

ISSN: 1412-033X
E-ISSN: 2085-4722

BIODIVERSITAS

Journal of Biological Diversity

V o l u m e 2 2 - N u m b e r 5 - M a y 2 0 2 1



Front cover: Plochoglottis javanica Blume
(PHOTO: RIANY ANDITA PUTRI KUSWANDI)

Published monthly

PRINTED IN INDONESIA

ISSN: 1412-033X

E-ISSN: 2085-4722



9 771412 033764



9 772085 472768

BIODIVERSITAS

Journal of Biological Diversity
Volume 22 - Number 5 - May 2021

ISSN/E-ISSN:

1412-033X (printed edition), 2085-4722 (electronic)

EDITORIAL BOARD:

Abdel Fattah N.A. Rabou (Palestine), **Agnieszka B. Najda** (Poland), **Ajay Kumar Gautam** (India), **Alan J. Lymbery** (Australia), **Annisa** (Indonesia), **Bambang H. Saharjo** (Indonesia), **Daiane H. Nunes** (Brazil), **Darlina Md. Naim** (Malaysia), **Ghulam Hassan Dar** (India), **Hassan Pourbabaie** (Iran), **Joko R. Witono** (Indonesia), **Kartika Dewi** (Indonesia), **Katsuhiko Kondo** (Japan), **Kusumadewi Sri Yulita** (Indonesia), **Livia Wanntorp** (Sweden), **M. Jayakara Bhandary** (India), **Mahdi Reyahi-Khoram** (Iran), **Mahendra K. Rai** (India), **Mahesh K. Adhikari** (Nepal), **Maria Panitsa** (Greece), **Mochamad A. Soendjoto** (Indonesia), **Mohib Shah** (Pakistan), **Mohamed M.M. Najim** (Srilanka), **Nurhasanah** (Indonesia), **Praptiwi** (Indonesia), **Rasool B. Tareen** (Pakistan), **Seyed Aliakbar Hedayati** (Iran), **Seyed Mehdi Talebi** (Iran), **Shahabuddin** (Indonesia), **Shahir Shamsir** (Malaysia), **Shri Kant Tripathi** (India), **Subhash C. Santra** (India), **Sugeng Budiharta** (Indonesia), **Sugiyarto** (Indonesia), **Taufiq Purna Nugraha** (Indonesia), **Yosep S. Mau** (Indonesia)

EDITOR-IN-CHIEF:

S u t a r n o

EDITORIAL MEMBERS:

English Editors: **Graham Eagleton** (grahameagleton@gmail.com), **Suranto** (surantouns@gmail.com); Technical Editor: **Solichatun** (solichatun_s@yahoo.com), **Artini Pangastuti** (pangastuti_tutut@yahoo.co.id); Distribution & Marketing: **Rita Rakhmawati** (oktia@yahoo.com); Webmaster: **Ari Pitoyo** (aripitoyo@yahoo.com)

MANAGING EDITORS:

Ahmad Dwi Setyawan (unsjournals@gmail.com)

PUBLISHER:

The Society for Indonesian Biodiversity

CO-PUBLISHER:

Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta

ADDRESS:

Jl. Ir. Sutami 36A Surakarta 57126. Tel. +62-271-7994097, Tel. & Fax.: +62-271-663375, email: editors@smujo.id

ONLINE:

biodiversitas.mipa.uns.ac.id; smujo.id/biodiv



Society for Indonesia
Biodiversity



Sebelas Maret University
Surakarta

GUIDANCE FOR AUTHORS

Aims and Scope *Biodiversitas*, *Journal of Biological Diversity* or abbreviated as *Biodiversitas* encourages submission of manuscripts dealing with all biodiversity aspects of plants, animals and microbes at the level of the gene, species, and ecosystem as well as ethnobiology.

Article types The journal seeks original full-length research papers, reviews, and short communication. Manuscript of original research should be written in no more than 8,000 words (including tables and picture), or proportional with articles in this publication number. Review articles will be accommodated, while, short communication should be written at least 2,000 words, except for pre-study.

Submission The journal only accepts online submission, through open journal system (<https://smujo.id/biodiv/about/submissions>) or email to the editors at unsjournals@gmail.com. Submitted manuscripts should be the original works of the author(s). The manuscript must be accompanied by a cover letter containing the article title, the first name and last name of all the authors, a paragraph describing the claimed novelty of the findings versus current knowledge. Submission of a manuscript implies that the submitted work has not been published before (except as part of a thesis or report, or abstract); and is not being considered for publication elsewhere. When a manuscript written by a group, all authors should read and approve the final version of the submitted manuscript and its revision; and agree the submission of manuscripts for this journal. All authors should have made substantial contributions to the concept and design of the research, acquisition of the data and its analysis; drafting of the manuscript and correcting of the revision. All authors must be responsible for the quality, accuracy, and ethics of the work.

Ethics Author(s) must obedient to the law and/or ethics in treating the object of research and pay attention to the legality of material sources and intellectual property rights.

Copyright If and when the manuscript is accepted for publication, the author(s) still hold the copyright and retain publishing rights without restrictions. Authors or others are allowed to multiply article as long as not for commercial purposes. For the new invention, authors are suggested to manage its patent before published.

Open access The journal is committed to free-open access that does not charge readers or their institutions for access. Readers are entitled to read, download, copy, distribute, print, search, or link to the full texts of articles, as long as not for commercial purposes. The license type is CC-BY-NC-SA.

Acceptance The only articles written in English (U.S. English) are accepted for publication. Manuscripts will be reviewed by editors and invited reviewers (double blind review) according to their disciplines. Authors will generally be notified of acceptance, rejection, or need for revision within 1 to 2 months of receipt. The manuscript is rejected if the content does not in line with the journal scope, does not meet the standard quality, inappropriate format, complicated grammar, dishonesty (i.e. plagiarism, duplicate publications, fabrication of data, citations manipulation, etc.), or ignoring correspondence in three months. The primary criteria for publication are scientific quality and biodiversity significance. **Uncorrected proofs** will be sent to the corresponding author by email as *.doc* or *.docx* files for checking and correcting of typographical errors. To avoid delay in publication, corrected proofs should be returned in 7 days. The accepted papers will be published online in a chronological order at any time, but printed in the early of each month (12 times).

A charge Starting on January 1, 2019, publishing costs waiver is granted to authors of graduate students from **Least Developed Countries**, who first publish the manuscript in this journal. However, other authors are charged USD 250 (IDR 3,500,000). Additional charges may be billed for language editing, USD 75-150 (IDR 1,000,000-2,000,000).

Reprints The sample journal reprint is only available by special request. Additional copies may be purchased when ordering by sending back the uncorrected proofs by email.

