Extracellular polysaccharides produced by the newly discovered source *Scopularis* spp.

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Background
Microorganisms are better and cheaper sources for the production of polysaccharides. Therefore, there has been an increasing interest in isolating and identifying new microbial polysaccharides.
Objective
The aim of this study was to produce new extracellular polysaccharides, with better rheological properties and varied applications, from the newly discovered fungal strain *Scopularis* spp., using different carbon sources.
Methods
Fourier transform infrared spectroscopy, carbohydrate analysis, and thin layer chromatography were the methods used for the preliminary characterizing of the produced polysaccharides.
Results
Among the 10 examined carbon sources, fructose, raffinose, sucrose, and maltose were found to produce an appreciable amount of extracellular polysaccharides (0.90, 0.87, 0.86, and 0.74 g/l, respectively), whereas arabinose, lactose, and mannitol produced a minimal amount of extracellular polysaccharides (0.22, 0.17, 0.12 g/l, respectively). However, all the tested sugars enhanced the growth of the fungal strain. The analytical method proved that the polymer was a heteropolysaccharide with six sugar moieties, all different in their relative ratios from one carbon source to another. Glucose was found to be the most abundant monosugar in all the polymer samples. Galactose, rhamnose, and glucuronic acid also appeared on the thin layer chromatography plate.
Conclusion
A new extracellular heteropolysaccharide was produced from the new source, *Scopularis* spp. The produced polysaccharide contained glucose, galactose, glucuronic acid, rhamnose, and two other unidentified sugars as indicated from the thin layer chromatography plate.

Keywords:
acid hydrolysis, carbon source, extracellular polysaccharides, *Scopularis* spp.

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Introduction

Polysaccharides are highly valued, biologically active polymers with many industrial applications in food, feed, textile, cosmetic, and pharmaceutical industries and are also used as depolluting agents [1]. Owing to their bioactive nature, they have many medicinal applications as anticancer, antiviral, antioxidant, antibacterial, anti-inflammatory, and prebiotic agents [2–8]. However, most of the commercial polysaccharides are produced from plants and algae and a small proportion is produced from microbial sources [9]. Fungi are currently an interesting source of biologically active compounds. Most of the mould-produced polysaccharides are obtained from mushrooms [8,10,11].

Microorganisms are better and cheaper sources for the production of polysaccharides compared with plants or algae because of their high growth rate, ability to grow in cheaper nutrient media within a few days, lower space requirement, and ease of manipulation [12]. Therefore, there has been an increasing interest in isolating and identifying new microbial polysaccharides that may compete with traditional polysaccharides.

Therefore, the aim of this study was to examine the ability of the new fungal strain *Scopularis* spp. to produce high yields of extracellular polysaccharides (EPS), with better rheological properties and varied applications, using different carbon sources.

Materials and methods

Microorganisms and media

The *Scopularis* spp. used in this study was obtained from the culture collection of the National Research Centre (Egypt). The strain was maintained by subculturing on

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potato dextrose agar slants monthly (PDA; Merck, Darmstadt, Germany). The slants were incubated at 28–30°C for 7 days before storage at 4°C.

The inoculum cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of sterilized medium comprising (g/l): lactose, 7.5; $NaN0_3$, 1.0; yeast extract, 1.5; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 1.0; KCl, 0.5; and $FeSO_4.7H_2O$, 0.01 at pH 5 [13] in a rotatory incubator shaker at 150 rpm and 28–30°C for 3 days before using.

Fermentation

Fermentation was carried out in 250 ml Erlenmeyer flasks containing 50 ml of the above mentioned medium with different sugars as the carbon source. The tested sugar solutions were sterilized separately and mixed aseptically with the other components before inoculation with 5% (v/v) of the inoculums. The flasks were incubated at 28–30°C in a rotary shaker at 150 rpm for 7 days.

Mycelial dry weight

The mycelial pellets were separated from the viscous liquid culture by centrifugation (6000 rpm, 20 min). After the removal of the supernatant, the mycelia was washed thoroughly with distilled water and dried to a constant weight to attain the mycelial dry weight.

