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RESEARCH ARTICLE

Isolation and characterization of 3D chitin from a mite species *Trachytes pauperior* (Parasitiformes: Uropodina)

Emel ÇAKMAK[1,2,4], Behlül KOÇ BİLİCAN[2,3]

[1] *Department of Plant and Animal Production, Güzelyurt Vocational School, Aksaray University, 68100 Aksaray, Turkey*
[2] *ASUBTAM-Aksaray University, Science and Technology Application and Research Center, 68100 Aksaray, Turkey*
[3] *Department of Biotechnology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Turkey*
[4] Corresponding author: emelcakmak@aksaray.edu.tr

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ABSTRACT: Chitin is the most abundant structural biopolymer after cellulose in terrestrial ecosystems. Until now, chitin isolation in powder or granule form has been carried out from many macro living groups (Arthropoda, Crustacea, Mollusca etc.). However, studies on chitin characterization of microscopic organisms have remained limited. In this study, three dimensional (3D) chitin extraction was performed for the first time from *Trachytes pauperior* (Berlese), a mite species. The obtained chitin was observed by light microscopy and characterized by FT-IR, and SEM analysis. Our findings suggest that chitin, which was obtained in high purity and constitutes a large part of the organism's body structure, could be a potential source for future studies.

Keywords: Acari, biopolymer, characterization, extraction, *Trachytes pauperior*.
Zoobank: http://zoobank.org/97CD8F14-CF8C-457D-ADB5-85613788CE7E

INTRODUCTION

Chitin is the most abundant biopolymer in nature after cellulose and is found in more than 70% of the living things in the world. Studies have shown that chitin can be isolated from the outer shells of many living organisms (such as crab, shrimp and lobster), from the cell wall of fungi and some algae and from the whole-body structure of coral, sponges and insects (Rudall, 1963; Wu et al., 2004; Ehrlich et al., 2007; Bo et al., 2012; Arbia et al., 2013; Rahman and Halfar, 2014). The study of Kaya et al. (2014a) showed that bat guano is also new chitin sources. Chitin, whose structure consists of β-1,4 linked N-acetyl D-glucosamine, has 3 defined allomorphs; alpha, beta and gamma. When previous studies examined, the most common form of chitin was determined as the alpha form and it has been identified in the branches of Arthropoda, Tardigrada, Bryozoa, Mollusca and the cell walls of Fungi (Brimacombe and Webber, 1964; Ehrlich et al., 2007; Al Sagheer et al., 2009). Considering the literature, it has been seen that there is no enough study on the isolation of the three dimensional (3D) chitin. Moreover, the isolated chitins were mostly obtained either powder or granule form of macro-organisms (Fadlaoui et al., 2019; Kardas et al., 2012).

Chitin is known to be non-toxic, biodegradable, edible, biocompatible, antioxidative, antimicrobial, thermally stable, antioncogenic and has a porous surface. These properties enable chitin and its derivatives to be used in numerous economically important applications in a wide variety of fields such as agriculture, medicine, food industry, textiles, and cosmetics (Jeon et al., 2000; Rinaudo, 2006; Krantz and Walter, 2009; Merzendorfer, 2009; Jayakumar et al., 2011; Anitha et al., 2014; Fernando et al., 2016; Hamed et al., 2016; Petrenko et al., 2017). Although chitin has such a wide area of use, the studies are clearly demonstrated that it is generally obtained from macro-sized organisms and microscopic creatures are neglected.