Manuscript preparation Manuscript is typed on A4 (210x297 mm²) paper size, in a single column, single space, 10-point (10 pt) Times New Roman font. The margin text is 3 cm from the top, 2 cm from the bottom, and 1.8 cm from the left and right. Smaller lettering size can be applied in presenting table and figure (9 pt). Word processing program or additional software can be used, however, it must be PC compatible and Microsoft Word based (*.doc* or *.rtf*; not *.docx*). **Scientific names** of species (incl. subspecies, variety, etc.) should be written in italic, except for italic sentence. Scientific name (genera, species, author), and cultivar or strain should be mentioned completely for the first time mentioning it in the body text, especially for taxonomic manuscripts. Name of genera can be shortened after first mentioning, except generating confusion. Name of the author can be eliminated after first mentioning. For example, *Rhizopus oryzae* L. UICC 524, hereinafter can be written as *R. oryzae* UICC 524. Using trivial name should be avoided, otherwise generating confusion. **Biochemical and chemical nomenclature** should follow the order of the IUPAC - IUB. For DNA sequence, it is better used Courier New font. Symbols of standard chemical and abbreviation of chemistry name can be applied for common and clear used, for example, completely written butilic hydroxyl toluene (BHT) to be BHT hereinafter. **Metric measurement** use IS denomination, usage other system should follow the value of equivalent with the denomination of IS first mentioning. Abbreviations set of, like g, mg, mL, etc. do not follow by dot. Minus index (m⁻², L⁻¹, h⁻¹) suggested to be used, except in things like "per-plant" or "per-plot". **Equation of mathematics** does not always can be written

down in one column with text, in that case can be written separately. **Number** one to ten are expressed with words, except if it relates to measurement, while values above them written in number, except in early sentence. The fraction should be expressed in decimal. In the text, it should be used "%" rather than "percent". Avoid expressing ideas with complicated sentence and verbiage, and used efficient and effective sentence.

Title of the article should be written in compact, clear, and informative sentence, preferably not more than 20 words. Name of author(s) should be completely written. **Name and institution** address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. Manuscript written by a group, author for correspondence along with address is required. First page of the manuscript is used for writing above information.

Abstract should not be more than 200 words. **Keywords** is about five words, covering scientific and local name (if any), research theme, and special methods which used; and sorted from A to Z. All important **abbreviations** must be defined at their first mention. **Running title** is about five words. **Introduction** is about 400-600 words, covering the background and aims of the research. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains for why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgments** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

Figures and Tables of maximum of three pages should be clearly presented. Title of a picture is written down below the picture, while title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; chart is preferred to use black and white images. Author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned source. **There is no appendix**, all data or data analysis are incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

References Author-year citations are required. In the text give the authors name followed by the year of publication and arrange from oldest to newest and from A to Z. In citing an article written by two authors, both of them should be mentioned, however, for three and more authors only the first author is mentioned followed by et al., for example: Saharjo and Nurhayati (2006) or (Boonkerd 2003a, b, c; Sugiyarto 2004; El-Bana and Nijs 2005; Balagadde et al. 2008; Webb et al. 2008). Extent citation as shown with word "*cit*" should be avoided. Reference to unpublished data and personal communication should not appear in the list but should be cited in the text only (e.g., Rifai MA 2007, pers. com. (personal communication); Setyawan AD 2007, unpublished data). In the reference list, the references should be listed in an alphabetical order (better, if only 20 for research papers). Names of journals should be abbreviated. Always use the standard abbreviation of a journal's name according to the **ISSN List of Title Word Abbreviations** (www.issn.org/2-22661-LTWA-online.php). The following examples are for guidance.

Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. *Biodiversitas* 7: 154-158.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) *Tropical Forest Community Ecology*. Wiley-Blackwell, New York.

Abstract:

Assaed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) *Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island*. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. *Mol Syst Biol* 4: 187. www.molecularsystemsbiology.com

Structural elucidation of the exopolysaccharide produced by *Curvularia lunata* isolate RJ01

JAY JAYUS^{1,2}, ROBBY AKROMAN³, NURHAYATI⁴, ARI SATIA NUGRAHA⁵, BAMBANG PILUHARTO⁶,
ROBERT JAMES SEVIOUR⁷

¹Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia. Tel.: +62-331-3302234, ✉email: jayus.ftp@unej.ac.id

²Center of Excellent for Industrial Plant Biotechnology, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.

³Department Graduate School of Biotechnology, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.

⁴Center for Development of Advanced Sciences and Technology, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.

⁵Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, Universitas Jember. Jl. Kalimantan 37, Jember 68121, East Java, Indonesia.

⁶Department of Chemistry, Faculty of Math and Natural Science, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.

⁷Biotechnology Research Centre, La Trobe University. P.O. Box 199, Bendigo, VIC 3552, Australia.

Manuscript received: 8 February 2021. Revision accepted: 20 April 2021.

Abstract. Jayus J, Akroman R, Nurhayati, Nugraha AS, Piluharto B, Seviour RJ. 2021. Structural elucidation of the exopolysaccharide produced by *Curvularia lunata* isolate RJ01. *Biodiversitas* 22: 2699-2705. An exopolysaccharide (EPS) was recovered from culture filtrates of the fungus *Curvularia lunata* isolate RJ01 prepared in Jember, Indonesia. Based on a prominent peak at 883 cm⁻¹, FTIR analysis suggested it is a α -D-glucan, a proposal confirmed by subsequent nuclear magnetic resonance (NMR) analysis which showed anomeric protons omit the signal at δ 4.37 and 4.8 ppm, and sugar proton signal in the region of 2.5 to 4.2 ppm. High-performance liquid chromatography (HPLC) analysis of the acid hydrolysates of this glucan revealed the presence of glucose (64%) and a mixture of galactose and mannose (36%). Digestion with (1 \rightarrow 3)- and (1 \rightarrow 6)-glucanase from *Acremonium* sp. IMI 383068 gave consistent linear products of (1 \rightarrow 3)-glycosidic linkages, since the glucan was digested by (1 \rightarrow 3)-glucanase only. The rheological behavior of aqueous EPS solution suggests it behaves as a pseudoplastic non-Newtonian fluid and thus has the potential for use as a thickening agent in foods. This study is the first report on the structural elucidation of EPS from *C. lunata*.

