Middle-East Journal of Scientific Research 23 (12): 2983-2986, 2015
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2015.23.12.102180

Characterization of Nanocrystals That Are Converted from Extracellular Biopolymer

[1]Amr A. El-Waseif and [2]Dina E. El-Ghwas

[1]Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt
[2]Department of Chemistry of Natural and Microbial products, National Research Center, Dokki, Egypt

Abstract: Crystallization is a very significant property affecting all mechanical and physical properties of Exopolysaccharide (EPS) just as for all kind of polymers. Nanocrystalline Exopolysaccharide (NCEPSs) was separated from EPS that *Lactobacillus plantarum NRRL B- 4496* produced by sulfuric acid hydrolysis–sonication treatment. Transmission electron microscopy (TEM), Ultraviolet (UV) spectrum and Fourier transform infrared (FTIR) spectrum, were conducted to investigate the NCEPSs with different morphologies and particle sizes showed different aggregation degrees. Results revealed that, NCEPSs under magnification of 200 nm was spherical in shape and there sizes ranging from 3.25 to 12.86 nm. Ultraviolet (UV) spectrum peak at 212 nm was characteristic of carbohydrates. Thus, this subject has taken in very good interest so far and it is believed that this interest will go on increasingly.

Key words: Nanocrystalline Exopolysaccharide · Sulfuric Acid Hydrolysis–Sonication Treatment · TEM · UV · FTIR

INTRODUCTION

Many microorganisms can synthesize exopolysaccharides (EPSs) and excrete them out of cell either as soluble or insoluble polymers. These EPS not only can protect the microorganisms but also can be applied in many biotechnological applications such as textile, pharmaceutical, cosmetics, food, metal mining, oil recovery and metal recovery. Common microbial EPSs such as xanthan and dextran have been used as commercial products for many years. Because of their novel properties, the bioactive microbial polysaccharides ?-D-glucans and bacterial cellulose have been used for immune modulation, tumor stasis and audio membrane. Thus, microbial EPSs have attracted more attentions from scientific and industrial communities [1, 2]. Exopolysaccharides (EPSs) are exocellular polymers present in the surface of many lactic acid bacteria. A number of strains of *Lactobacillus* and *Bifid bacterium*, the most common probiotics included in commercial products, are able to produce these biopolymers [3]. Some of the EPS producing strains are currently used in the manufacture of fermented dairy products as a source of natural thickeners and stabilizing ingredients. Positive health effects have been attributed to EPSs [4]. However, the physiological role that these polymers play in the bacterial ecology of probiotic lactic acid bacteria still remains uncertain. It has been suggested that EPSs from other bacteria can act as protective agents against desiccation, antimicrobial compounds, bacteriophage attack and phagocytosis [5]. They can also be involved in adhesion to surfaces and biofilm formation, such as oral biofilm [6]. In recent years, the use of nanofibular materials as a scaffold to prepare metallic nanoparticles has merited substantial attention owing to their important potential applications in the fields of catalysis, electronic nanodevices, optoelectronics, sensors, biomedical and nanocomposites [7, 8]. Distinct from conventional stabilizers the physical size of the nanofibular materials and embedded particles are both in the submicrometer or nanometer range, thus the characteristic large surface area of nanofibular stabilizer and nanoparticles is maintained [9]. Furthermore, these hybrids can possess both the advantages of nanofibers, such as light weight, flexibility and mold ability and of inorganic particles such as exceptional functionality, high strength and thermal stability. EPS nanocrystals, which are typically crystalline rod-like particles, can be easily extracted from a variety of renewable sources by controlled acid hydrolysis of EPS. They have some

Corresponding Author: Amr A. El-Waseif, Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. Tel: +201006543350. E-mail: amrelwaseif@yahoo.com.

notable properties, such as large aspect ratio, good dissolvability in water, excellent mechanical properties and a high capacity for absorption of guest molecules [10]. EPS nanocrystals have found applications in material science, for instance, the reinforcement of polymers [11]. However, they have low thermal stability, which limits their applications as reinforcements. The use of inorganic nanoparticles may enhance the thermal properties of EPSNCs which can be useful for high performance applications [12]. The present work is an attempt to biosynthesize of nanocrystals that are converted from extracellular biopolymer and characterization of these crystals using UV-vis spectroscopy, transmission electron microscopy (TEM) and Fourier transform-infrared spectroscopy (FTIR).

