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ORIGINAL ARTICLE

Endophytic fungus *Neopestalotiopsis clavispora* AUMC15969: biosynthesis and characterization of exopolysaccharides and biodiesel production

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Abstract
Exopolysaccharides (EPS) are bioactive metabolites with high molecular weight and are produced by several microorganisms such as endophytic fungi. Thus, the aim of the present study was designed to explore the biosynthesis and characteristics of EPS by the endophytic fungus *Neopestalotiopsis clavispora* AUMC15969 and then investigate some environmental and nutritional factors that affect their production. Moreover, we estimated an additional value, namely the production of biodiesel. Maximum production of EPS was 7.86 g/L when *N. clavispora* was grown on lactose as the sole carbon source and peptone as the nitrogen source, respectively, and pH 7 at 35 °Cfor 10 days. The produced EPS had a total sugar content of 0.93 g/g where protein content was 0.076 g/g. It revealed a strong antioxidant activity that improved with increasing sample concentration, with the optimum concentration of 10 mg/mL producing 83.1% DPPH radical scavenging activity with an IC_{50} equal to 3.89 mg/mL. The extracted lipid from the fungal mycelia at the end of the fermentation process was 31.76% w/w. The biodiesel produced from the transesterification of lipids was 87.4% total fatty acid methyl esters. The present study demonstrated the potential production of EPS and lipid biopolymers in one-pot fermentation which could use as a resource for industrial technologies.

Keywords *Neopestalotiopsis clavispora* · Exopolysaccharide · Biodiesel · Endophytic fungi

1 Introduction

Polysaccharides are highly hydrated polymers composed of repeated monosaccharides connected by glycosidic bonds. They are made up of an extremely wide range of compounds due to not only the various potential monosaccharide units but also how these units are linked together [1]. Recently, the production of exopolysaccharides (EPS) from microbes became highly interesting due to their industrial applications in different fields of food and medicine and health, especially from endophytic microbes where endophytic microbes have the ability to produce many bioactive compounds, such as antiviral, anticancer, antibiotics, and antidiabetic agents [2–4]. EPS can be divided into two types depending on the composition of monosaccharides. One is the heteropolysaccharide composed of a variety of monosaccharides with significant structural diversity, such as xanthan gum. The other is the homopolysaccharide composed of the same monosaccharides, such as cellulose and glucan, which can be further subdivided into HoPS and HePS [5, 6]. Microorganisms of different taxonomic groups have the capability to synthesize exopolysaccharides with interesting physical and chemical properties, such as high viscosity, good gelating properties, and synergistic effect when interacting with

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other polysaccharides [7]. Yeasts belonging to the *Cryptococcus*, *Hansenula*, *Rhodotorula*, *Lipomyces*, *Bullera*, *Aureobasidium*, and *Sporobolomyces* genera can synthesize exopolysaccharides including mannans, glucans, glucomannans, galactomannans, and phosphomannans [8].

The exopolysaccharides produced from yeasts are very attractive for large-scale production as the downstream process for exopolysaccharide recovery is simple and easy. Plant endophytic fungi are microorganisms that live in the interior tissues of living plants and do not cause any obvious negative consequences or symptoms [9]. Endophytes are considered potential producers of unique and biologically active compounds such as aliphatic compounds, alkaloids, flavonoids, peptides, isocoumarins derivatives, and steroids [10–12]. Recently, the production of polysaccharides from endophytes has been reported in many studies [12–15]. For example, *P. polymyxa* EJS-3 can generate EPS with the concentration of 22.82 g/L when using sucrose and yeast extract as carbon and nitrogen sources under the optimal culture conditions of 24°C and pH 8 for 60 h, and has strong scavenging activity against superoxide and hydroxyl free radicals [3, 4, 16]. Fungal EPS compounds have many advantages, such as their production is not affected by seasonal changes and they are easier to recycle and purify than EPS from plant and algal sources [2]. Microbial exopolysaccharides have multifarious industrial applications as thickeners, emulsifiers, and stabilizers in the food industry and others exhibit biological activity with therapeutic and pharmaceutical potential [17].

The production of polysaccharides by specific organisms could be affected by different environmental factors surrounding the organisms and/or the growth medium. The new trend in biotechnological processes is the production of several products simultaneously. In addition to the production of EPS, fungal mycelium can be reutilized as a source of valuable products such as reserved lipids which present as inclusion bodies inside it that could use as a feedstock for biodiesel. Microbial lipids from microorganisms are considered a prospective feedstock to increase the world bio-oil production that would cause low ecosystem impact [18]. Oleaginous yeasts and fungi have also been considered potential oil sources for biodiesel production because they accumulate large amounts of lipids. Filamentous fungi hold promise in this respect. They can fairly rapidly (within 96–130 h) accumulate much biomass and produce a wide range of biologically active compounds applicable in farming and medicine [19]. Biodiesel fuel is fatty acid monoalkyl (methyl or ethyl) esters produced from renewable sources such as vegetable oils or animal fats by the transesterification process of such triglycerides [20]. Biodiesel is rather an attractive alternative fuel to conventional petroleum diesel fuels because of its biodegradable, non-toxic, and clean sustainable source [21].