Keywords: *Curvularia lunata*, fungal exopolysaccharide, α -glucan, rheological and functional properties

INTRODUCTION

Fungal EPSs are chemically diverse and present a number of possible functions used widely in foods, equo gkeu."cpf" ofgkpkpgu" *Quk umc-Jaroszuk et al. 2020). They exhibit diverse bioactive properties including immunomodulatory, antioxidant, antimicrobial (Mohan et al. 2019), hypolipidemic, hypoglycemic, hepatoprotective and antitumor actions (Wan et al. 2020). An EPS from the fungus *Ganoderma lucidum* has been shown to have multiple biological properties including hepatoprotective, immunomodulating, antitumor, and antioxidant substances (Zhou et al. 2014). Meanwhile, *Sanghuangporus vaninii* polysaccharide has been reported as having antitumor properties only (Wan et al. 2020). Fungal polysaccharides which have immunostimulating effects have been detected in *Cordyceps sinensis* (Wu et al. 2014) and *Grifola frondosa* fruiting bodies (Meng et al. 2017), as well as in other fungi reviewed by Loukotová et al. (2018). Moreover, fungal EPSs also possess antioxidative activities, such as polysaccharides from *Radix pseudostellariae* (Chen et al. 2013), *Auricularia auricula* (Hao 2014), and *Cordyceps sinensis* mycelium and *Ganoderma lucidum* (Zhonghui et al. 2014). However, the relationship between chemical structure and functional properties is still not well understood. For example,

Lachnum sp. YM26 produces a glucan linked by the (1 \rightarrow 3)-glycosidic bond and has anti-aging properties (Ye et al. 2012) while the As1-1 from *Aspergillus ur* [38"ykvj" (1 \rightarrow 8)"cpf" (1 \rightarrow 2) linkages has strong antioxidant potential (Chen et al. 2011). Furthermore, the tjc opqicncevcp"nkpmgf"d{" -*3 6.8"cpf" -*3 4+nkpmcigu" from *Fusarium solani* SD5 has anti-allergic and anti-inflammatory properties (Mahapatra and Banerjee 2012). The *Rhodotorula glutinis* EPS ykvj" -*3 5+"cpf" -*3 6+" linkages can act as an anti-viral (Ibrahim et al. 2012), and *Pseudozyma* sp. NIK" 2:387" GRU" nkpmgf" d{" -*3 5+" jcu" potential for use in thickeners, plasticizers, and emulsifiers (Sajna et al. 2013). Moreover, the exploration and exploitation of fungal polysaccharides show some advantages over other polysaccharides resources, such as distinct and reproducible production parameters to overcome environmental constraints, relatively higher yield, and high quality and purity of the final product (Moscovici 2015).

Fungal exopolysaccharides (EPSs) are carbohydrate polymers that in certain cases are secreted through cell walls and often form a protective layer (Yu et al. 2014; Chen et al. 2013) or, as with many fungal EPSs, are excreted into the environment as mucilaginous slime (Llamas et al. 2012). The polysaccharide monomer units can vary, and include mannose (Chen et al. 2011), glucose

(Guo et al. 2013), galactose (Chen et al. 2010), and rhamnose (Mahapatra and Banerjee 2012), generating either homo- or hetero-polysaccharides that can contain other inorganic or organic compounds (Llamas et al. 2012). Some of these polysaccharides consist of monomers linked by diverse alpha- and beta-glucosidic linkages (Sharmila et al. 2014). Although some are linear, most fungal glucans have a branched (1-3)- β -linked backbone of glucose units with branches of short glucose chains linked to the backbone by (1-6)- β linkages in which the frequency of branching can vary (Chen and Seviour 2007; Xiao et al. 2020). Those with beta-linked monomers appear to be less flexible than those whose monomers are linked with alpha glucosidic linkages similar to pullulan (Castellane et al. 2015). However, there is much still to understand with regard to how the structure of EPSs determines their physico-chemical properties. In recent decades research has focused on the relationship between the structure and activity of polysaccharides and their detailed modes of action, with the aim of understanding and using their functional properties.

The use of fungal EPSs in food processing has attracted interest due to their immunostimulating or therapeutic properties, such as hypocholesterolemic and hypoglycemic actions, which may lead to the development of novel functional foods or nutraceuticals (Giavasis 2013). In the food industry, many fungal EPSs have been used for many years, mainly as purified food additives and ingredients for human diet obtained from naturally occurring foods or recovered from metabolites of fungal culture fermentation (Schilling et al. 2020). Due to their diverse and modifiable properties, the spectrum of fungal EPSs applications is wide and includes gelling agents, thickeners, texturizers, suspension stabilizers, and Pickering emulsifiers. Although the physicochemical and biological characteristics and the applications of a number of fungal EPSs, such as pullulan and scleroglucan, have already been well-developed and commercialized, some less industrialized biopolymers, including mushroom polysaccharides such as ganoderan from *Ganoderma* sp., zymosan, lentinan from *Lentinus edodes*, grifolan and epiglucon from *Epicoccum* sp., are still being explored (Toukach and Egorova 2016). As a result, many new fungal polysaccharides continue to appear as the exploration of polysaccharide-producing strains continues. Observation of the roles of polysaccharides in fungi and a deep understanding of the relationship between structure and bioactivity will assist efforts to develop biomaterials both as antitumor drugs and for vaccine production (Barbosa 2020).

Curvularia is an endophytic fungus (Avinash et al. 2015) that is pathogenic to many plants, especially grasses (Manamgoda et al. 2012). In humans, this fungus was found in a person who had persistent fungal endophthalmitis (Alex et al. 2013). Several metabolites of commercial value as antimicrobial agents have been isolated from *C. lunata*, including curvularic acid and lunatin (Abdel-Ghany et al. 2015), ethyl acetate extract (Avinash et al. 2015) and anthraquinones (Jadulco et al., 2002), curvularin (Mondol et al. 2017), and perylenequinones (Cruz et al. 2020). *C. lunata* strain RJ01

has recently been reported to produce an EPS (Akroman et al. 2019), with potency as a food additive. In this paper, we investigate the chemical and physical properties of the EPS produced by *C. lunata*. To the best of our knowledge, this is the first report on the structural elucidation and physicochemical attributes of an EPS from this fungus.

MATERIALS AND METHODS

Production and purification of exopolysaccharide from *Curvularia lunata* isolate RJ01

Curvularia lunata isolate RJ01 with accession number MK629001.1. was grown in Czapek Dox broth under the same conditions as those described by Ramirez (2016). Biomass was removed by centrifugation and EPS harvested in the supernatant was precipitated using 96% ethanol and this precipitate was harvested by centrifugation at 5000 rpm for 5 minutes (Akroman et al. 2019; Ramirez 2016). The EPS was then dialyzed for 24 hours against water to remove any remaining low-molecular-weight compounds, and freeze-dried using a Virtis Advantage Plus freeze-dryer (SP Scientific, Warminster, USA).

Fourier transform infrared (FTIR) spectra analysis

Infrared spectra of the EPS were generated using an ATR alpha Fourier transform infrared spectrometer (Bruker Optics Inc., USA) in a chemistry laboratory at the Faculty of Pharmacy, University of Jember, Indonesia. The FTIR spectroscopic analysis began when 5 μ l dry EPS was mixed with KBr powder, then pounded and pressed into 1 mm pellets and placed on a silicone container. The container was then placed on a micro reader unit. Measurement of transmittance was conducted within the wavelength of 4000 to 600 cm^{-1} .