MATERIALS AND METHODS

Production of Exopolysaccharide: The exopolysaccharide (EPS) was produced by *Lactobacillus plantarum NRRL B- 4496.* The culture was propagated for EPS production using growth medium MRS broth medium containing (g/l) [13] Peptone, 10.0; Meat extract, 8.0; yeast extract, 4.0; D-Glucose, 20.0; Dipotassium hydrogen phosphate, 2.0; Sodium acetate trihydrate, 5.0; Triammonium citrate, 2.0; Magnesium sulfate heptahydrate, 0.2 and Magnesium sulfate tetrahydrate, 0.05; the pH was adjusted to 6.2 under static condition at 30°C for 24 hours.

Preparation of Nanocrystalline Exopolysaccharide (NCEPSs): The NCEPSs were prepared according to pervious study with some modification [14]. Exopolysaccharide (EPS) (0.11 gm) was hydrolyzed by diluted sulfuric acid (64 %, 250 ml) and incubated at room temperature for 24 hours under static conditions. At the end of the incubation period, the color of the suspension becomes dark brown. Then the EPS suspension was diluted with de-ionized water to stop the hydrolysis reaction, allowed to settle overnight until the suspensions were layered and the clear top layer was decanted off. After that, the supernatant washed with de-ionized water until no layered was found by centrifuge at 5000 rpm for 10 min. The supernatant was taken and the previously step was repeated (Several times). The final washed was conducted using dialysis page with de-ionized water for several days until the water pH remained constant. Afterwards, ultrasonication was conducted for 20 min at an output power of 1200W. Finally, the NCEPSs suspension sample was subjected to freeze-drying. All processings were done under dark conditions.

Characterization of the Nanocrystalline Exopolysaccharide (NCEPSs)

Transmission Electron microscopy (TEM): The NCEPSs was evaluated for their surface and shape characteristics by transmission electron microscopy. The TEM image was carried out using: Electron probe micro-analyzer JEOL-JXA 840A, Model Japan

Ultraviolet (UV) Spectrum: The UV spectrum analysis was carried out using: T80+UV/VIS Spectrometer, PG Instrument Ltd. Range: 190-1000 nm.

FTIR Spectrum Measurement: IR-spectra (4000-400 cm^{-1}) were recorded on Nexus 670 FTIR spectrophotometer (Iclet Co., USA), using KBr disc.

RESULTS AND DISCUSSION

Characterization of Nanocrystalline Exopolysaccharide (NCEPSs)

Transmission Electron microscopy (TEM): The results of transmission electron microscope (TEM) showed that, the NCEPSs in the reaction mixture has a uniform spherical shape and showing varying sizes as observed in Figure 1. Under magnification of 50 nm the size of NCEPSs were ranging from 14.22 to 37.33 nm, under magnification of 100 nm the size were 11.36 to 27.75 nm and under magnification of 200 nm the NCEPSs were ranging from 3.25 to 12.86 nm.

Ultraviolet spectrum of Nanocrystalline Exopolysaccharide (NCEPSs): The colorless solution was turned into dark brown color indicating the formation of NCEPSs. Also, the NCEPSs were characterized by measuring the surface plasmon resonance (SPR) band using UV-vis spectroscopy. This technique has proved to be very useful for the analysis of nanoparticles [15]. The UV absorption spectrum of the purified NCEPSs revealed that the peak at 212 nm is characteristic of carbohydrates especially ketose. Several scientists reported that, the polysaccharide showed two peaks at 212 and 228 nm. The peak at 212 nm is characteristic of carbohydrates [16]. It is worth mentioning that purified EPS before crystallization recorded two peaks at 212 and 228 nm in pervious study [17].

FTIR spectrum of Nanocrystalline Exopolysaccharide (NCEPSs): The FTIR spectrum measurement was done to determine the possible functional groups of NCEPSs. As shown in Figure 3, the peak at 3406.64 cm^{-1} can be attributed to the stretch vibration of (O–H) stretch group.