N. clavispora has a wide host distribution, including in China, Thailand, Malaysia, North Queensland, and Australia, and it is pathogenic to plants [22]. *N. clavispora* ASU1 was found to have the potential to remove Cd and Zn from water in intermittent systems [23]. However, there were few reports about EPS and lipid production from *N. clavispora*. Thus, the objectives of this study were to investigate the ability of *N. clavispora* AUMC15969 to produce EPS. Firstly, single factor experiments were performed to obtain the optimal culture conditions (including cultivation types, cultivation period, carbon source, nitrogen source, temperature, and initial pH) on the EPS production. Then, the EPS by measuring total sugar and protein content, FTIR, and antioxidant activity were characterized. The lipid content of fungal cell biomass as inclusion bodies at the end of the fermentation process and obtained biodiesel from lipid transesterification was also estimated.

2 Materials and methods

2.1 Microorganism and EPS biosynthesis

N. clavispora AUMC15969 was isolated from Avocado fruits as an endophytic fungus which was undertaken in our previous paper [23]. In 250-mL conical flasks, 100 mL of the production medium containing 50 g/L glucose, 3 g/L malt extract, and 1 g/L K_2HPO_4 were autoclaved at 121 °C for 20 min and inoculated with 2 mL of *N. clavispora* spore suspension (10^6 spore/mL) for exopolysaccharide and lipid production. The flasks were then incubated at 30 °C for 7 days in a rotatory shaker (120_{rpm}). At the end of the incubation period, the fungal culture was filtered and the viscous filtrate was used for the separation of EPS and the dried fungal biomass was collected for determination of lipid accumulation as inclusion bodies (Fig. 1).

2.2 Isolation and EPS determination

A filtration technique under vacuum was used to obtain the fungus cell-free filtrate from the culture according to [24] and then purified to obtain purified EPS [5]. Exopolysaccharide was precipitated by adding 3 volumes of cold ethanol and allowed to stand overnight at 4 °C. After centrifugation, the EPS precipitate was washed twice with absolute ethanol. EPS fraction dissolved in ethanol and partially purified by dialyzed by cellophane membrane against distilled water for 48h, then the mixture was centrifuged to obtain EPS [25]. Redissolving of EPS in distilled water and re-precipitated by cold ethanol was carried out to eliminate other undissolved materials that adhered to EPS. The EPS precipitates were collected by centrifugation (6000 rpm) for 10 min and then dried and weighed as grams per liter.

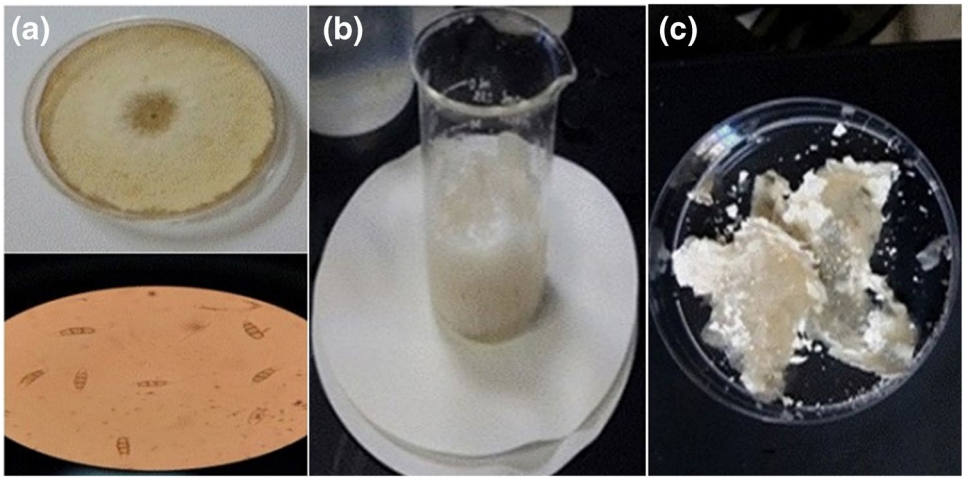

Fig. 1 Growth of *N. clavispora* AUMC15969 on PDA medium, optical microscope image of fungal conidia (**a**), crude EPS (**b**), dried EPS (**c**)

2.3 Physiological factors affecting EPS biosynthesis

2.3.1 Cultivation method

Two different cultivation methods were used where *N. clavispora* AUMC15969 was cultivated on static and submerged (shaking at 120 rpm) cultures using a production medium as described before at an incubation period of 7 days, 30 °C, and pH 7. EPS production and fungal dry weight were measured as previously described.

