J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 4 (1): 17 - 28, 2013

Degradation of natural and synthetic rubber by *Gordonia alkanivorans* strain E1

Ibrahim, E. M. A. and T. M. El-ameen
Dept. of Genetic, Fac. of Agric., South Valley Univ., Qena, Egypt.

ABSTRACT

A bacterium isolated from soil sample contaminated by old tire rubber in Qena governorate, Egypt. It was shown to be able to utilize natural rubber (NR) latex, synthetic poly (*cis*-1, 4-isoprene) and other isopronoid compounds as a sole carbon source and energy. The strain was aerobic, Gram-positive and produced very short elementary branching hyphe which disintegrated into rod/cocci-like elements. Taxonimic characterization of this isolate by 16S rDNA gene sequence analysis showed highest similarities to the 16S rDNA gene sequence of *Gordonia alkanivorans* (99,8%), which is mesophilic *actinomycetes.* Bacterial isolate was, therefore, referred to as *Gordonia alkanivorans* strain E1. *Gordonia alkanivorans* strain E1 grows well at 25, 30, and 35 °C but it did not grow at 45 °C. It was able to utilize a wide variety of carbon source such as glucose, fructose, hexadecane, acetate, starch and skim milk. Degradation behaviour indicated that the strain grow adhesively and depends on direct contact with the rubber substrate so belongs to CMN-group (*Corynebacterium*, *Mycobacterium*, *Nocardia*). The capability of rubber degradation was confirmed by mineralization experiments. Degradation of isoprenoid compounds and related compounds namely, squalane, squalene, phytol, acetonylacetone, geranylacetone citronellal and citronellic acid was recognized indicating that the bacterium has the metabolic capability to utilize some isoprenoid compounds as carbon and energy sources.

INTRODUCTION

Waste rubber is becoming a worldwide waste disposal problem (Liu et al., 2000). One particular concern is the huge quantity of natural and synthetic rubber product produced and discarded annually and caused a potential environmental hazard especially when natural rubber (NR) stock pile catch on fire (Smith and Klingensmith, 1991). Consequently, it is very important and worth trying to develop a microbial process for waste NR disposal (Jang, et al., 1998). Natural rubber is mainly consisting of *cis*-1,4-polyisoprene and it is synthesized by more than 2000 plant species belonging mostly to the Euphorbiaceae. NR is still produced in large amounts (~ 10^8 tons / year) from the rubber tree *Hevea brasiliensis*. The *cis*-1,4-polyisoprene, with an average molecular mass about 10^6 Da, is the main constituent (> 90% of dry weight) of NR. Many reports on the isolation and characterization of rubber-degrading microorganisms have been published among them (Rook, 1955; Leaflang, 1963; Tsuchii et al., 1985; Heisey and Papadatos, 1995; Linos et al., 2000; Arneskotter et al., 2004; Ibrahim et al., 2006; Schulte et al., 2008; Bröker and Steinbüchel, 2009). With regard to the decomposition strategies, two different groups of rubber-degrading bacteria ccould be distinguished (Linos et al., 2000; Arneskotter et al., 2004). While bacteria forming clear zone (Translucent halos) on latex-containing mineral agar have been repeatedly described by (Jendrossek et al., 1997),where only few representive of the second, adhesive growing group were so far isolated and

described by (Tsuchii et al., 1985; Linos et al., 2000; Ibrahim et al., 2006) who classifiedthem into the so-called CMN-group (*Corynebacterium*, *Mycobacterium*, *Nocardia*). *Gordonia*, *Mycobacterium*, and *Nocardia* were identified as non clear zone forming rubber degrading bacteria that are dependent on direct contact to the substrate (Linos et al., 2000). Compared to clear zone forming rubber-decomposing actinomycetes, the adhesively growing bacteria represent the more powerful rubber-degrading bacteria.

In this study, a bacterium that is able to utilize NR latex and synthetic poly (*cis*-1,4-isoprene) as a sole carbon source was isolated and characterized. Furthermore, the degradation of bacterium isolate on treated as well as nontreated NR-latex gloves was investigated. Special emphasis was given to the potency of the *Gordonia alkanivorans* strain E1 to utilize synthetic rubber materials and some isoprenoids compounds and related compounds namely, Squalane, squalene, phytol, Acetonylacetone, geranylacetone citronellal and citronellic acid. It also suggests that rubber-degrading bacteria might be useful for the disposal of discarded rubber products. Identification and development of rubber metabolizing microorganisms potentially could provide a biotechnological solution to this problem.