Nuclear magnetic resonance (NMR) spectroscopy analyses

^1H NMR spectra were recorded at 20 $^{\circ}\text{C}$ using a 500 Mhz JEOL ECS-500 spectrometer carried out at Tropical Disease Diagnostic Centre, Airlangga University Indonesia, in which the chemical shifts were expressed in ppm. The sample was prepared by dissolving freeze-dried EPS (10 mg) in methanol- d_4 .

EPS linkage- α -(1 \rightarrow 3)- β -glucanase from *Acremonium* sp.

Dqj" Gzq" *3 5+- cpf" *3 8+- -glucanases were purified from culture media of *Acremonium* sp. IMI 383068 grown on pustulan as substrate, as described by Jayus et al. (2001) and Jayus et al. (2002), and purified with FPLC (AKTA, Amersham Pharmacia Biotech, Sweden) using an anion exchange column (Sigma) as described by Jayus et al. (2004). EPS hydrolysis was performed with each enzyme in sodium acetate buffer (50 mM pH 5.0) containing the EPS (2 mg/mL) as a substrate at 40 $^{\circ}\text{C}$ for 30 min. The reducing sugars released were then measured using the DNS method (Miller 1959).

High-performance liquid chromatography (HPLC) analysis

The monomer composition of EPS was determined using Knauer HPLC, RI detector type 1260 (Berlin, Germany), Metacharb 87C column using H₂O as eluent, a flow rate of 0.5 mL/min, and temperature of 85 °C, a technique conducted at the Integrated Testing and Research Laboratory, University of Gajah Mada, Indonesia. A 10 mg sample of EPS was hydrolyzed by 2 M H₂SO₄ at 100 °C for 3 h. The hydrolysate was then neutralized with calcium acetate and injected into an HPLC with an injection volume of 20 µL.

Rheological analysis of *Curvularia lunata* EPS

The rheological behavior of the EPS was analyzed using a Brookfield RVDV-II+ Pro meter with 3 spindles, located at the laboratory of Food and Agricultural Products Process Techniques, Faculty of Agricultural Technology, University of Brawijaya, Indonesia. Flow behavior was measured at 30 °C of 1% EPS by comparing shear stress (Pa) and shear rate (1/s).

Statistical analysis

The replicated data are expressed as mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Monosaccharide composition of EPS from *Curvularia lunata* isolate RJ01

The results of the HPLC analysis of EPS hydrolyzed using acid showed that the EPS of *Curvularia lunata*

isolate RJ01 is a hetero-polysaccharide type. This is indicated by the presence of several EPS sugar constituents including glucose, galactose, and mannose. The relative sugar ratio in EPS is 6.4: 3.6 (glucose to galactose + mannose, with retention time values of 10.504 and 11.279 minutes, respectively) (Figure 1). This EPS composition is similar to that of *Pseudozyma* EPS and *Rhizobium* sp. PRIM-18, the relative proportions of sugar of which are 2.4 to 5.0 to 2.6 and 6.1 to 1.8 to 1, respectively (glucose to galactose to mannose) (Sajna et al. 2013; Priyanka et al. 2015). Both of these EPSs have high viscosity with pseudoplastic-type non-Newtonian fluid properties. The bioactive properties of similar sugar constituent types have been reported as potentially accelerating wound healing process *in vitro* (Priyanka et al. 2015). Whether or not the EPS from *C. lunata* isolate RJ01 possesses bioactivities has not yet been examined further. The more complex sugar constituent polysaccharide from fungus *Sanghuangporus vaninii* (Wan et al. 2020) consists of not only glucose, galactose, and mannose, but also rhamnose, glucuronic acid, galacturonic acid, glucosamine, galactosamine, xylose, arabinose, and fucose also revealed an antitumor activity. The presence of secreted fungal polysaccharides can be in the form of a layer over the surface of the microorganism, and they can be distinguishable from any polysaccharides occurring within the cell. The major functions of EPSs are thought to be as general physical protection avoiding access to any harmful compounds; they may also prevent dehydration. Phagocytoses by other microorganisms may also be prevented by this kind of capsular polysaccharides (Lei and Edmund 2017). Fungal polysaccharides may also play a crucial role in the reproduction of fungi (Barbosa 2020).