One of the most abundant invertebrates living in forest ecosystems are mites and more than 50,000 species have been described (Manu et al., 2018). Especially, Uropodina mites are one of the most widespread and diverse groups of mites living in soil (Kontschán, 2010, 2013; Kontschán et al., 2013). Besides, soil mites are known to play an important ecological role in the forest. For instance, they are reported to participate in soil formation processes and affect productivity (Manu et al., 2018). In the genus *Trachytes* Michael within the family Trachytidae, up to 30 species have been identified, 23 of which are from Central European countries such as Germany, the Czech Republic, Austria, Poland, Slovakia, Hungary, Ukraine and Romania (Masan, 2003). *Trachytes pauperior* has a wide ecological tolerance and lives in a variety of habitats (Masan, 2003). This species has been recorded from Europe and Siberia (Masan, 2003), and also reported form Turkey in a PhD dissertation by Bal (2002) and later in MSc dissertation by Özen (2012), but these dissertations have not been published yet. Until now, taxonomic and ecological studies have been conducted on the genus *Trachytes* in general (Pecina, 1970a,b, 1980; Hutu, 1973, 1982; Zirngiebl-Nicol, 1973; Bloszyk, 1980, 1999; Masan, 2003), but no study has been carried out on the content of chitin. In the current study, chitin isolation was performed for the first time from *Trachytes pauperior* (Berlese), which was followed out without damaging the 3D structure.

MATERIALS AND METHODS

Sample Collection

The mite specimens identified as *Trachytes pauperior* were extracted from soil and litter under *Quercus* sp., Pülümür Valley, TURKEY, 39°35'18.72''N 39°51'09.35''E, 1459 m a.s.l., 30 January 2019, using Berlese funnels.

Chitin Extraction

In total, 100 specimens were washed with distilled water for chitin extraction, any remaining particles were removed and dried at 60 °C for 3 days for extraction. It was treated at 60 °C in 2M 250 mL HCl solution for 6 hours to remove the minerals in the structure. At the end of this period, the samples were washed with pure water until they reached neutral pH by keeping their shape. Subsequently, samples were treated with 2M NaOH solution at 85 °C for 8 hours to remove protein residues in their structure. At the end of the treatment, the samples were washed again with pure water until they reached neutral pH and dried for 3 days at 60 °C.

Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectra of the chitin isolates obtained from the species *T. pauperior* in 3D were recorded using Perkin Elmer Spectrometer in the range 4000-650 cm^{-1} at 8 cm^{-1} resolution in the wavelength range of 600-4000 cm^{-1}. Besides, the 64 scans were averaged to improve the signal-to-noise ratio.

Scanning Electron Microscopy (SEM) and Light microscopy image

The surface morphology of the isolates of the 3D chitin was demonstrated with the ZEISS LS-10 Life Science Scanning Electron Microscopy device. To get better images, the material was gold-plated before the analysis via Cressington sputter-coated 108 Auto, TED PELLA, INC. The 3D chitin produced from *T. pauperior* was also observed by light microscopy in 5X-60X magnification (Leica DM4000 B LED). The chitin was imaged in ambient conditions using glass slide. Besides, the natural *T. pauperior* structure (without chitin isolation) was examined by light microscopy (Leica Z6 APO).

RESULTS

FTIR

FTIR spectrum of chitin isolates obtained from the species *T. pauperior* in 3D was given in Figure 1 and Table 1. It is clearly observed that the Amide I band is divided into two peaks in the FTIR spectrum of the 3D chitin isolate from *T. pauperior* (Fig. 1 and Table 1), which shows the compatibility of this chitin with α-form. The second important band for the chitin is the Amide II band, which is found around 1552 cm^{-1} for a pure chitin. The recorded Amide II band for the 3D chitin isolate from *T. pauperior* is 1556 cm^{-1}. Thus, the Amide II band was also compatible with the literature and reveals the purity of the 3D chitin obtained from *T. pauperior* as an alpha chitin.

SEM and Light microscopy image

SEM images recorded to illuminate the surface morphology of the chitin isolated from *T. pauperior* in 3D were shown in Figure 2. It was clearly seen in the recorded images that the 3D structure was preserved during the isolation of the chitin and formed a large part of the organism's structure (Figs 2A-C). Looking at the images recorded at different magnifications, it was clearly demonstrated that the structure consists of tubercles with nanofibers and pores, which looks like the pattern of the cuticular cover is the nanoscale images of the chitin inside the cuticle. The same phenomenon can be observed using white light microscopy. The chitin isolated from *T. pauperior* as described above, possess 3D arrangement (Figs 3A, B). It is worth noting that these specific structures of mite chitin are extremely sensitive to drying at room temperature. Thus, to prevent degradation of 3D morphology, the samples were immediately scanned.