MATERIALS AND METHODS

Culture medium:

Cultivation was carried out in Erlenmeyer flasks containing mineral salts medium (MS medium) as described by Schlegel et al. 1961 and rubber as sole carbon source. The synthetic poly (*cis*-1,4-isoprene) rubber (IR) and natural rubber (NR) latex were added in concentration of 0.5% (wt/v). Natural rubber (NR) latex gloves were cut into small pieces, and added either untreated or after extraction with acetone or chloroform in concentration of 0.5% (wt/v). The other carbon sources such as hexadecane, acetate, glucose and fructose were added to liquid MS medium in concentration of 0.2% (wt/v), the entire media was autoclaved. All cultures were inoculated with cell obtained from 3-4 days precultured in nutrient broth which were washed twice with sterile saline before use. During incubation at 30 °C, the cultures were agitated at 130 rpm on a rotary shaker. Squalane or squalene was added to the medium at final concentrations of 0.5% (wt/v). To test growth on other isoprenoids compounds namely; Squalane, squalene, phytol, Acetonylacetone, geranylacetone citronellal and citronellic acid, cells were exposed to a vapor of the respective compound delivered from sterile filter paper containing 100 µl of this compound and placed in the lid of Petri plate. Inoculation plates were incubated in an inverted position with the lid at the bottom at 30 °C, separately and in closed containers as indicatd by (Berekaa and Steinbuchel, 2000).

Microorganisms:

The bacterial used in this study was isolated from soil sample contaminated by old tire rubber in Qena governorate, Egypt. Two gram of soil sample contaminated by old tire rubber was diluted with 100 ml sterile saline

and vortex for 2 min. 1 ml of the dilution was inoculated within Erlenmeyer flasks containing 100 ml MS medium and 0.5% (wt/vol) synthetic poly (*cis*-1,4-isoprene) as the sole carbon source and energy and incubated at 30°C. If growth was macroscopically detected within the first 3 weeks, 1 ml of the respective enrichment culture was transferred into 100 ml of fresh MS medium containing 0.5% (wt/vol) synthetic poly (*cis*-1,4-isoprene), and the cells were incubated again under the same conditions until an increase of the optical density was obtained. This procedure was repeated one more time. At the end serial dilutions were made and inoculated on solid Standard I (St-I) medium, subculture on solid St-I medium for purification. Single pure colonies were subsequently tested for growth on MS medium with synthetic poly (*cis*-1,4-isoprene) rubber as sole carbon source. The bacterium was characterized morphologically, physiologically as described in Bergey's Manual of Systematic Bacteriology (Goodfellow, 1986) and 16S rDNA was amplified and sequenced (Rainey, et al., 1996).

Polymers:

Three types of poly (*cis*-1,4-isoprene) were used in this study:

(i)Purified natural rubber latex from *Hevea brasiliensis* was obtained from Weber & Schaer (Hamburg, Germany).

(ii) Synthetic poly (*cis*-1,4-isoprene) rubber was obtained from Continental AG (Hanover, Germany).

(iii) NR-latex gloves were purchased from Roth (Karlsruhe, Germany).

Determination of mineralization rates:

Evidence for biodegradation of the poly (*cis*-1,4-isoprene) hydrocarbon chain to CO_2 was obtained by determination of respiratory CO_2 evolution during cultivation of cells in presence of poly (*cis*-1,4-isoprene) as sole carbon source. Determination was carried out in tightly closed Erlenmeyer flasks by using the property of Ba $(OH)_2$ to precipitate CO_2 as $BaCO_3$. The flasks containing 50 ml MS medium cultures, the rubber substrate (synthetic poly (*cis*-1,4-isoprene) or NR latex) and a test tube containing 15 ml of a 0.2 M $Ba(OH)_2$ solution were inoculated with 0.3% (vol/vol) of a well grown preculture that was washed twice with sterile saline solution 0.9% (w/v) before inoculation and incubation at 30 °C on a rotary shaker at 130 rpm. Cells of *Nocardia farcinica* E1 which able to degraded NR latex and Synthetic poly (*cis*-1,4-isoprene) rubber (Ibrahim et al.,2006) were cultivated at 50 °C. At each measurement, the flasks were aerated and the test tubes were replaced by new tubes containing fresh $Ba(OH)_2$ solution. Consumption of carbonate by precipitation of CO_3^{2-} as $BaCO_3$ was determined for each period by titration with HCl and compared to a non-inoculated control for different time intervals (Linos and Steinbüchel, 1998). Mineralization rates of the mesophilic adhesive growing isolate strain were compared to the rates obtained from thermophilic adhesive growing strain *Nocardia farcinica* E1 which isolated previously by Ibrahim et al., 2006 and able to degraded NR latex and synthetic poly (*cis*-1,4-isoprene).