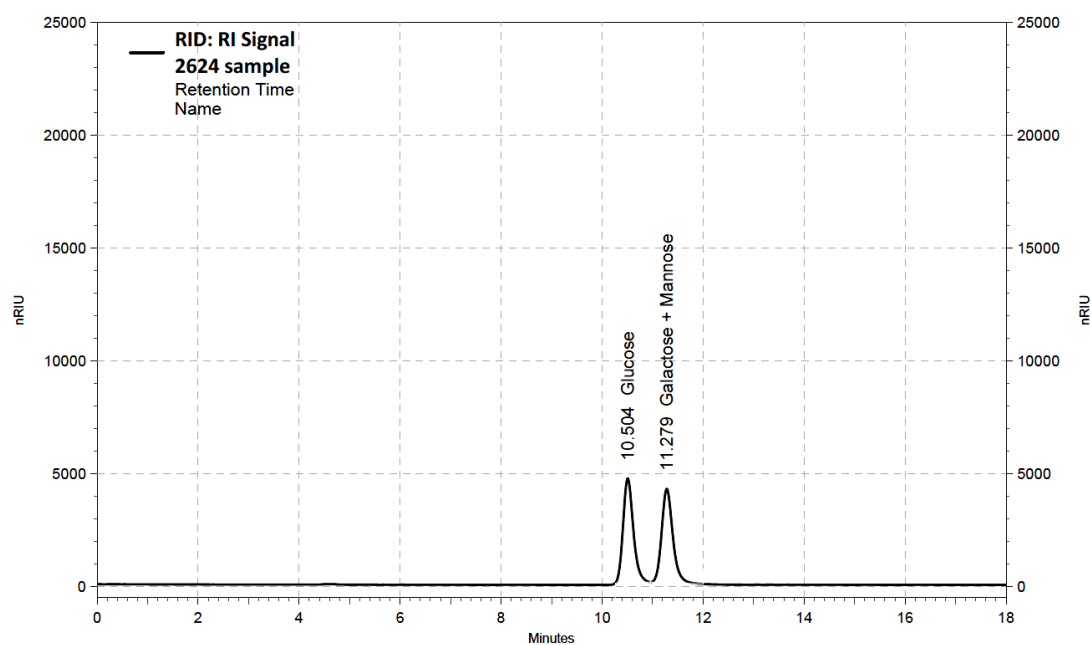


Figure 1. HPLC analysis of *Curvularia lunata* RJ01 EPS

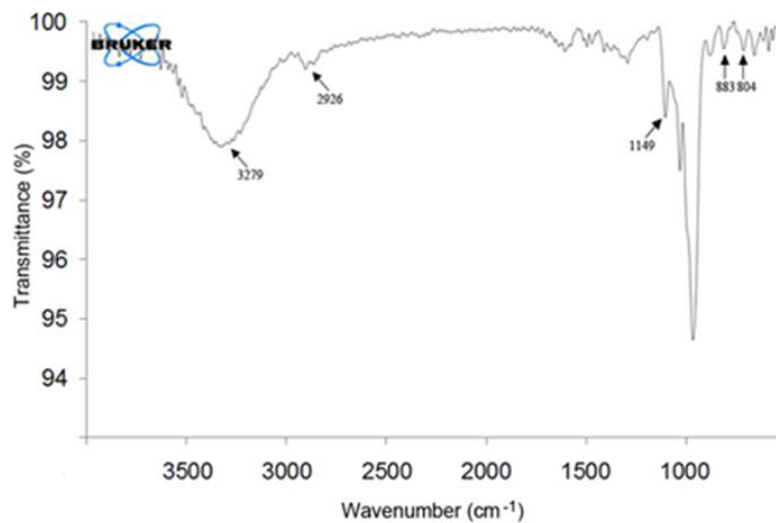


Figure 2. The FTIR spectrum of *Curvularia lunata* RJ01 EPS

Fourier transform infrared spectra of EPS

Our results showed that the functional groups in EPS *C. lunata* were on absorption bands including 3279 cm^{-1} , 1149 cm^{-1} and 2926 cm^{-1} and were in the form of hydroxyl (OH), ketones, or ether (COC) and alkanes (CH) (Figure 2). With the existence of these functional groups, EPS may belong to the group of carbohydrate compounds. Similar results were found in EPS from *Rodotorula* (Ramirez 2016), with its EPS consisting of several functional groups including hydroxyl groups, ketones and alkanes. However, the *Rodotorula* EPS not only contained high total sugar composition made up of glucose and mannose, but also low protein content (Ramirez 2016). The 3279 cm^{-1} hydroxyl absorption band in IR spectra of *C. lunata* showed the presence of polar functional groups of carbohydrate rings which may contribute to the solubility of EPS to water, as reported by Karbowski et al. 2011. The *C. lunata* EPS spectra showed an absorption band at 883 cm^{-1} , indicating

Vjg"j{ftqn{uku"rtqfwev"qh"GRU"wukpi" -glucanases assay

Vjg" *3 5+- -glucanase assay on the *C. lunata* EPS released reducing sugar as the hydrolysis product, kpfkcvkpi" vjcv" vjg" GRU" eqpukuvu" qh" *3 5+- -glycosidic linkages. Meanwhile, in the EPS hydrolysis product fkiqvgf" d{" *3 8+- -glucanase, no reducing sugar was fgvevgf" cu" vjg" *3 8+- -glucanase was not able to hydrolyze the EPS. It can thus be assumed that the EPS is a nkpqct" -glucan, different to the other branched fungal -glucan and fungal cell wall reported by Xiao et al. (2020), and to a rod-shaped polysaccharide such as schizophyllan from the fungi of *Schizophyllum commune* and scleroglucan from *Sclerotium rolfisii*. The *C. lunata* EPS may be similar to a linear random coil type structure such as pullulan from the fungus *Aureobasidium pullulans* (Lei

2016). Given vjg" rtgugpeg"qh"*3 5+- -glucan bonds, the 3 glucose monomers, mannose and galactose detected in the HPLC analysis indicate that this EPS structure is linear in shape with no branching. Even though the molecular weight (MW) of the *C. lunata* EPS has not yet been examined, many fungal polysaccharides have been reported as tending to have higher MW (Barbosa 2020). If this is the case with the *C. lunata* EPS, the EPS may have the potential to be used as an adsorber. Linear chain polysaccharides exhibit a greater adsorption ability than branched ones (Lei 2016). A linear neutral polysaccharide with larger MW appears to be adsorbed more easily smaller type. Fungal polysaccharides from *Ganoderma applanatum* and *Abortiporus biennis* have also been reported to be used as water-adsorbing materials (Bancerz et al. 2018).

Nuclear magnetic resonance spectroscopy of EPS

Analysis of the $^1\text{H-NMR}$ spectrum of *C. lunata* isolate RJ01 EPS showed the presence of proton signals in the region of 2.5 to 4.2 ppm (Figure 3). The proton signal showed a chemical shift of the sugar proton signals (Nugraha et al. 2015). Chen et al. (2016) and Ge et al. (2013) also observed several EPS proton signals on the spectrum of water-soluble polysaccharides from *Alternaria* sp. and *Phellinus baumii* Pilát that appeared in the region of 3.4 to 4.3 ppm. Additionally, 3 signals of anomer protons appeared at 5.24, 5.18 and 4.20 ppm (labeled as a, b and c, Figure 3). In addition, only an anomeric proton at 4.20 ppm indicated a clear coupling constant value of 6.4 Hz which represents the - configurations, as also observed by Zha et al. (2015) and Zhang et al. (2016). The peak labeled a and b did not provide a well-resolved multiplicity as compound with numbers of hydroxyl moiety (i.e sugars) produced less resolved proton spectra under methanol-*d* as solvent.