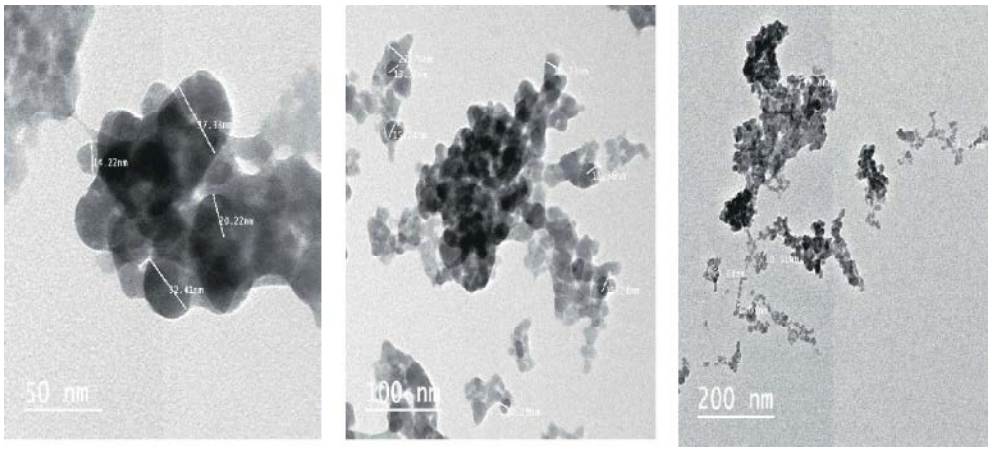

Fig. 1: Transmission electron microscopy (TEM) of NCEPSs under different magnifications

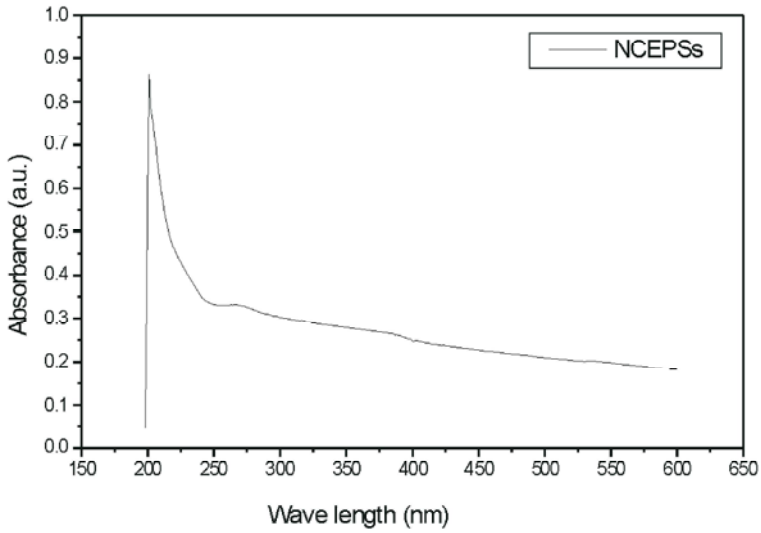

Fig. 2: UV-Vis Spectra absorbance of synthesis NCEPSs

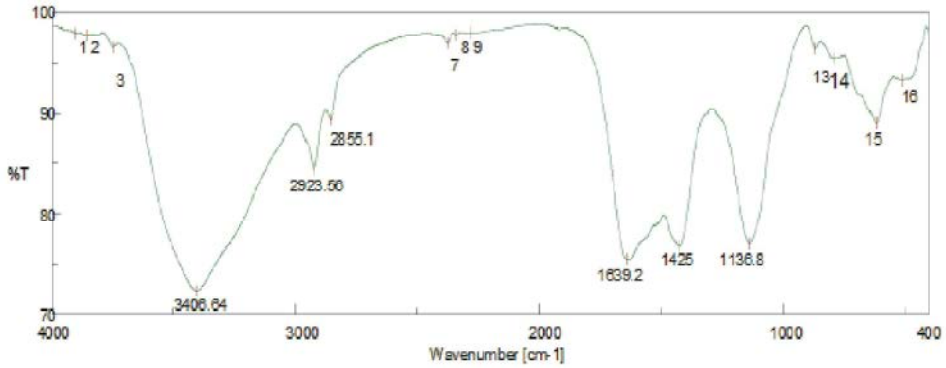

Fig. 3: The FTIR spectra of NCEPSs

Also, the peaks at about 2923.56 and 2855.1 cm^{-1} is assigned to the (C–H) stretching group. On the other hand, the peak at 1639.2 cm^{-1} is attributed to (C=O) carbonyl and carboxyl stretching group. Furthermore, the peak at 1425.14 is assigned to (O–H) bend group. Moreover, the absorption band at 1136.83 cm^{-1} is ascribed to (C–O–C) stretching mode from the glucosidic units [18].