2.3.2 Incubation periods

The endophytic fungus *N. clavispora* AUMC15969 was grown on a production medium, we used lactose as the best carbon source instead of glucose in that medium. The fermented culture was sampled at different incubation periods (2, 4, 6, 8, 10, 12, and 14 days) for EPS and biomass production at 30 °C, pH 7 on a rotary shaker at 120 rpm.

2.3.3 Carbon sources

The effect of various carbon sources on the EPS production by *N. clavispora* AUMC15969 was examined by substituting in production medium with carbon sources namely, glucose, fructose, galactose, sucrose, lactose starch, and cellulose at the same concentration (50 g/L). The submerged cultures were incubated at 30 °C and pH 7 for 7 days. The EPS production and fungal dry weight were measured.

2.3.4 Nitrogen sources

The effect of different organic and inorganic nitrogen sources on EPS production by the tested endophytic fungus was investigated by substituting peptone in the production medium containing lactose as a carbon source with equivalent weights of various nitrogenous compounds. Namely; peptone, yeast extract, glycine, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, and $Ca(NO_3)_2$ have been utilized and then incubated at 35 °C, pH 7 for 10 days in submerged culture.

2.3.5 Temperatures

N. clavispora AUMC15969 was grown on a production medium with lactose as a carbon source and incubated at different temperatures (20, 30, 35, and 40 °C) under shaking at 120 rpm, pH 7 for 10 days for EPS and biomass production.

2.3.6 pH values

The effect of pH values on EPS production performed by the cultivation of the tested fungus on production medium contains lactose as carbon source and peptone as nitrogen source at different pH values (4, 5, 6, 7, 8, and 9) and incubation carried out at 35 °C, 120 rpm shaking for 10 days.

2.4 EPS characterization

2.4.1 Determination of total sugar and protein content

The 3 mL of partially purified EPS solution was dialyzed against distilled H_2O for 3 days to remove any residual monosaccharides from the culture medium which may be attached with precipitated EPS. The EPS of the dialyzed filtrate was then quantified colorimetrically by the anthrone method [26, 27] and protein content was quantified by Bradford et al. [26].

2.4.2 Fourier transform infrared spectroscopy

The functional groups of dried exopolysaccharide were analyzed using the KBr pressed disk technique (Thermo Scientific Nicolet iS10 FT-IR Spectrometer, USA) in the Chemistry Department of the Faculty of Science at Assiut University.

2.4.3 Antioxidant activity assay by DPPH free radical scavenging assay

The antioxidant activity of exopolysaccharide was investigated based on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity [28, 29]. The 2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were obtained from Sigma-Aldrich Chemicals Co., Germany. DPPH solution (10×10^{-5} M) was prepared by dissolving 0.04 g of DPPH in 1000 mL ethanol. DPPH (2 mL) (0.1 Mm) was added to an aliquot (0.2 mL of each sample) with appropriate dilutions. The mixture was shaken vigorously and then incubated in a dark place for 30 min at 25°C. The reaction can be represented by discoloration of the DPPH solution and measured spectrophotometrically at 517 nm. DPPH solution (2.0 mL) plus ethanol (0.2 mL) was used as a control. The experiments were carried out in triplicate using ascorbic acid as a reference standard and DPPH radical scavenging activity was calculated by using the formula [30]. At these experimental conditions, the scavenging capacity of the positive control (vitamin C, 10 mg/mL) reached 98%.

$$DPPH\ radical\ scavengng\ activity = \frac{absorbance\ ofcontrol - absorbance\ ofsample}{absorbance\ ofcontrol} \times 100$$

2.5 Lipid production and biodiesel characterization

Lipid accumulation was estimated in dried fungal biomass by extraction protocol and determined colorimetrically using DIAMOND Diagnostics kits, Egypt [31]. The residual fungal biomass after extraction of exopolysaccharide was utilized for biodiesel production. Biodiesel synthesis from each fungal biomass was performed by direct acid esterification technique as reported by [32]. The produced biodiesel (fatty acid methyl esters, FAME) was extracted by n-hexane. Then, a 2 µL of the FAMEs (top layer) were collected and analyzed using GC/MS, Agilent Model 6890N/5975B [Column DB 5ms, Agilent form (30, 0.25 mm, and 0.25 mm)] in the Chemistry Department, Faculty of Science, Assiut University. The percent of FAME (biodiesel) yield was estimated by comparing the FAME peak area of the internal standard at the particular retention time.

2.6 Statistical analysis

Statistical analysis was performed using Statistica IBM SPSS 26, and analysis of variance was used in the analysis. Homogeneous groups were determined using the Tukey test, at a significance level of $p \leq 0.05$. The results were presented as averages from three independent experimental series.