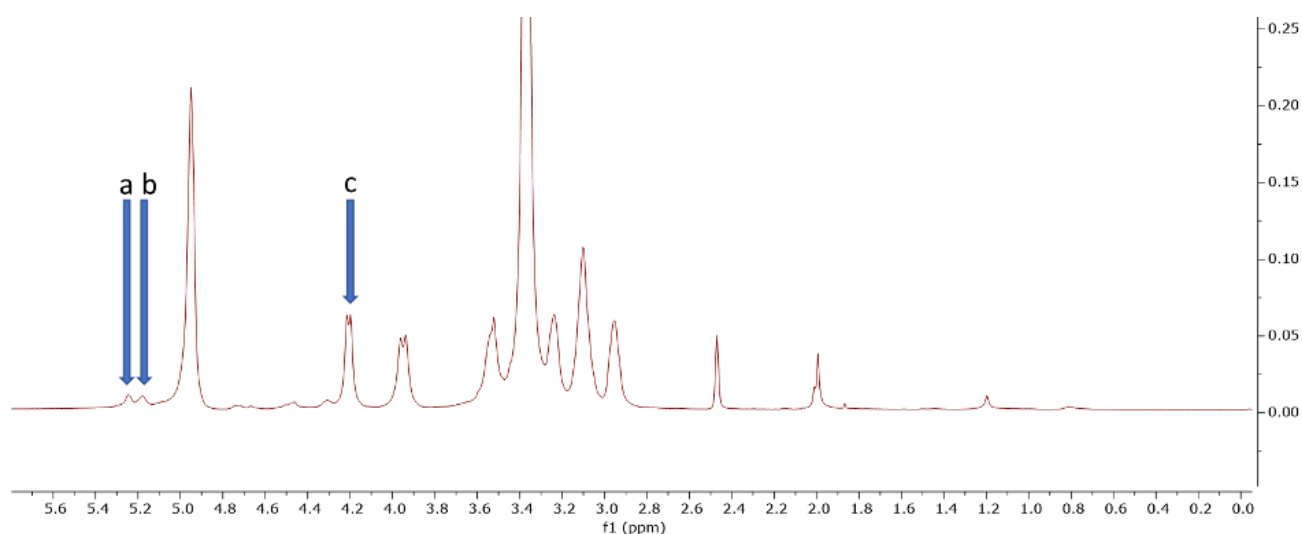


Figure 3. $^1\text{H-NMR}$ spectrum of *Curvularia lunata* RJ01 EPS (methanol, 500 MHz)

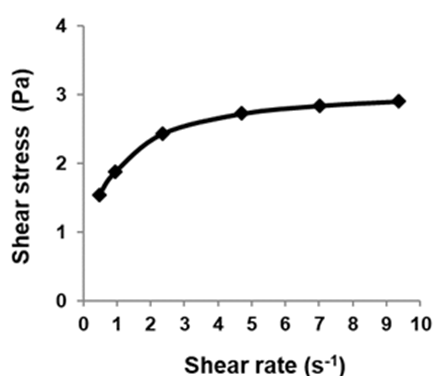


Figure 4. The rheological behavior of *Curvularia lunata* RJ01 EPS

The rheology of *C. lunata* isolate RJ01 EPS

Based on the rheological test of *C. lunata* isolate RJ01 EPS in relation to the shear rate and shear stress at a temperature of 30 °C, it can be seen that solution viscosity decreased with the increasing rate of shear stress (Figure 4), thus indicating pseudoplastic-type non-Newtonian fluid properties. The EPS was also highly viscous in water (2.23 poise), with a rating higher than that of pullulan (1.79 poise) (Schilling et al. 2020, Tsujisaka and Mitsuhashi 1993) and *Adansonia digitata* mucilage (2.1 poise) in the same concentration of 1% (w/v) (Deshmukh et al. 2013). The high rheological nature of linear *C. lunata* isolate RJ01 EPS in terms of the behavior of non-Newtonian pseudoplastic fluids shows high potential for use as a thickener and gelling agent in various industries. Sajna et al. (2013) also reported that EPS from *Pseudozyma* has potential as a thickener and gelling agent due to its high rheological properties. Some linear β -glucans from bacteria which have been widely used in food and industrial fields, such as curdlan and gellan, are industrially important

because of their ability to inhibit syneresis and to stabilize emulsions in dairy products, as well as their ability to control the rheology of water-based systems in other liquid product (Lei and Edmund 2017). The length of the linear chain of polysaccharides will also affect their viscosity in aqueous solutions. As reported by Khan et al. (2017), various molecular weights of β -glucans released by edible mushrooms *Pleurotus ostreatus*, *Agaricus bisporus*, and *Coprinus atrimentarius* exhibit different rheological properties.

In summary, we successfully isolated and identified *C. lunata* isolate RJ01 and its EPS from specimens drawn from agricultural areas in the city of Jember, Indonesia. Based on the identification results derived from FTIR, NMR, HPLC, and enzyme digestion, the EPS is found to have a sugar composition of glucose, galactose, and β -glucosidic linkage. This EPS is non-toxic and has a high rheological value and pseudoplastic type, potentially rendering it suitable for use in the food industry as thickeners, emulsifiers, and/or gelling agents. Future work is needed to investigate the bio-functional properties of *C. lunata* isolate RJ01 EPS.

ACKNOWLEDGEMENTS

The authors gratefully thank the University of Jember, Indonesia, for supporting the funding of this research through Islamic Development Bank (IsDB) programs.

REFERENCES

- Abdel-Ghany TM, Shater ARM, Negm ME, Al Abboud MA, Elhussieny NI. 2015. Efficacy of botanical fungicides against *Curvularia lunata* at molecular levels. *J Plant Pathol Microb* 6: 289. DOI:10.4172/2157-7471.1000289