Extract antimicrobial substances from NR-latex gloves:

To extract antimicrobial substances from NR-latex gloves, the material was treated with acetone or chloroform as follows: 1 g of latex gloves

was extracted with 100 ml acetone or chloroform for 10-12 h. During this period the solvent was replaced one to two times with fresh acetone or chloroform. The treated material was left to dry and was subsequently used as carbon source (Tsuchii et al., 1996).

DNA Extraction and determination of 16S rDNA gene:

The total DNA from the isolate strain was prepared by the versatile quick-prep method for gram-positive bacteria as described by Pospiech and Neumann, 1995. PCR-mediated amplification of 16S rDNA genes, using *Taq* DNA polymerase and the primers 27f (5-GAGTTTGATCCTGGCTCAG-3_) and 1525r (5-AGAAAGGAGGTGATCCAGCC-3_). For amplification of 1489 bp segment, the PCR was performed in a final volume of 50 µl containing 10 mM trais-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at concentration of 0.2 mM, 1.25 IU of Taq DNA polymerase, each primer at a concentration of 0.2 mM and 2 µl of the DNA template. The thermal cycling parameters included an initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 30 sec, and primer extension at 72 °C for 2 min. finally the reaction mixture was heated to 72 °C for 10 min and subsequently cooled to 4 °C. five µl of amplified mixture was then analyzed using 1.5% 0.5xTBE agarose gel electrophoresis. The gel was stained with ethidium bromide, visualized under UV light, and photographed. Purification of the PCR products were carried out as described previously (Rainey, et al., 1996).

Determination of 16S rDNA gene sequence:

PCR-mediated amplification of 16S rDNA genes, using *Taq* DNA polymerase and the primers 27f (5-GAGTTTGATCCTGGCTCAG-3) and 1525r (5- AGAAAGGAGGTGATCCAGCC-3), and purification of the PCR product were carried out as described previously (Rainey, et al., 1996). The purified 16S rDNA gene fragment was direct sequenced. DNA sequences were determined with the primers 27f (5-GAGTTTGATCCTGGCTCAG-3), 343r (5-CTGCTGCCTCCCGTA-3), 357f (5-TACGGGAGGCAGCAG-3), 519r (5-G[T/A]ATTACCGCGGC[T/G]GCTG-3), 536f (5-CAGC(C/A)GCCGCGGTAAT[T/A]C3), 803f(5ATTAGATACCCTGGTAG-3_), 907r (5_-CCGTCAATTCATTTGAGTTT-3), 1114f (5_-GCAACGAGCGCAACCC-3), 1385r (5-CGGTGTGT[A/G]CAAGGCCC-3), and 1525r (5-AGAAAGGAGGTGATCCAGCC-3), using a SequiTherm EXCEL II Long-Read L-C kit (Epicenter, Madison, WI) and a Li-COR model 4200 sequencer (LI-COR Biosciences, Lincoln, NE). Sequences were aligned manually with published sequences from representative actinomycetes obtained from EMBL. BlastN was used to determine the percentages of nucleotides identical to 16S rDNA gene sequences in the GenBank databases.

RESULTS AND DISCUSSION

Isolation and characterization of the rubber-degrading microorganism:

The rubber degrading isolate was enriched and isolated from soil sample contaminated by old tire rubber material from Qena governorate, Egypt (material and method). Morphologically, the cells produced very short

elementary branching hyphe which disintegrated into rod/cocci-like elements. It showed the typical rod-coccus growth cycle which is usually found among *Gordonia* and related taxa like *Rhodococcus* (Goodfellow, 1986, 1992; Goddfellow and Lechevalier, 1989). The cells exhibited creamy beige colonies which changed to pastel orange when cultivated in the presence of light. The cells were Gram-positive, slightly acid fast and non-motile. No spore could be detected. The strain was able to utilize a wide variety of carbon source such as hexadecane, acetate, glucose, fructose, starch and skim milk (Table 1).