3 Results and discussion

3.1 The influence of cultivation conditions on the EPS biosynthesis

3.1.1 Cultivation types

In the current study, *N. clavispora* AUMC15969 was cultivated on static and submerged culture for EPS and biomass production (Fig. 2). The result shows that submerged cultivation was better than static one, which was consistent with previous studies [33, 34]. The production of EPS and fungal biomass where reached 2.8 and 18 g/L, respectively (Fig. 3a). It was known that submerged culture provides a more homogeneous distribution of oxygen and food for fungal growth than static. When oxygen is available in the medium, EPS production by fungi normally reached its optimum level in shaking conditions [33]. In the study of the production of EPS by *Aspergillus parasiticus* in agitated and static fermentation, the concentration of EPS increased in agitated than static fermentation from 0.18 to 0.41 g/L, respectively [34].

3.1.2 Incubation periods

The optimum period for the highest concentration of biomass was 19.06 g/L was 8 days, while the maximum concentration of EPS was 4.42 g/L produced after 10 days (Fig. 3b). The optimum incubation period for the maximum growth of *A. wentii* Ras101 was 7 days [35]. The reported data that the highest EPS concentration produced by *Alternaria alternate* was 11.96 mg/mL produced after 9 days [36]. While 4 days incubation period is the best for EPS production with *Hirsutella* sp. [37]. Also, 4 days was the favorite incubation period for producing the largest amount of EPS which was 17.6 mg/L by endophytic *Diaporthe* sp. JF766998 [24]. In another study that the maximum amount of EPS was 10.13 g/L produced after 4 days while the maximum amount of biomass was 5.343 g/L produced after 5 days by endophtic fungal isolate *Colletotrichum alatae* LCS1 [15].

3.2 The influence of nutrient conditions on the EPS biosynthesis

3.2.1 Carbon sources

It was reported that different carbon sources had different influences on catabolic repression on secondary metabolism [38]. Sucrose is the suitable carbon source for EPS and biomass

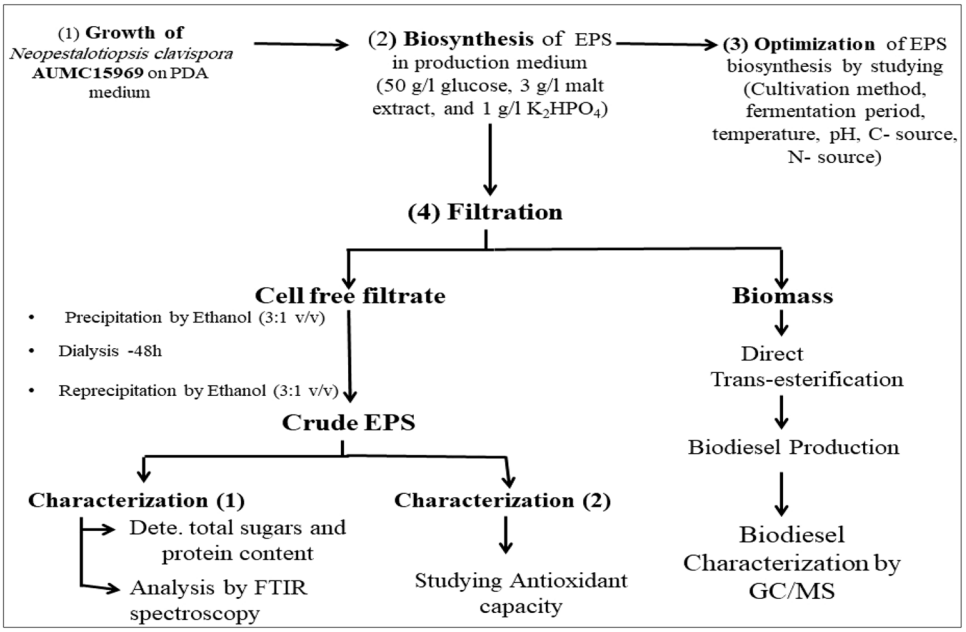

Fig. 2 A diagrammatic design summarizing the study protocol of EPS and lipid production by *N. clavispora* AUMC15969

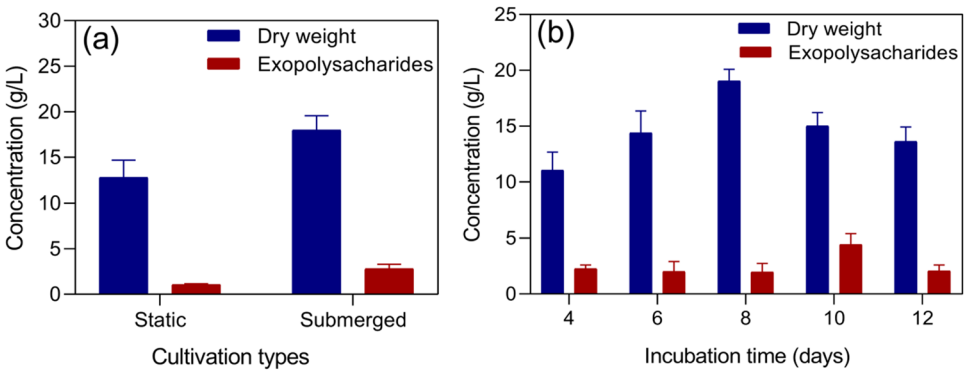

Fig. 3 The effect of different cultivation types (**a**) and incubation periods (**b**) on biomass and EPS produced by *N. clavispora* AUMC15969. Error bars indicate mean±standard deviation