- Akroman R, Nurhayati N, Suwasono S, Jayus J. 2019. Phenotypic and genotypic characteristics of exopolysaccharide-producing fungi as a source of food additives. *Biodiversitas* 20 (9): 2468-2474. DOI: 10.13057/biodiv/d200906
- Alex D, Li D, Calderone R, Peters SM. 2013. Identification of *Curvularia lunata* by polymerase chain reaction in a case of fungal endophthalmitis. *Med Mycol Case Rep* 2: 137-140. DOI: 10.1016/j.mmcr.2013.07.001
- Avinash KS, Ashwini HS, Babu HNR, Krishnamurthy YL. 2015. Antimicrobial potential of crude extract of *Curvularia lunata*, an endophytic fungi isolated from *Cymbopogon caesius*. *J Mycol* 2015: 1-4 DOI: 10.1155/2015/185821
- Depegt|"T."Quk umc-Jaroszuk M, Jaszek, Sulej J, Wiater A, Matuszewska A, Rogalski J. 2018. Fungal polysaccharides as a water-adsorbing material in esters production with the use of lipase from *Rhizomucor variabilis*. *Int J Biol Macromol* 118: 957-964. DOI: 10.1016/j.ijbiomac.2018.06.162
- Barbosa JR. 2020. Occurrence and possible roles of polysaccharides in fungi and their influence on the development of new technologies. *Carbohydr Polym* 246: 116613. DOI: 10.1016/j.carbpol.2020.116613
- Castellane TCL, Otoboni AMMB, Lemos EGD. 2015. Characterization of exopolysaccharides produced by rhizobia species. *Rev Bras Cienc Solo* 39 (6): 1566-1575. DOI: 10.1590/01000683rbscs20150084
- Chen J, Seviour R. 2007. Medicinal importance of fungal beta-³ 5+³ 8+-glucans. *Mycol Res* 111: 635-652. DOI: 10.1016/j.mycres.2007.02.011
- Chen YT, Yuan Q, Shan LT, Lin MA, Cheng DQ, Li CY. 2013. Antitumor activity of bacterial exopolysaccharides from the endophyte *Bacillus amyloliquefaciens* sp. isolated from *Ophiopogon japonicus*. *Oncol Lett* 5: 1787-1792. DOI: 10.3892/ol.2013.1284
- Chen Y, Mao W, Tao H, Zhu W, Qi X, Chen Y, Li H, Zhao C, Yang Y, Hou Y, Wang C, Li N. 2011. Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus *Aspergillus* sp. Y16. *Bioresource Technol* 102: 8179-8184. DOI: 10.1016/j.biortech.2011.06.048
- Chen Y, Guo S, Xu J, Chen Y, Li H, Qi X. 2010. Studies on exopolysaccharides from a marine endogenous fungus of the sponge (*Alternaria* sp.). *Periodical Ocean Univ China* 5: 1-4.
- Chen Y, Mao WJ, Yan MX, Liu X, Wang SY, Xia Z, Xiao B, Cao SJ, Yang BQ, Li J. 2016. Purification, chemical characterization, and bioactivity of an extracellular polysaccharide produced by the marine sponge endogenous fungus *Alternaria* sp. SP-32. *Mar Biotechnol (NY)* 18 (3): 301-313. DOI: 10.1007/s10126-016-9696-6
- Chen Z, Li S, Wang X, Zhang CL. 2013. Protective effects of *Radix pseudostellariae* polysaccharides against exercise-induced oxidative stress in male rats. *Exp Ther Med* 5: 1089-1092. DOI: 10.3892/etm.2013.942
- Cheng KC, Demirci A, and Catchmark JM. 2011. Pullulan: Biosynthesis, production, and applications. *Appl Microbiol Biotechnol* 92: 29-44. DOI: 10.1007/s00253-011-3477-y
- Cruz HJM, Boffo EF, Geris R. 2020. Perylenequinones from *Curvularia lunata*. *Biochem Syst Ecol* 92: 104086. DOI: 10.1016/j.bse.2020.104086
- Deshmukh SS, Katare YS, Shyale SS, Bhujbal SS, Kadam SD, Landge DA, Shah DV, Pawar JB. 2013. Isolation and evaluation of mucilage of *Adansonia digitata* Linn. as a suspending agent. *J Pharmaceutics*. DOI: 10.1155/2013/379750
- Ge Q, Mao JW, Zhang AQ, Wang YJ, Sun PL. 2013. Purification, chemical characterization, and antioxidant activity of a polysaccharide from the fruiting bodies of sanghuang mushroom (*Phellinus baumii* Pilat). *Food Sci Biotechnol* 22: 301-307. DOI: 10.1007/s10068-013-0081-1
- Giavasis I. 2013. Production of microbial polysaccharides for use in food. Woodhead Publishing Limited 413-468. DOI: 10.1533/9780857093547.2.413
- Guo S, Mao W, Li Y, Tian J, Xu J. 2013. Structural elucidation of the exopolysaccharide produced by fungus *Fusarium oxysporum* Y24-2. *Carbohydrate Res* 365: 9-13. DOI: 10.1016/j.carres.2012.09.026
- Hao HT. 2014. Effects of *Auricularia auricula* polysaccharides on exhaustive swimming exercise-induced oxidative stress in mice. *Trop J Pharm Res* 13: 1319-1326. DOI: 10.4314/tjpr.v13i11.11
- Ibrahim GS, Mahmoud MG, Asker MMS, Ghazy EA. 2012. Production and biological evaluation of exopolysaccharide from isolated *Rhodotorula Glutinins*. *Aust J Basic Appl Sci* 6: 401-408.
- Jadulco R, Brauers G, Edrada RU, Ebel R, Wray V, Sudarsono, Proksch P. 2002. New metabolites from sponge derived fungi *Curvularia lunata* and *Cladosporium herbarum*. *J Nat Prod* 65: 730-733 DOI: 10.1021/np010390i
- Jayus J, McDougall BM, Seviour RJ. 2001. Purification and properties of c³*3 8+-glucanase from *Acremonium* sp. IMI 383068. *Enzyme Microb Technol* 29: 194-200. DOI: 10.1016/S0141-0229(01)00381-7
- Jayus J, McDougall BM, Seviour RJ. 2002. Factors affecting the synthesis qh³*3 5+cpf³*3 8+-glucanases by the fungus *Acremonium* sp. IMI 383068 grown in batch culture. *Enzyme Microb Technol* 31: 289-299. DOI: 10.1016/S0141-0229(02)00106-0
- Jayus J, McDougall BM, Seviour RJ. 2004. Purification and ejctceygtk|cvkqpp³qh³vjg³*3 5+-glucanases from *Acremonium* sp. IMI 383068. *FEMS Microbiol Lett* 230: 259-264. DOI: 10.1016/S0378-1097(03)00913-3.
- Karbowiak T, Ferret E, Debeaufort F, Voilley A, Cayot C. 2011. Investigation of water transfer across thin layer biopolymer films by infrared spectroscopy. *Int J Membr Sci* 370: 82-90. DOI: 10.1016/j.memsci.2010.12.037
- Khan AA, Gani A, Masoodi FA, Mushtaq U, Naik AS. 2017. Structural, tjgqkicekn."cpkqzfcpr."cpf³ hwpvqpcn³ rtrgrt³kgu³ qh³ óglucan extracted from edible mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Coprinus atramentarius*. *Bioact Carbohydrates Diet Fibre* 11: 67-74. DOI: 10.1016/j.bcdf.2017.07.006
- Lei S. 2016. Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. *Int J Biol Macromol* 92: 37-48. DOI: 10.1016/j.ijbiomac.2016.06.100
- Lei S, Edmund TF. 2017. Polysaccharides, Microbial. Elsevier Inc.
- Llamas I, Amjres H, Mata JA, Quesada E, Béjar V. 2012. The potential biotechnological applications of the exopolysaccharide produced by the halophilic bacterium *Halomonas almeriensis*. *Molecules* 17: 7103-7120. DOI: 10.3390/molecules17067103
- Loukotová L, Konefař R, Venclíková K, Machová D, Janou-ková O, Rabyk M, Netopilík M, Mázl CE, ^{††} pánek P, Hrubý M. 2018. Hybrid thermoresponsive graft constructs of fungal polysaccharide β-glucan: Physico-chemical and immunomodulatory properties. *Eur Polymer J* 106: 118-127. DOI: 10.1016/j.eurpolymj.2018.07.004
- Mahapatra S, Banerjee D. 2012. Structural elucidation and bioactivity of a novel exopolysaccharide from endophytic *Fusarium solani* SD5. *Carbohydr Polym* 90 (1): 683-689. DOI: 10.1016/j.carbpol.2012.05.097
- Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD. 2012. A phylogenetic and taxonomic re-evaluation of the bipolaris - cochliobolus - curvularia complex. *Fungal Diversity* 56 (1): 131-144. DOI: 10.1007/s13225-012-0189-2
- Meng M, Cheng D, Han L, Chen Y, Wang C. 2017. Isolation, purification, structural analysis and immunostimulatory activity of water-soluble polysaccharides from *Grifola frondosa* fruiting body. *Carbohydr Polym* 157: 1134-1143. DOI: 10.1016/j.carbpol.2016.10.082
- Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31: 426-428. DOI: 10.1021/ac60147a030
- Mohan K, Ravichandran S, Muralisankar T, Uthayakumar V, Chandrasekar R, Seedeve P, Rajan DK. 2019. Potential uses of fungal polysaccharides as immunostimulants in fish and shrimp aquaculture: A review. *Aquaculture* 500: 250-263. DOI: 10.1016/j.aquaculture.2018.10.023
- Mondol MAM, Farhouse J, Islam MT, Schuffler A, Laatsch H. 2017. Metabolites from the endophytic fungus *Curvularia* sp. M12 acts as motility inhibitors against *Phytophthora capsici* zoospores. *J Nat Prod* 80 (2): 347-355. DOI: 10.1021/acs.jnatprod.6b00785
- Moscovici M. 2015. Present and future medical applications of microbial exopolysaccharides. *Front Microbiol* 6: 1012. DOI: 10.3389/fmicb.2015.01012.
- Nugraha AS, Hilou A, Vandegraaff N, Rhodes DI, Haritakun R, Keller PA. 2015. Bioactive glycosides from the African medicinal plant *Boerhavia erecta* L. *Nat Prod Res* 29: 1954-1958. DOI: 10.1080/14786419.2015.1013470
- Quk umc-Jaroszuk M, Sulej J, Jaszek M., Jaroszuk- ekug[†] L[†] 4242[†] Applications of fungal polysaccharides. Reference Module Life Sci. DOI: 10.1016/B978-0-12-809633-8.21092-3
- Priyanka P, Arun AB, Ashwini P, Rekha PD. 2015. Versatile properties of an exopolysaccharide R-PS18 produced by *Rhizobium* sp. PRIM-18. *Carbohydr Polym* 126: 215-221. DOI: 10.1016/j.carbpol.2015.03.017
- Ramirez MAJ. 2016. Characterization and safety evaluation of exopolysaccharide produced by *Rhodotorula minuta* BIOTECH 2178. *Int J Food Eng* 2 (1): 31-35. DOI: 10.18178/ijfe.2.1.31-35