Table 1. Phsiological characterization of *Gordonia alkanivorans* strain E1.

Characteristic	*Gordonia alkanivorans* strain E1
Gram staining	+
pH range	6-8.5
LAAP -test	-
KOH -test	-
Spor forming	-
Glucose	+
Fructose	+
Gluconate	+
Na-citrate	+
Na-succinate	+
Hexadecane	+
Na-acetate	+
Pentane	-
Hexane	-
Skim milk	+/-
Casein	-

Growth was qualitatively estimated as follows: (+) growth; (+/-) poor growth; (-) no growth. *Gordonia alkanivorans* strain E1 were cultivated on MS medium agar plates with different concentration of carbon source as described in Materials and methods.

The bacterium was catalase and oxidase-positive. The isolate grows well at 25, 30, and 35 °C but do not grow at 45 °C and the Optimum temperature was 30 °C. The almost complete 16S rDNA sequence isolate E1 was determined as described in Material and Methods and were aligned with sequences deposited in GenBank to determine closely related species. The 16S rDNA gene sequence strain E1 showed highest similarities to the 16S rDNA gene sequence of *Gordonia alkanivorans* (Kummer et al., 1999) and *G. nitida* (Yoon et al., 2000) (99.8% and 99.6% respectively), which is mesophilic *actinomycetes*. Fig. 3 shows the phylogentic position of the isolate strain within the genus *gordonia* and *Rhodococcus*. The results also indicated that the bacterium could be classified among the first CMN group of bacterium that shows adhesive growth on rubber material and grow in direct

contact to the rubber substrate (Linos et al., 2000). Isolate E1 was therefore referred to as *Gordonia alkanivorans* E1.

Mineralization of Natural and synthetic Rubber:

The capacity of the *Gordonia alkanivorans* strain E1 for rubber degradation was quantified as described in Materials and Methods by measuring the carbon dioxide released during growth on synthetic poly(*cis*-1,4-isoprene) and NR latex. The mineralization rates were compared to the mineralization rates obtained for thermophilic adhesively growing *Nocardia farcinica* E1. The results presented in Fig. 1 A showed that mesophilic adhesive growing isolated strain *Gordonia alkanivorans* strain E1 were able to degrade and mineralize synthetic poly (*cis*-1,4-isoprene) and NR-latex demonstrating the metabolic conversion of polymer to carbon dioxide. As expected, the poly (*cis*-1,4-isoprene) contained in NR latex served as a more favourable carbon source than synthetic poly (*cis*-1,4-isoprene) as indicated by higher mineralization values obtained, which might be due to the fact that the main composition of NR latex consists of more than 90% of poly (*cis*-1,4-isoprene) (Mw 10^6 Da) and less than 10% of non rubber constituents (Subramaniam, 1995). It was recognized that the mineralization level of NR-latex by mesophilic adhesively growing bacteria *Gordonia alkanivorans* strain E1 and thermophilic adhesively growing bacteria *Nocardia farcinica* E1 was approximately the same (Fig. 1 B).).