production by *Stemphylium* sp. strain and glucose is the most suitable carbon source for *C. alatae* LCS1 [15, 39]. The effect of different carbon sources (such as sucrose, glucose, fructose, galactose, starch, cellulose, and lactose) on the production of fungal biomass and EPS was studied in this experiment (Fig. 4a). *N. clavispora* AUMC15969 was able to grow on all carbon sources. The largest amount of biomass was 30.73 g/L in the case of fructose and lactose provided the maximum EPS of 3.9 g/L which indicated that these sugars may be more suitable to metabolize by the tested fungus.

3.2.2 Nitrogen sources

N. clavispora AUMC15969 used all tested nitrogen sources which were peptone, yeast extract, glycine, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, and $Ca(NO_3)_2$ as shown in Fig. 4 b. Peptone was the most favorable nitrogen source for maximum biomass 28.8 g/L and EPS 5.46 g/L, which is consistent with the results of previous studies. The maximum amount of EPS was 13.97 g/L which was produced by the endophytic strain *Berkleasmium* sp. Dzf12 when used peptone as N-source [14]. Yeast extract was the best nitrogen source for *Stemphylium* sp. which provides the maximum EPS and biomass amount of 1.4 and 9 g/L, respectively when cultivated for 7 days at 30°C [39]. The yeast extract supports the maximum amount of EPS and biomass produced by *C. alatae* LCS1 which were 15.01 and 8.43 g/L, respectively [15].

3.3 The influence of physical parameters on the EPS biosynthesis

3.3.1 Temperatures

The effect of culture temperature on exopolysaccharide production by *N. clavispora* AUMC15969 was studied at variable temperatures (10-50 °C). The obtained results revealed that EPS synthesis enhanced dramatically with increasing culture

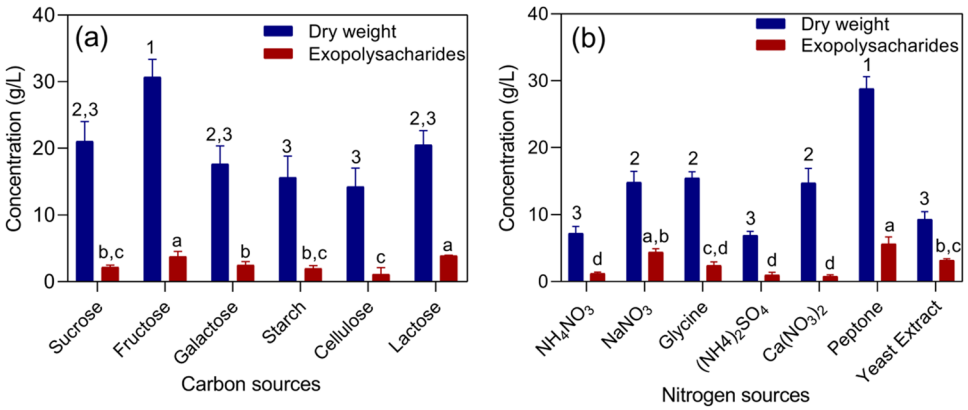

Fig. 4 The effect of different carbon sources (**a**) and nitrogen sources (**b**) on biomass and EPS produced by *N. clavispora* AUMC15969. The presented same letters and numbers indicate no significant differences at ($p < 0.05$)

temperature over the range of 10-35 °C. However, above 35 °C, there was a significant decrease in EPS amount where it was 1.21 g/L at 40 °C, and the maximum EPS amount was 5.11 g/L at 35 °C. The cultivation at 30 °C was the best for a maximum biomass amount of 21.46 g/L (Fig. 5a). It was reported that a range between 30 °C and 33 °C was found to be suitable for EPS production by *G. lucidum* [40]. In another study, the most of fungal strains produced maximum EPS within a temperature range of 22 °C to 30 °C [41]. The cultivation of *C. alatae* LCS1 at 26 °C provided the highest amount of EPS and biomass 12.13 and 7.863 g/L, respectively [15].