Isolation of the extracellular polysaccharides

The viscous supernatant obtained from the above mentioned step were collected and dialyzed against tap water for 2 days using a 10 000–12 000 MWCO membrane (VWR Scientific, Spectrum Companies, Goshen Parkway, West Chester, USA), changing it three times daily; it was then dialyzed against distilled water in the same way, after which the solution was centrifuged again as indicated above. The dialyzed cultures were mixed with three volumes of chilled absolute ethanol (v/v) with stirring. The precipitated polysaccharide was collected together as viscous filaments and could easily be separated from the liquid and the other compact particles that settled quickly to the bottom. The collected EPS were washed with a water : ethanol mixture (1 : 1, v/v) to remove the residue of the liquid culture; it was then dried and weighted as crude EPS.

All the experiments were conducted in triplicate and the results are the averages of these three independent trials.

Monosaccharide composition analysis

Acid hydrolysis of the crude polysaccharides was carried out according to the procedure described by Fischer and Dorfel [14]. In brief, 0.05 g of the crude EPS was mixed with 0.5 ml of 80% sulfuric acid and left overnight at room temperature; it was then diluted with 6.5 ml of distilled water and boiled in a water bath for almost 6 h. The mixtures were cold neutralized with excess $BaCO_3$ and subjected to thin layer chromatography for primary investigation.

Thin layer chromatography

Silica gel plates (Merck) were used to identify the composition of the hydrolyzed polysaccharides. The samples were spotted onto the plates along with different standard monosugars. The plates were developed at room temperature in a saturated chamber containing *n*-propanol : water (85 : 15, v/v). The sugars were detected by spraying the dried plates with 3% phenol reagent, followed by incubation at 100°C in an oven for 10 min [15].

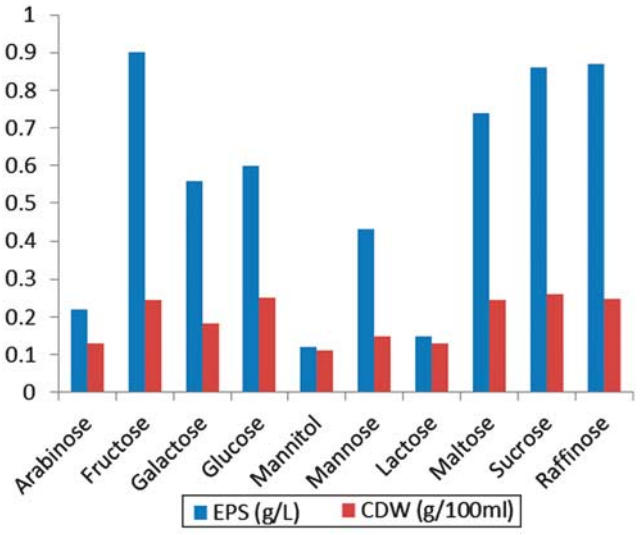
Fourier transform infrared analysis

The crude polysaccharide was mixed with KBr powder, ground, and pressed into 1 mm pellets for Fourier transform infrared (FTIR 6100; Jasco, HoChi Minh City, Japan) spectroscopy in the frequency range of 4000–400 cm^{-1}.