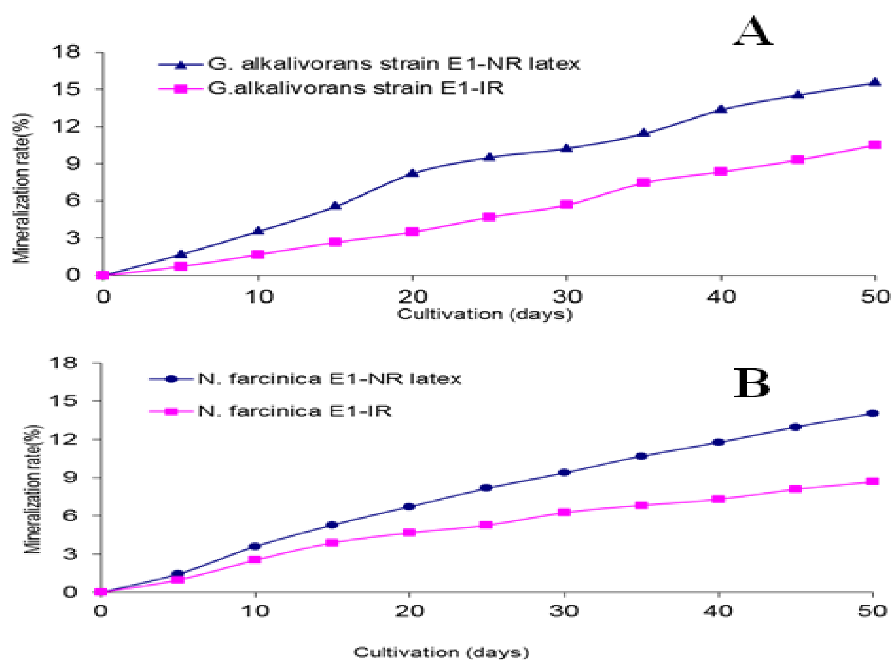

Fig. 1: Mineralization of synthetic poly(*cis*-1,4-isoprene) and natural rubber latex. (A) *Gordonia alkanivorans* strain E1 and (B) *Nocardia farcinica* E1 were cultivated at 30 and 50 °C respectively, in MS medium containing 0.5% (wt/vol) synthetic poly (*cis*-1,4-isoprene) or 0.5% (vol/vol) NR latex.

However, *Gordonia alkanivorans* strain E1 mineralized about 10.52% (wt/wt) of the synthetic poly (*cis*-1,4-isoprene) after 50 days. In contrast, adhesively growing thermophilic bacteria *Nocardia farcinica* E1 metabolized in the same period only about 8.5% of synthetic poly(*cis*-1,4-isoprene). These results correspond well to the results obtained from *Nocardia* sp. strain 835A (Tsuchii et al., 1996; 1997) and *G. polyisoprenivorans* VH2 (Linos et al., 2000

Enhanced mineralization of pretreated NR-latex gloves:

Manufacture of rubber products from raw NR and synthetic rubber (IR) are usually accompanied by the addition of compounding ingredients. Many of these substances are known to prevent microbial growth (Zyska, 1981). In case of NR-latex gloves, no information about these ingredients was available from the manufactures. Interestingly, organic extraction of NR-latex gloves revealed one possibility to improve the biodegradation process with respect to potential biotechnological treatment of the rubber wastes. Beside the ability of *Gordonia alkanivorans* strain E1 to degrade natural and synthetic rubbers, the degradation of vulcanized rubber products such as NR-latex gloves was investigated. Moreover, the degradation of NR-latex gloves after removal of additives by extraction with organic solvents such as acetone or chloroform was also studied. In this experiment, the ability of *Gordonia alkanivorans* strain E1 to degrade NR-latex gloves was investigated. For this purpose, 250 mL Erlenmeyer mineralization flasks with screw and containing 50 mL MS medium were prepared and the rubber substrate was added at concentration of 0.5% (wt/v). NR-latex gloves (treatment and nontreatment) were cut in the form of small pieces and added before addition the MS medium and autoclaving. The flasks were inoculated with 1 mL of 3 days old preculture of *Gordonia alkanivorans* strain E1 cells that was previously washed with sterile saline solution 0.9% (wt/v) and the mineralization was estimated at different time intervals. Results shown in Fig. 2 clearly demonstrated the enhancement of NR-latex gloves degradation after treatment of the glove material with organic solvents such as acetone or chloroform. This treatment led to a more than 2 to 3-fold increase of the mineralization of rubber expressed by % CO_2 released during growth. The extraction of antimicrobial substances from NR-latex gloves was also done by Tsuchii and his co-workers (1996) in order to remove chemicals with microbicidal activities.

Degradation of structurally analogous isoprenoid compounds:

In order to study the potency of the rubber-degrading isolate, *Gordonia alkanivorans* strain E1, used to degrade structurally analogous isoprenoid compounds and other related compounds, where their utilization was investigated. The results shown in Table 2 indicated that the keto and acidic forms of the isoprenoids compounds such as acetonylacetone, geranylacetone, geranic acid, and citronellic acid were easily utilized by *Gordonia alkanivorans* strain E1.