3.3.2 pH values

The hydrogen ion concentration (pH) in the medium plays an important role in microbial cell activities, where the external pH of the medium may affect the plasma membrane permeability [42]. They mention that in general, fungal strains are more tolerant to acidic than alkaline pH whereas, pH values 5–6 were found to be the suitable pH for most fungal growth. Other studies found that the effect of pH value on the growth kinetics of microorganisms was an important environmental factor affecting cell growth and product formation [43, 44]. The effect of different pH values (4, 5, 6, 7, 8, and 9) on the production of biomass and EPS by *N. clavispora* AUMC15969 was studied which showed in Fig. 5 b. The highest concentration of biomass and EPS were 26.4 g/L and 7.86 g/L at pH 7 and they decreased below and above this value.

The pH 5.5 is the optimal pH for EPS production by several fungi, including *Diaporthe* sp. JF766998, *Hirsutella* sp. and *P. pulmonarius*. For example, the endophytic fungal isolate *Diaporthe* sp. JF766998 had a biomass concentration of 17.6 g/L at pH 5.5 [24, 37, 45]. However, for some other fungi, pH 6.0 is optimal. The maximum mycelial growth was 9 g/L and EPS was 1.4 g/L produced by *Stemphylium* sp. at pH 6.0 when cultivated for 7 days at 30 °C [39]. The maximum amount of EPS and biomass produced by endophytic isolate *C. alatae* LCS1 at pH 6 were 7.896 and 13.03 g/L, respectively [15].

3.4 EPS characterization

3.4.1 Determination of total sugar and protein content

The biochemical characterization of the EPS produced in the production medium by *N. clavispora* AUMC15969 was evaluated using the determination of total sugars and protein contents as well as FTIR. The obtained results of the total sugar contents showed that 0.93 g/g of the produced EPS comprises sugars. Protein content was recorded as 0.076 g/g of the extracted EPS, which supposed as impurities so the obtained biopolymer was

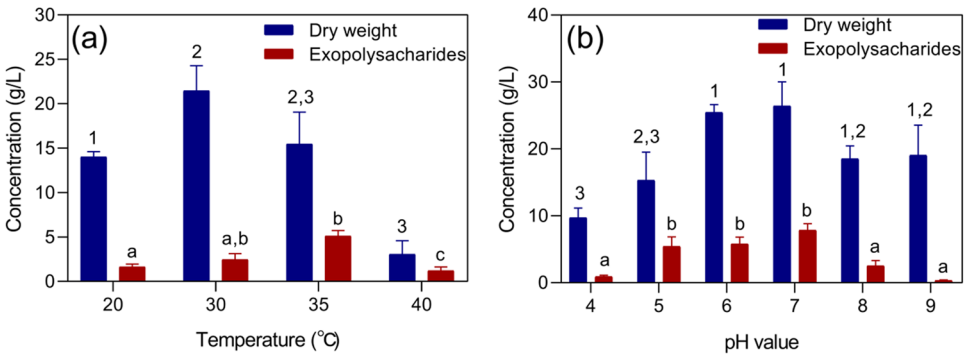

Fig. 5 The effect of different temperatures (**a**) and pH values (**b**) on biomass and EPS produced by *N. clavispora* AUMC15969. The presented same letters and numbers indicate no significant differences at ($p < 0.05$)

fitted to be designated as expolysaccharide. Interestingly, reducing sugar contents, total sugars, and protein concentration of *Neopestalotiopsis* sp. strain SKE15 bioactive exopolysaccharide was 39.1%, 95.3%, and 1.6% w/w, respectively which illustrates the presence of biopolymer side chains, and indeed the product is categorized as exopolysaccharide whenever compared to protein content [46]. The results obtained in this study and comparison with previously reported studies on the conditions and productions of EPS by different endophytes are shown in Table 1.

3.4.2 Fourier transform infrared spectroscopy

Various peak bands of the EPS infrared spectrum, functional groups, and their absorbance are shown in Fig. 6 and Table S1. The observed fungal groups in test exopolysaccharides by (FTIR) spectroscopy demonstrate a prominent peak at 3369.66 cm^{-1} corresponding to O-H groups present in carboxylic acid, which is joined by bands at 2927.27 cm^{-1} referring to H stretching in the carboxylic group [47]. The 1648.18 cm^{-1} might be attributed to the stretching vibration of the polysaccharide's carbonyl group (C=O) [52]. A small peak at 1546.88 cm^{-1} corresponding to an amino group was also observed in the spectrum of EPS [53]. Another peak of 1453.13cm^{-1} was due to the methyl group (CH_3) [54]. The band 1370.45 cm^{-1} identifies the vibration stretching of alkyl hydrogen (CH_2 -CH_3) in the aliphatic alkyl group (R-CH_2 - CH_3) [47]. They stated that the peak at 1080.58 cm^{-1} is assigned to the stretching vibration of (C-O, alcohol, ester,

Table 1 Summary of the results obtained in this study and comparison with previously reported work on conditions and productions of EPS by different endophytes