Results and discussion

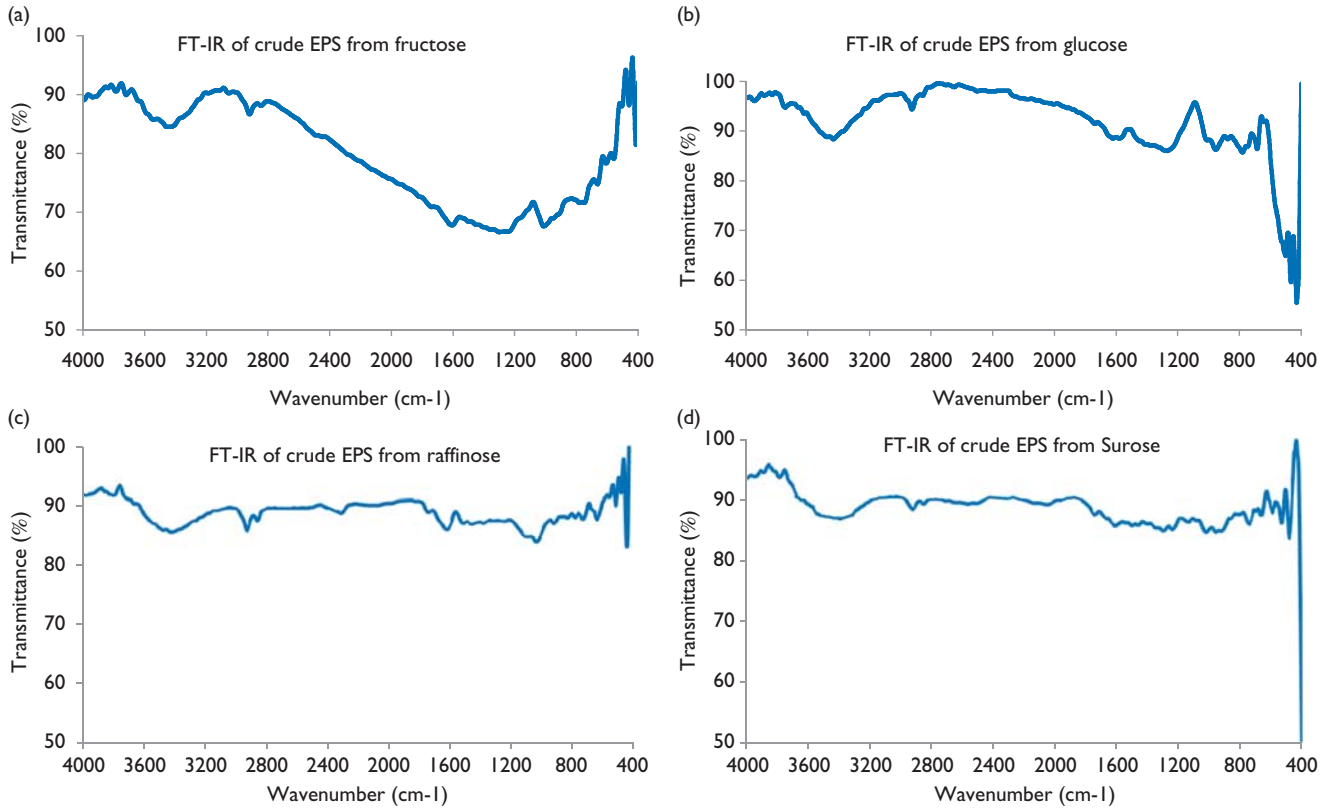
Carbohydrates are very important nutritional requirements for the growth and development of all fungi. However, different fungal species vary in their ability to utilize different carbon sources. The results shown in Fig. 1 indicate that the tested fungus had the ability to grow in all the used carbon sources, but the production of the EPS was quite distinct for each sugar used. Among the 10 sources examined, fructose, raffinose, sucrose, and maltose enhanced the production of EPS (0.90, 0.87, 0.86, and 0.74 g/l, respectively), whereas arabinose, lactose, and mannitol produced minimal amounts of EPS (0.22, 0.17, 0.12 g/l, respectively). The effect of the different carbon sources on the amount of polysaccharides produced was recorded by all the researchers and was found to be related to the microorganism used. The mycelial growth did not parallel with the production of EPS; this has also been reported by other researchers [16–18]. It has been observed that the production of EPS increased with an increase in the concentration of the sugars used and when the morphology of the fungal growth was in the form of pellets rather than fibers (unpublished data). The amount of EPS produced during

Figure 1



The effect of different sugars on the production of extracellular polysaccharides (EPS) and cell growth [expressed as constant dry weight (CDW)].