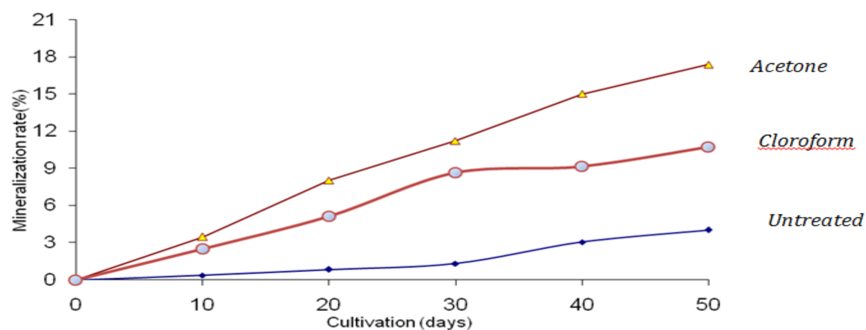

Fig. 2: Mineralization of NR-latex gloves by *Gordonia alkanivorans* strain E1. The degree of mineralization is shown as per cent of carbon released as CO_2 during the time course of the experiment using untreated (-♦-), chloroform-treated (-❍-) or acetone-treated (-Δ-) NR-latex glove material as sole carbon source.

Similarly, good growth on saturated-branched hydrocarbons (squalane) and moderate growth on unsaturated-branched isoprenoid compounds phytol and squalene was also recorded. On the other hand, no growth was recognized when Citronellal was used as carbon and energy source. Utilization of similar compounds by rubber-degrading bacterium *Mycobacterium fortuitum* NF4 was also recorded by Berekaa and Steinbuchel (2000). These results collectively indicated that the bacterium has the metabolic capability to utilize some isoprenoid compounds as carbon and energy sources.

Table 2. Growth of *Gordonia alkanivorans* strain E1 on isoprenoid compounds and related compounds.

Compound tested	Growth of *Gordonia alkanivorans* strain E1 on the respective carbon source
Phytol	++
Acetonylacetone	+++
Geranylacetone	+++
citronellic acid	+++
Geranic acid	+++
Squalane	+++
Squalene	++
Citronellal	-

Growth was qualitatively estimated as follows: (-) no growth, (++) moderate growth and (+++) good growth. Test of isoprenoid compounds and related compounds: The isolate were cultivated in MS medium agar plates and were exposed to the vapor of the tested compound (100 µl) as described in Materials and methods.

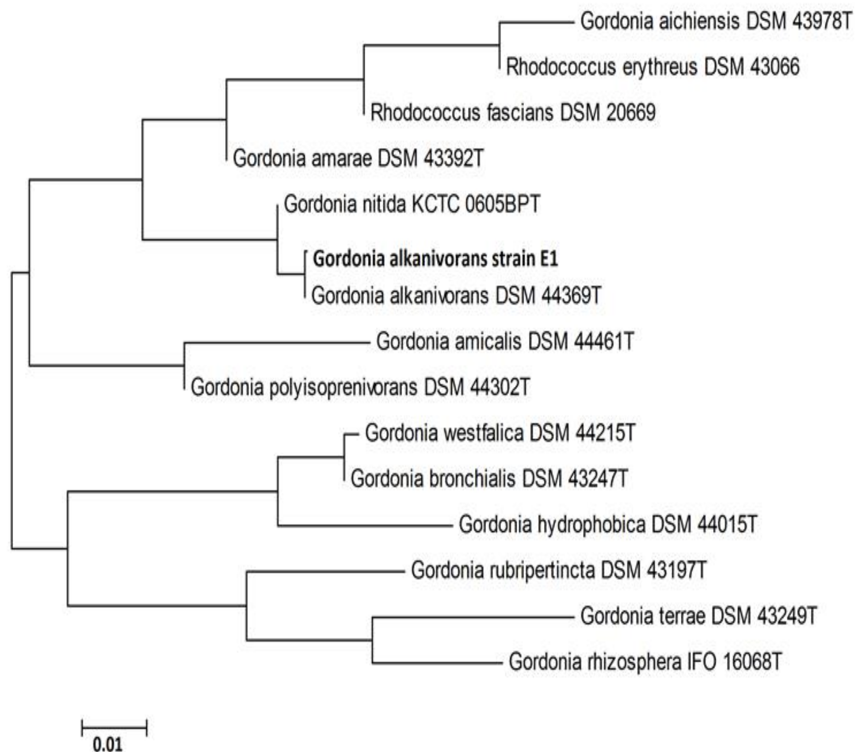

Fig. 3: Phylogenetic dendrogram, obtained by distance-matrix analysis, showing the position of *Gordonia alkanivorans* strain E1 among members of the genus *Gordonia* and *Rhodococcus*. Bar, 0.01 substitutions per nucleotide position.