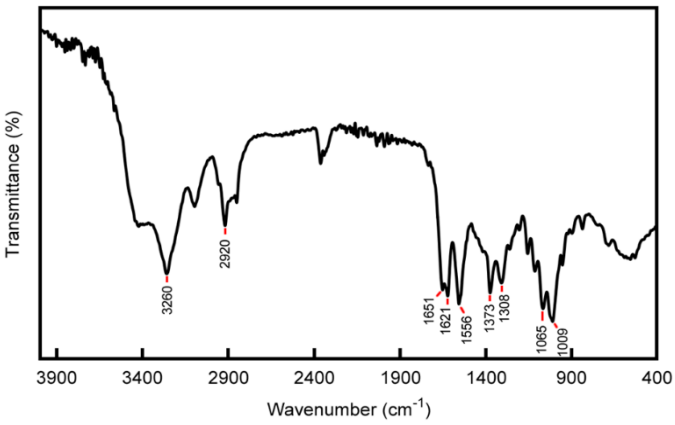

Figure 1. FTIR spectrum of the 3D chitin isolate from *T. pauperior*.

DISCUSSION

For the first time, 3D chitin was successfully isolated from the body structure of the species *T. pauperior* in the current study. As a result, it has been demonstrated that the 3D isolated chitin constitutes a large part of the organism's body structure. The presence of chitin in the peritrophic membrane of *Acarus siro* (Acari: Acaridae) has only been confirmed so far by Sobotnik et al. (2008). However, the study of Sobotnik et al. (2008) was not conducted on the characterization of chitin, only showing that the presence of chitin in the peritrophic membrane provides an opportunity for the application of chitin effectors as acaricides. As it is known, one of the pathways of chitin synthesis in Acari is found in the hypodermis under the cuticle (Mothes and Seitz, 1981). The mite cuticle consists of a wax-containing epicuticle and a thin procuticle (Mothes and Seitz, 1982, 1984). In other words, the nano-sized imaging of the 3D chitin was obtained from the procuticle region of the cuticle layer of the integument.

FTIR spectroscopy is one of the powerful tools for structural analysis of poly-saccharides, including the chitin (Żółtowska-Aksamitowska et al., 2018). Vibration spectra are also sensitive to intramolecular and intermolecular

Table 1. FTIR spectra of the chitin obtained from *Trachytes pauperior*.

Functional groups and vibration modes	Classification	Wavenumber (cm^{-1}) frequency	
		Chitin from *Trachytes pauperior*	Commercial α-chitin (Kaya et al., 2017)
O–H stretching	-	3421	3433
N-H stretching		3260	3104 -3260
CH3 sym. stretch and CH2asym. stretch	Aliphatic compounds	2920	2940
CH3 sym. stretch	Aliphatic compound	2852	2875
C=O secondary amide stretch	Amide I	1651	1652
C=O secondary amide stretch	Amide I	1621	1620
N–H bend, C–N stretch	Amide II	1556	1552
CH2 bending and CH3 deformation	-	1417	1420
CH bend, CH3 sym. deformation	-	1373	1375
CH2 wagging	Amide III, components of protein	1308	1307
Asymmetric bridge oxygen stretching		1154	1154
Asymmetric in-phase ring stretching mode		1112	1112
C–O–C asym. stretch in phase ring	Saccharide rings	1065	1067
C–O asym. stretch in phase ring	-	1009	1008
CH3 wagging	along chain	951	951
CH ring stretching	Saccharide rings	896	892

interactions as well as to the geomolecular molecule. Recently, this method has been successfully applied to identify three known isomorphs of chitin (α, β and γ) (Kaya et al., 2017). There are two characteristic peaks for the chitin. The first one is the Amide I band. Looking at the information in the literature, if this peak is an undivided peak around 1640 cm^{-1}, the chitin is called β-form, however; the two sharp peaks divided around 1660 and 1620 cm^{-1} are called α-form (Jang et al., 2004; Kaya et al., 2017). In the FTIR analysis, it was detected that the isolated chitin both was in α-form and included peaks that were quite compatible with the literature. The Amide I band can be sharply shown at 1651 and 1621 cm^{-1} in the spectrum of the chitin isolate, almost completely consistent with the literature (Table 1).