- Sajna KV, Sukumaran RK, Gottumukkala LD, Jayamurthy H, Dhar KS, Pandey A. 2013. Studies on structural and physical characteristics of a novel exopolysaccharide from *Pseudozyma* sp. NII 08165. *Int J Biol Macromol* 59: 84-89. DOI: 10.1016/j.ijbiomac.2013.04.025
- Schilling C, Badri A, Sieber V, Koffas M, Schmid J. 2020. Metabolic engineering for production of functional polysaccharides. *Curr Opin Biotechnol* 66: 44-51. DOI: 10.1016/j.copbio.2020.06.010.
- Sharmila K, Thillaimaharani K A, Durairaj R, Kalaiselvam M. 2014. Production and characterization of exopolysaccharides (EPS) from mangrove filamentous fungus *Syncephalastrum* sp. *Afr J Microbiol Res* 8 (21): 2155-2161. DOI: 10.5897/AJMR12.2341
- Synnytsya A, Novak M. 2014. Structural analysis of glucans. *Ann Transl Med* 2: 1-14. DOI: 10.3978/j.issn.2305-5839.2014.02.07
- Toukach PV, Egorova KS. 2016. Carbohydrate structure database merged from bacterial, archaeal, plant and fungal parts. *Nucleic Acids Res* 44: D1229-D1236. DOI: 10.1093/nar/gkv840
- Tsujisaka Y, Mitsuhashi M. 1993. Pullulan. In: Whistler RL, BeMiller JN (eds) *Industrial gums. Polysaccharides and their derivatives*, 3rd ed. Academic Press, San Diego.
- Wan X, Jin X, Xie M, Liu J, Gontcharov AA, Wang H, Lv R, Liu D, Wang Q, Li Y. 2020. Characterization of a polysaccharide from *Sanghuangporus vaninii* and its antitumor regulation via activation of the p53 signaling pathway in breast cancer MCF-7 cells. *Int J Biol Macromol* 163: 865-877. DOI: 10.1016/j.ijbiomac.2020.06.279
- Wu DT, Meng LZ, Wang LY, Lv GP, Cheong KL, Hu DJ, Guan J, Zhao J, Li SP. 2014. Chain conformation and immunomodulatory activity of a hyperbranched polysaccharide from *Cordyceps sinensis*. *Carbohydr Polym* 110: 405-414. DOI: 10.1016/j.carbpol.2014.04.044
- Xiao Z, Zhou W, Zhang Y. 2020. Fungal polysaccharides. *Adv Pharmacol* 87: 277-299. DOI: 10.1016/bs.apha.2019.08.003
- Ye M, Chen W, Qiu T, Yuan R, Ye Y, Cai J. 2012. Structural characterization and anti-ageing activity of extracellular polysaccharide from a strain of *Lachnum* sp. *Food Chem* 132 (1): 338-343. DOI: 10.1016/j.foodchem.2011.10.087
- Yu J, Sun R, Zhao Z, Wang Y. 2014. *Auricularia polytricha* polysaccharides induce cell cycle arrest and apoptosis in human lung cancer A549 cells. *Int J Biol Macromol* 68: 67-71. DOI: 10.1016/j.ijbiomac.2014.04.018
- Yu W, Chena G, Zhanga P, Chen K. 2016. Purification, partial characterization and antitumor effect of an exopolysaccharide from *Rhizopus nigricans*. *Int J Biol Macromol* 82: 299-307. DOI: 10.1016/j.ijbiomac.2015.10.005
- Zha XQ, Lu CQ, Cui SH, Pan LH, Zhang HL, Wang JH, Luo JP. 2015. Structural identification and immunostimulating activity of a *Laminaria japonica* polysaccharide. *Int J Biol Macromol* 78: 429-438. DOI: 10.1016/j.ijbiomac.2015.04.047
- Zhang BW, Xu JL, Zhang H, Zhang Q, Lu J, Wang J. H. 2016. Structure elucidation of a polysaccharide from *Umbilicaria esculenta* and its immunostimulatory activity. *PLoS One* 11 (12): e0168472. DOI: <https://doi.org/10.1371/journal.pone.0168472>.
- Zhonghui Z, Xiaowei Z, Fang F. 2014. *Ganoderma lucidum* polysaccharides supplementation attenuates exercise-induced oxidative stress in skeletal muscle of mice. *Saudi J Biol Sci* 21: 119-23. DOI: 10.1016/j.sjbs.2013.04.004
- Zhou H, Liu G, Huang F, Wu X, Yang H. 2014. Improved production, purification and bioactivity of a polysaccharide from submerged cultured *Ganoderma lucidum*. *Arch Pharm Res* 37: 1530-1537. DOI 10.1007/s12272-014-0391-8.