Acknowledgments

We would like to thank Dr. Alexander Steinbuechel (Institute of Molecular Microbiology and Biotechnology, Münstr - Germany) for sequencing 16S rDNA.

REFERENCES

Arenskötter, M., D. Bröker and A. Steinbüchel. (2004). Biology of the metabolically diverse genus *Gordonia*. Appl. Environ. Microbiol. 70: 3195-3204

Berekaa, M. M. and A. Steinbüchel. (2000). Microbial degradation of the multiply branched alkene 2,6,10,15,19,23-hexamethyltetracosane (squalene) by *Mycobacterium fortuitum* and *Mycobacterium ratisbonense*. Appl. Environ. Microbiol. 66: 4462-4467

Bröker, D. and A. Steinbüchel. (2009). Megaplasmid pKB1 of the rubber-degrading bacterium Gordonia westfalica strain Kb1. In: E. Schwartz (Ed.) Megaplasmids, Volume 11 of Microbiology Monographs (Ed. A. Steinbüchel), Springer, Heidelberg, pp. 297-309

Goodfellow, M. (1986). Genus *Rhodococcus* Zopf 1891, 28[AL]. In *Bergey's Manual of Systematic Bacteriology*. 4: 2362-2371. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Goodfellow, M. and M. P Lechevalier. (1989). Genus *Nocardia*. Trevisan 1899, 9AL. In *Bergey's Manual of Systematic Bacteriology*, vol. 4; 2350-2361. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Goodfellow, M. (1992). The family *Nocardiaceae*. In *The Prokaryotes*, 2nd edn, pp. 1188-1213. Edited by A. Balows, H. G. Tru$ per, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Heisey, R. M. and S. Papadatos. (1995). Isolation of microorganisms able to metabolize purified natural rubber. Appl. Environ. Microbiology 61: 3092-3097

Ibrahim, E. M. A, M. Arenskötter, H. Luftmann and A. Steinbüchel. (2006). Identification of poly (*cis*-1,4-isoprene) degradation Intermediates during growth of moderately thermophilic actinomycetes on rubber and cloning of a functional lcp homologue feom Nocardia farcinica strain E1. Appl. Environ. Microbiol., 72:3375-3382

Jang, J.W., T. S. Yoo, J. H. Oh and I. Iwasaki. (1998). Discarded tire recycling practices in the United States, Japan and Korea. – Research Conservation Recycle 22: 1-14

Jendrossek, D., G. Tomasi and R. Kroppenstedt. (1997). Bacterial degradation of natural rubber: a privilege of actinomycetes? FEMS Microbiol. Lett. 150:179-188

Kummer, C., P. Schumann and E. Stackebrandt. (1999). *Gordonia alkanivorans* sp. nov., isolated from tar-contaminated soil. *Int J Syst Bacteriol* 49: 1513-1522

Leeflang, K. W. H. (1963). Microbial degradation of rubber. J. Amer. Water Works Assoc. 55:1523-1535

Linos, A. and A. Steinbuchel. (1998). Microbial degradation of natural and synthetic rubbers by novel bacteria belonging to the genus Gordonia. Kautsch. Gummi kunstst. 51: 496-499

Linos, A., M. M. Berekaa, R. Reichelt, U. Keller, J. Schmitt, H. C. Flemming, R. M. Kroppenstedt and A. Steinbüchel (2000). Biodegradation of *cis*-1,4-polyisoprene rubbers by distinct actinomycetes: microbial strategies and detailed surface analysis. Appl. Environ. Microbiol. 66:1639-1645

Liu, H. S., J. L. Mead and R. G. Stacer. (2000). Environmental effects of recycled rubber in land-fill applications.- Rubber Chemistry Technology 73: 551-564

Pospiech, A. and B. Neumann. (1995). A versatile quick-prep of genomic DNA from gram-positive bacteria. Trends Genet. 11:217–218.

