelicolor* AY280745, and *S. flaveolus* AY520425) were determined and the phylogeny of these strains were constructed (Fig. 3). Results show that the sequence similarity between the strain ASU-03

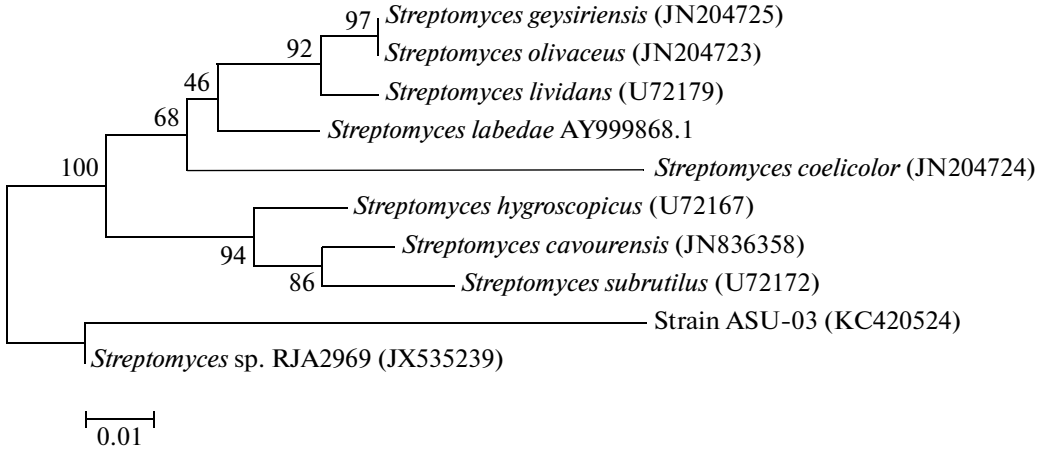

Fig. 2. Phylogenetic relationship between the *Streptomyces* strain ASU-03 and other 16S rDNA sequences of published strains. In the phylogenetic tree, ASU-03 and *Streptomyces* sp. were clustered together as one clade.

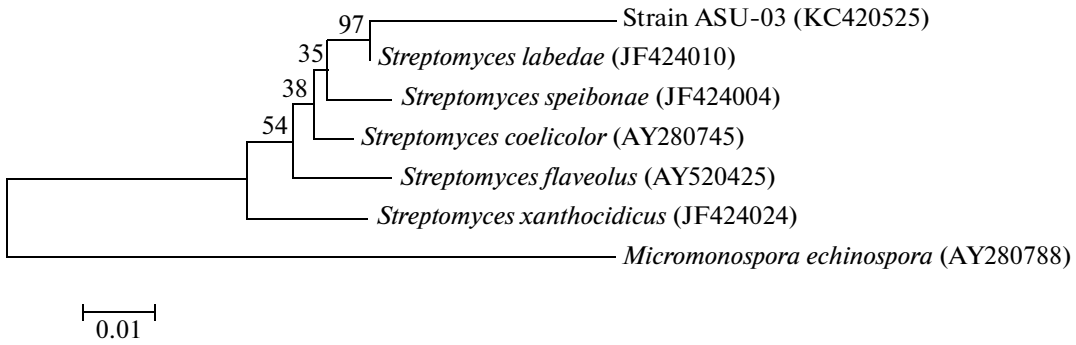

Fig. 3. Neighbor-joining tree showing relationships between *Streptomyces* strain ASU-03 and representatives of the genus *Streptomyces* based on partial nucleotide sequences (321 bp) of the *rpoB* gene. The scale bar indicates 0.01 nucleotide substitutions per nucleotide position. GenBank accession numbers are given in parentheses. *M. echinospora* was used as an outgroup.

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ASU-03   24  GGTCCGTACGGGTCTCGCCCGTATGGAGCGCGTCGTGCGCGAGCGCATGACCACCCAGGA  83
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. labe.  3  GGTCCGTACGGGTCTCGCCCGTATGGAGCGCGTCGTGCGCGAGCGCATGACCACCCAGGA  62

ASU-03   84  CGTCGAGGCGATCACGCCGCAGACGGTGATCAACATCCGGCCGGTCGTCGCCTCCATCAA  143
             |||||||||||||||||||||||  |||||||||||||||||||||||||||||||||||
S. labe.  63  CGTCGAGGCGATCACGCCGCAGACCCTGATCAACATCCGGCCGGTCGTCGCCTCCATCAA  122

ASU-03  144  GGAGTTCTACGGCACCAGCCAGCTGTCCGAGTTCATGGACCAGTACAACCCGCTGTCGGG  203
             |||||||| ||||||||||||||||||| |||||||||||| ||||||||||||||||||
S. labe. 123  GGAGTTCTTCGGCACCAGCCAGCTGTCCCAGTTCATGGACCAGAACAACCCGCTGTCGGG  182

ASU-03  204  GCTGACGCACAAGTGTCGTCTGAACGCCCTCGGCCCGGGTAGCCTCTCCCGTGAGCGGGC  263
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. labe. 183  GCTGACGCACAAGCGTCGTCTGAACGCCCTCGGCCCGGGTGGCCTCTCCCGTGAGCGGGC  242

ASU-03  264  AGGCTTCGAGGTCCGTGACGTGCACCCCTCGCACTACGGCCGCATGTGCCCGATCGA     320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S. labe. 243  CGGCTTCGAGGTCCGTGACGTGCACCCCTCGCACTACGGCCGCATGTGCCCGATCGA     299
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Fig. 4. *rpoB* gene sequence of *Streptomyces* strain ASU-03 compared with *Streptomyces labedae* JF424010.

and the reference strains ranged from 94 to 97%, whereas higher sequence similarity for strain ASU-03 was observed with the *S. labedae* JF424010 reference strain (Fig. 4). Phylogenetic tree based on the partial *rpoB* sequences demonstrated that strain ASU-03 and *S. labedae* shared a one cluster. Therefore, the identification of strain ASU-03 was confirmed as *S. labedae*. The result demonstrates that analysis of *rpoB* gene can be used for the identification of *Streptomyces* strains at species level that because of phylogenetic analysis based on partial *rpoB* gene sequences has some advantages over 16S rDNA; firstly, because *rpoB* is single-copy gene [33], direct sequence analysis targeting this gene can be applied universally to *Streptomyces* strains. In contrast, 16S rDNA is a multicopy gene and, though rare, some *Streptomyces* strains possess multiple gene copies with different sequences [34]. In this case, application of direct sequencing is not possible because of the ambiguous results produced by the different sequences. Secondly, *rpoB* is a protein encoding gene. Therefore, deduced amino acids, in addition to DNA sequences, can be used for the delineation of groups or species within the genus *Streptomyces*. Many researchers have been used *rpoB* gene analysis for the confirm identification of the family *Streptomycetaceae* [21, 35].

CONCLUSIONS

S. labedae ASU-03, a strain isolated from Egyptian soil, was able to degrade natural rubber effectively and produce clear zone during growth on opaque mineral salts agar with latex. It used the hydrocarbon of natural rubber as the sole source of carbon and energy. It was able to produce degradation products with low molecular weights. The results of increase in protein content, the decrease of viscosity and molecular weight, the detection of growth by SEM, and production of *keto* group by Schiff's reagent strongly confirmed the degradation of natural rubber by *Streptomyces* ASU-03. The sequences of 16S rRNA gene identified the strain as *Streptomyces* sp. and the specific name, *S. labedae*, was clarified by *rpoB* gene sequencing and phylogenetic analysis. It is also concluded that the use of a *rpoB* gene-based method for the phylogeny gives a better resolution in the species level identification.

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