Fungus	Cultivation types	Cultivation times (days)	Carbon source	Nitrogen source	Temperature (°C)	pH	EPS production (g/L)	Reference
P. polymyxa EJS-3	ND	2.5	Sucrose	Yeast extract	24	8.0	22.82	[3]
A. *wentii Ras101*	Static culture	7	Glucose	ND	28	6.0	ND	[35]
Hirsutella sp.	ND	4	Glucose	Peptone	ND	5.5	2.17	[37]
Diaporthe sp. JF766998	Submerged culture	4	Glucose	ND	28	ND	0.154	[24]
N. sp. SKE15	Submerged culture	9	ND	ND	28	5.2	2.02	[46]
B. subtilis SH1	ND	4	Glucose	Peptone	37	9.0	24	[47]
H. coralloides	Submerged culture	7	Maltose	Soy peptone and yeast extract	22	6.5	0.13	[48]
P. brevicompactum NRC 829	Static culture	6	Glucose	Bacto-peptone	28	6.0	ND	[49]
C. militaris NG3	Submerged culture	4	Sucrose	Corn steep powder	30	8.0	3.4	[50]
G. lucidum CAU5501	Submerged culture	7	Glucose	Peptone	30	ND	1.723	[51]
N. clavispora AUMC15969	Submerged culture	10	Lactose	Peptone	35	7.0	7.86	This study

ND not detected

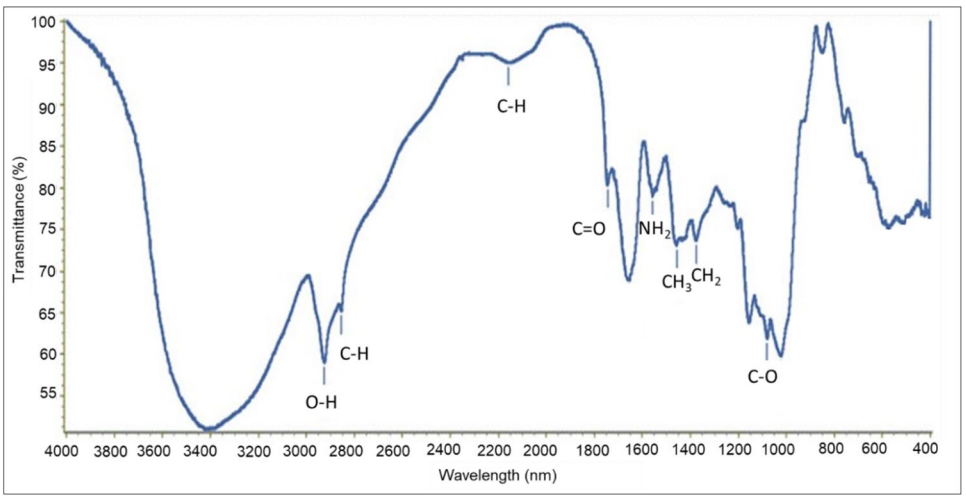

Fig. 6 Infrared spectrum of the EPS produced by *N. clavispora* AUMC15969

ether, and phenol) groups. The FTIR spectra of EPS showed the presence of carboxyl and hydroxyl groups, which usually act as binding sites for divalent cations [55]. The presence of acidic sugars in EPS may have a critical function in the polymer's heavy metal-binding capacities [56]. The presence of sugar molecules in fungal EPS implies that they can be used extensively in fungal biosorption assays and heavy metal sequestration from mangrove estuary waters [57].

3.4.3 Antioxidant activity of EPS

The exopolysaccharide showed the highest antioxidant activity, especially at concentrations (10 and 5 mg/mL) with a high percentage of inhibition equal to 83.1 and 77.3% in comparison with ascorbic acid. The antioxidant activity of EPS is influenced by the concentration of EPS in the solution, and the standard antioxidant compound (vitamin C) has a similar correlation (Fig. 7). IC_{50} of the sample was 3.89 mg/mL known as the concentration of the sample which scavenge 50% of DPPH free radical. This result was consistent with the EPS produced by Endophytic *Fusarium culmorum* Strains which has high antioxidant activity, most likely associated with the highest content of phenolic compounds in this EPS [58]. Furthermore, a high percentage of DPPH scavenging reached to 80% which referred to the high antioxidant capacity of EPS produced by the mushroom *Hericium coralloides* [48].

3.5 Lipid synthesis and biodiesel composition

3.5.1 Lipid production

Lipids produced by microbial biosynthesis have the potential to replace plant-based oil in the creation of biodiesel [59]. Endophytic fungi have recently been extensively explored for their vast range of benefit applications involving the biosynthesis of bioactive metabolites such as nutraceuticals and medicines, agricultural application to increase crop performance, and oil accumulation for biofuels [60]. At the end of the fermentation process, the lipid which accumulated in the fungal mycelium was extracted and recorded as 31.76% w/w. That lipid is considered an additional value to EPS which is produced by the selected fungal strain that has been used as a source of biodiesel in the current study.