Generally, the chitin can be grouped in four different ways according to the surface morphology (Kaya et al., 2014b). The first form is a smooth surface morphology without any nanofibers and pores. The second form consists of nanofibers but does not contain pores. The third form has nanofibers and co-pores. The fourth form exhibits two types of pores of different sizes (one large and one small) in combination with nanofibers. In this study, the SEM analysis revealed that the 3D chitin isolated by consists of nanofibers and nanopores, which consistent with the other Arachnida species (Kaya et al., 2016). Similarly, it has been previously stated in the literature that the surface morphology of α-chitin consists of nanofibers and natural pores (Al Sagheer et al., 2009; Ifuku et al., 2011; Kaya et al., 2013; Mushi et al., 2014). In the study of Seyyar and Demir (2020), the chitin from external skeleton of an opilionid species, *Phalangium opilio* (Arachnida: Opiliones) was firstly extracted, and the chitin has been found to have nanofiber and nanoporous surface and alpha form. Additionally, the chitin characterization of two spider specimens demonstrated similar surface morphology (Kaya et al. 2014b). In 2020, Machałowski and coworkers obtained chitin from spider source retaining its unique shapes, including the 3D tubular architecture.

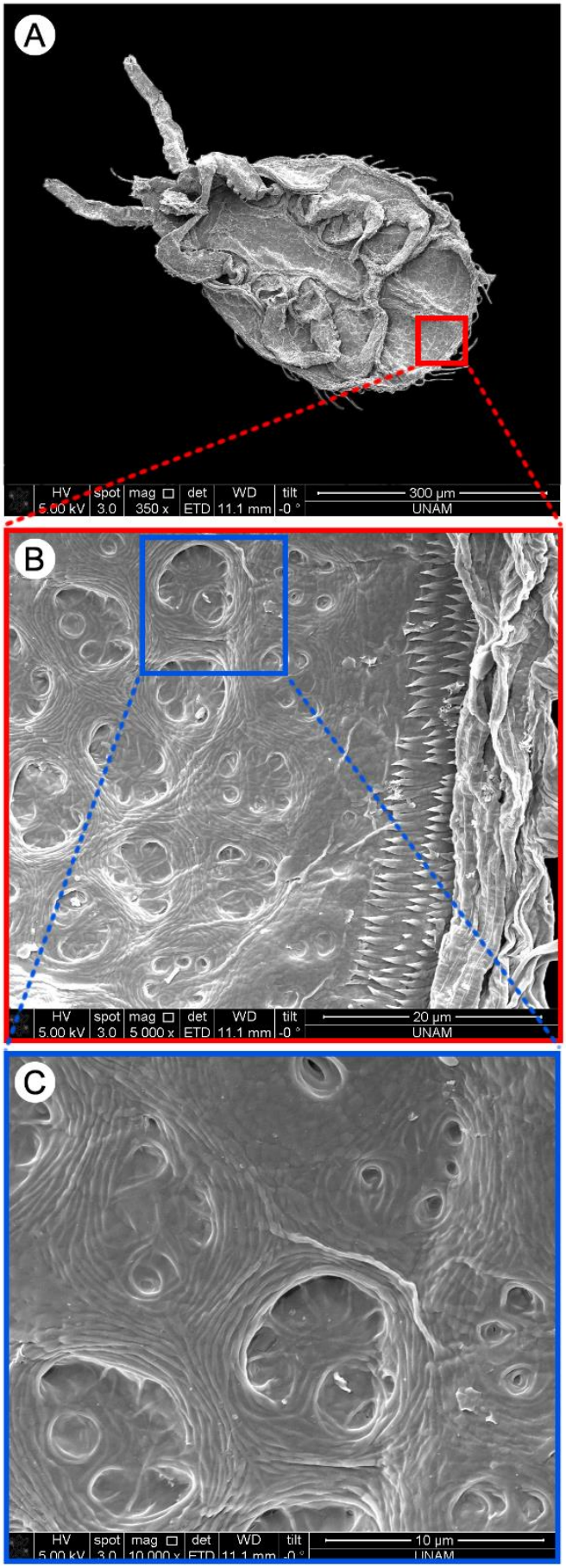