Rainey, F. A., N. Ward-Rainey, R. M. Kroppenstedt and E. Stackebrandt. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int. J. Syst. Bacteriol. 46:1088–1092

Rook, J. J. (1955). Microbial deterioration of vulcanized rubber. Appl. Microbiol. 3:302-309

Schlegel, H. G., H. Kaltwasser and G. Gottschalk. (1961). Ein Submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstumsphysiologische Untersuchungen. Arch. Mikrobiol. 38:209–222

Schulte, C., M. Arenskötter, M. M. Berekaa, Q. Arenskötter, H. Priefert and A. Steinbüchel. (2008). Possible involvement of an extracellular superoxide dismutase (SodA) as radical scavenger in poly(*cis*-1,4-isoprene) degradation. *Appl. Environ. Microbiol.* 74: 7643-7653

Smith, F. G. and W. B Klingensmith (1991). Tires as a consumer and source of recycled material. – Rubber chemistry Technology 64: 122-130.

Subramaniam, A. (1995). The chemistry of natural rubber latex. Immunol. Allergy Clin., 15: 1-30

Tsuchii, A., T. Suzuki and K. Takeda. (1985). Microbial degradation of natural rubber vulcanizates. Appl. Environ. Microbiol. 50:965-970.

Tsuchii, A., K. Takeda, T. Suzuki and Y. Tokiwa (1996). Colonization and degradation of rubber pieces by Nocardia sp. Biodegradation, 7: 71-84

Tsuchii, A., K. Takeda and Y. Tokiwa. (1997). Biodegradation, 7: 405-413

Yoon, J. H., J. J. Lee, S. S. Kang, M. Takeuchi, Y. K. Shin, S. T. Lee, K. H. Kang and Y.-H. Park. (2000). *Gordonia nitida* sp. nov., a bacterium that degrades 3-ethylpyridine and 3-methylpyridine. *Int. J. Syst Evol Microbiol* 50: 1203-1210

Zyska, B. J. (1981). Rubber, pp. 323–385. *In* A. H. Rose (ed.), Microbial biodeterioration, economic microbiology, vol. 6. Academic Press, London, England

تحليل المطاط الطبيعى والصناعى بواسط بكتيريا *Gordonia alkanivorans* strain E1

عبيد محمد احمد ابراهيم و ثروت محمد الامين
قسم الوراثة – كلية الزراعة – جامعة جنوب الوادى

البكتريا التى تم عزلها من عينة تربة ملوثة بواسطة إطارات سيارات من المطاط القديم فى محافظة قنا بمصر اتضح مقدرتها على تحليل كلا من المطاط الطبيعى والصناعى وبعض مركبات الايزوبرونيد الاخرى بإستخدامها كمصدر للكربون والطاقة. وتتميز هذه البكتريا بانها تنمو هوائيا وموجبة الجرام وتنمو على شكل هيفات اولية صغيرة متشابكة التى تتحول الى الشكل العصوى او الكروى عند تحللها. التصنيف الوراثى لهذة البكتريا بواسطة تحليل تتابع الـ 16S rDNA أوضح أنها على درجة عالية من التشابه مع بكتيريا الـ *Gordonia alkanivorans* (٩٩.٨%) لذلك سوف نطلق عليه اسم *Gordonia alkanivorans* strain E1. هذه البكتيريا تنمو جيدا على درجة حرارة ٢٥ و ٣٠ و ٣٥ درجة مئوية ولا يوجد نمو لها على درجة حرارة ٤٥ درجة مئوية. كما ان هذه البكتيريا لها مجال واسع فى تحليل مصادر الكربون المختلفة مثل الجولوكوز والفركتوز والهكساديكان والاسيتات والنشا ولبن الفرز واستخدامها كمصدر للكربون والطاقة. سلوك تحليل المطاط بواسطة هذه البكتيريا يعتمد على الاحتكاك المباشر لمواد المطاط ولذلك فهى تنتمى الى مجموعة الـ CMN- group ومقدرة هذه البكتيريا على التحليل تم دراسته بواسطة تجارب الـ Mineralization على كلا المطاط الطبيعى والصناعى. كما وجد ان هذه البكتيريا لها المقدرة على تحليل مركبات الايزوبرونيد والمركبات المشابهه لها تركيبيا مثل الاسكوالين والاسكوالان والفيتول والسترونيلال والفرنيسول واستخدامها لتلك المركبات فى النشاط الميتابولزمى كمصدر للكربون والطاقة.

قام بتحكيم البحث

أ.د / على ماهر العدل	**كلية الزراعة – جامعة المنصورة**
أ.د / محمد يونس حسين	**كلية الزراعه – جامعة اسيوط**