The amount of lipid collected by the tested strain *N. clavispora* AUMC15969 (*P. clavispora)* could be related to its isolation process from the endophytic niche of avocado fruit, which is characterized by having a very high lipid content, which is only exceeded by the fruits of palm and olive trees [61]. As a result, this ecological niche may lead to a probable horizontal gene transfer between the Avocado plant and the fungus, or the endophytic fungus may have gained experience in boosting lipid accumulation in order to complete and survive in these settings. Fungi that survive in oily habitats and on oil-producing host plants have a greater capacity to synthesize fungal lipids that account for 35% of the dry weight of the biomass [62]. They reported that the yield of lipids supplied by endophytic *N. Surinamensis* was 13.6% w/w when grown on potato dextrose broth medium at 27 °C for 3 weeks under static conditions.

3.5.2 Biodiesel composition

Microbial oils are now believed as a promising potential feedstock for biodiesel production due to their similar composition of fatty acids to that of vegetable oils [32]. In this study, the composition of fatty acid methyl ester (biodiesel) after the transesterification process of dry fungal biomass waste was studied by GC/MS analysis. Data obtained from the GC/MS analysis revealed that octadecanoic acid, hexadecanoic acid, 9,12-octadecadienoic acid, cis-13-octadecenoic acid, and methyl 2-hydroxy-tetracosanoate were the most

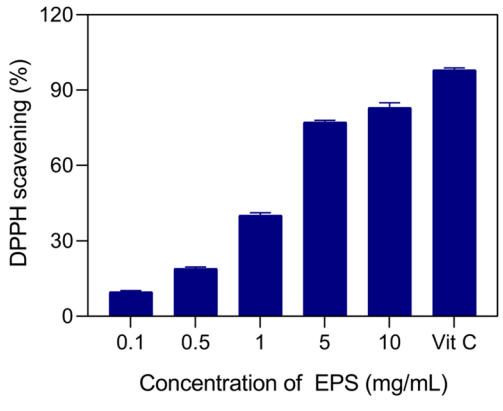

Fig. 7 Antioxidant activity (DPPH assay) of different EPS concentrations and Vit C as standard at (10 mg/mL). Error bars indicate mean±standard deviation

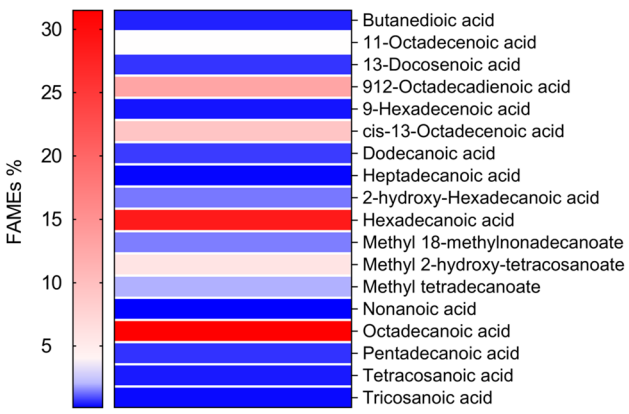

Fig. 8 The fatty acid methyl esters (FAMEs) obtained from the transesterification process of *N. clavispora* AUMC15969

common fatty acid methyl esters recording 31.48, 28.49, 12.82, 9.27, and 5.75% of total esters, respectively (Fig. 8). On the other hand, the other fatty acids were detected in the GC/MS profile in variable amounts; namely, butanedioic acid, 11-octadecenoic acid, 13-docosenoic acid, 9-hexadecenoic acid, dodecanoic acid, heptadecanoic acid, 2-hydroxyhexadecanoic acid, methyl 18-methylnonadecanoate, methyl tetradecanoate, pentadecanoic acid, tetracosanoic acid, tricosanoic acid and nonanoic acid (Fig. 8).

4 Conclusion

The tested endophytic *N. clavispora* AUMC15969 produced a high quantity of EPS 7.86 g/L at optimal conditions which include pH 7 at 35°C and lactose as the carbon source, peptone as nitrogen source, for 10 days. The produced EPS has 0.93 g/g sugar content and 0.076 g/g protein content. EPS showed strong antioxidant activity at a concentration of 10 mg/mL with an IC_{50} of 3.89 mg/mL. Also, *N. clavispora* AUMC15969 produced a high yield of lipid 31.76% w/w, the lipid used to produce biodiesel which has 87.4% total fatty acid methyl esters. The production of EPS and biodiesel was carried out in one pot fermentation process which helped to save time, and money and reduce the risk of environmental contamination by fungal hypha wastes.

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Data availability The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any authors.

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