CONCLUSIONS

Nanocrystalline Exopolysaccharide (NCEPSs) was separated from EPS that *Lactobacillus plantarum NRRL B- 4496* produced by sulfuric acid hydrolysis–sonication treatment. NCEPSs under transmission electron microscopy were spherical in shape and there is a ranging in sizes. Ultraviolet (UV) spectrum peak at 212 nm was characteristic of carbohydrates. Thus, this subject has taken in very good interest so far.

REFERENCES

1. Satpute, S.K., I.M. Banat, P.K. Dhakephalkar, A.G. Banpurkar and B.A. Chopade, 2010. Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol. Adv., 28: 436-450.
2. Staudt, A.K., L.G. Wolfe and J.D. Shrout, 2011. Variations in exopolysaccharide production by Rhizobium tropici. Arch Microbiol., 194: 197-206.
3. Andaloussi, S.A., H. Talbaoui, R. Marczak and R. Bonaly, 1995. Isolation and characterization of exocell*ular polysaccharides p*roduced by *Bifidobacterium longum*. Appl. Environ. Biotechnol., 43: 995-1000.
4. Rozen, R., D. Steinberg and G. Bachrach, 2004. *Streptococcus mutans* fructosyltransferase interactions with glucans. FEMS Microbiol. Lett., 232: 39-43.
5. Lo´pez, R. and E. Garcı´a., 2004. Recent trends on the molecular biology of pneumococcal capsules, lytic enzymes and bacteriophage. FEMS Microbiol. Rev., 28: 553-580.
6. Ruas-Madiedo, P., J. Hugenholtz and P. Zoon, 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. Int. Dairy J., 12: 163-171.
7. Liu, R.L., Y.X. Huang, A.H. Xiao and H.Q. Liu, 2010. Preparation and photocatalytic property of mesoporous ZnO/SnO_2 composite nanofibers. J. Alloys Compd, 503: 103-110.
8. Yin, Z.Y., S. Sun, T. Salim, S.X. Wu, X. Huang, Q.Y. He, Y.M. Lam and H. Zhang, 2010. Organic photovoltaic devices using highly flexible reduced graphene oxide films as transparent electrodes. ACS Nano, 4: 5263-5268.
9. Patel, A.C., S.X. Li, C. Wang, W.J. Zhang and Y. Wei, 2007. Electrospinning of porous silica nanofibers containing silver nanoparticles for catalytic applications. Chem. Mater, 19: 1231-1238.
10. Liu, H., D. Wang, Z. Song and S. Shang, 201. Preparation of silver nanoparticles on cellulose nanocrystals and the application in electrochemical detection of DNA hybridization. Cellulose, 18: 67-74.
11. Dong, H., K.E. Strawhecker, J.F. Snyder, J.A. Orlicki, R.S. Reiner and A.W. Rudie, 2012. Cellulose nanocrystals as a reinforcing material for electrospun poly (Methylmethacrylate) fibers: Formation, properties and nanomechanical characterization. Carbohydr. Polym, 87: 2488-2495.
12. Drogat, N., R. Granet, V. Sol, A. Memmi, N. Saad, C. K. Koerkamp, P. Bressollier and P. Krausz, 2011. Antimicrobial silver nanoparticles generated on cellulose nanocrystals. J. Nanopart. Res., 13: 1557-1562.
13. De Man, J.D., M. Rogosa and M.E. Sharpe, 1960. A Medium for the Cultivation of Lactobacilli. J. Appl. Bact, 23: 130-135.
14. Beck-Candanedo, S., M. Roman and D.G. Gray, 2005. Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. Biomacromolecules, 6(2): 1048-1054.
15. Morones, J.R., J.L. Elechiguerra, A. Camacho and J.T. Ramirez, 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353.
16. Yun, U.J. and H.D. Park, 2003. Physical properties of an extracellular polysaccharide produced by *Bacillus* sp. CP912. Letters in Applied Microbiology, 36: 282-287.
17. Haroun, B.M., B.M. Refaat, H.A. El-Menoufy, H.A. Amin and A.A. El-Waseif, 2013. Structure Analysis and Antitumor Activity of the Exopolysaccharide from Probiotic *Lactobacillus plantarum* NRRL B-4496 *In vitro* and *In vivo*. Journal of Applied Sciences Research, 9(1): 425-434.
18. Soheyla, H., 2013. Green Synthesis of Silver Nanoparticles Induced by the Fungus *Penicillium citrinum*. Tropical Journal of Pharmaceutical Research, 12(1): 7-11.