Figure 2. SEM images of chitin isolates from *T. pauperior* in 3D.

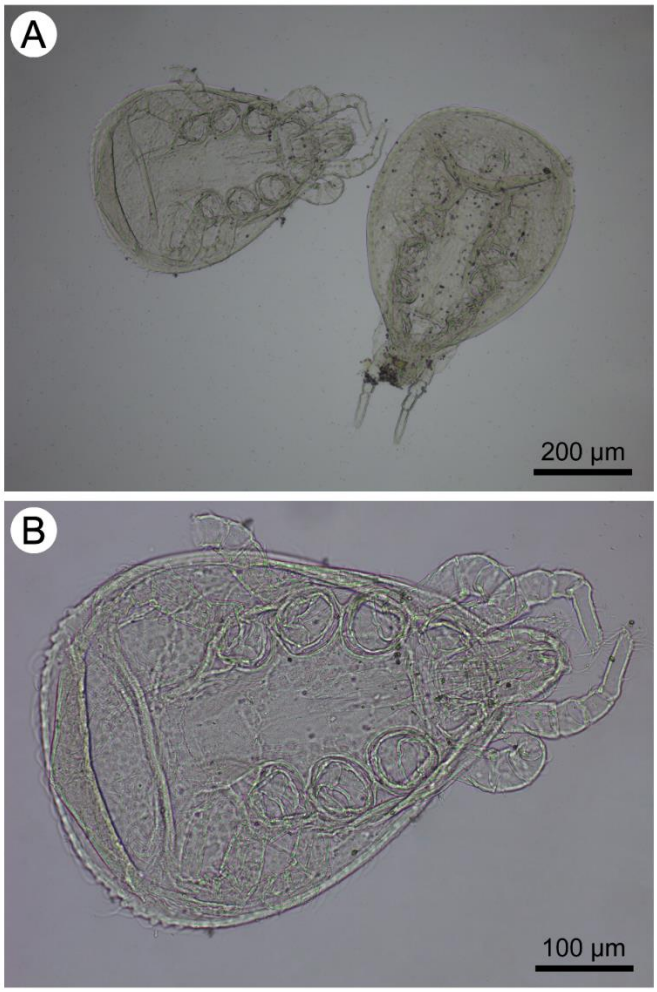

Figure 3. Purified chitin of *T. pauperior* of light microscopy modus (0-60X).

In addition to SEM images, the light microscopy clearly showed that the 3D structure was preserved without damaging. These part that looks like the pattern of the cuticular cover is the nanoscale images of the chitin inside the cuticle. Although we could not give the ratio of the chitin because it is very small organism, we predict that the content of the chitin is close to the Arthropoda, since we can isolate the 3D structure without deterioration. Thus, the results of the current study demonstrated that as a microscopic organism, *T. pauperior*, could be an alternative source of chitin for future applications. We suggest that the discovery of chitin within other representatives of the mite species is the next step.

Authors' contributions

Emel Çakmak: Investigation (equal), formal analysis (equal), methodology (equal), visualization (lead), writing - original draft (supporting), writing - review & editing (lead). **Behlül Koç-Bilican**: Investigation (equal), formal analysis (equal), methodology (equal), visualization (supporting), writing - original draft (lead), writing - review & editing (supporting).

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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