Figure 2



(a) Fourier transform infrared (FTIR) of the crude extracellular polysaccharides (EPS) produced from fructose as the carbon source in the culture medium. (b) FTIR of the crude EPS produced from glucose as the carbon source in the culture medium. (c) FTIR of the crude EPS produced from raffinose as the carbon source in the culture medium. (d) FTIR of the crude EPS produced from sucrose as the carbon source in the culture medium.

our study is within the range that has been published by other authors [17–19].

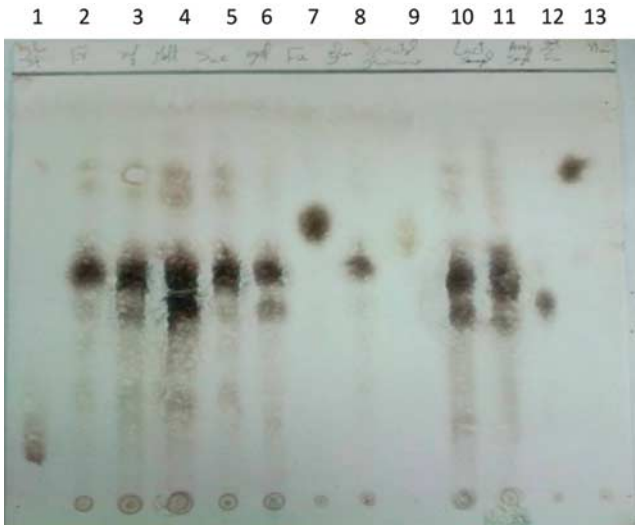
Fourier transform infrared spectroscopic analysis

The configuration of the crude EPS produced from four different sugars has been shown in Fig. 2a–d.The spectra clearly indicate that all the samples had a broad band around 3400 cm^{-1} representing a large number of hydroxyl groups and a sharp band at 2922 cm^{-1} for the C–H bending vibration of the CH_2 groups, and the two bands are characteristic of carbohydrate polymers. The bands near 1736 cm^{-1} and those around 1250 cm^{-1} may be attributed to the stretching vibration of the C = O and C–O–C of the acyl groups. The bands at 1420 cm^{-1} and those around 1606–1621 cm^{-1} have been suggested to represent the carboxyl groups of acids, whereas the bands in the range of 820–955 cm^{-1} represent the linkages between the mono sugars. All the data were within the range that has been reported by other authors [5,6,20–23].

Effect of different carbon sources on the composition of the extracellular polysaccharides

The monosugar composition of the crude EPS produced from the different carbon sources was identified using thin layer chromatography as shown in Fig. 3. The plates indicate the presence of more than six distinguishable spots in most of the samples. However, the relative ratio

Figure 3



Thin layer chromatography plate for the different samples: 1, 7, 8, 9, 12, and 13 for glucuronic acid, fucose, glucose, *N*-acetyl glucosamine, galactose, and rhamnose standards, respectively, and 2, 3, 4, 5, 6, 10, and 11 for fructose, raffinose, maltose, sucrose, glucose, lactose, and arabinose samples, respectively.

of the monosugars was entirely different. All the samples mainly contain glucose, galactose, glucuronic acid, and rhamnose. Although glucose is the main monosugar component of the produced EPS, neither glucose nor

its isomer galactose gave the highest yield of the produced EPS, when used in the culture medium as the carbon source. However, both glucose and galactose gave an appreciable amount of EPS (0.6 and 0.56 g/l, respectively).The influence of the carbon source on the production and composition of the EPS has been reported by other authors as well [5,17,24]. The presence of different sugar moieties suggests that the produced EPS was a heteropolysaccharide.

Conclusion

A new extracellular heteropolysaccharide was produced from a newly discovered source, *Scopularis* spp. The strain has the ability to grow and produce EPS in the presence of all the tested sugars. The produced polysaccharide contains glucose, galactose, glucuronic acid, rhamnose, and two other unidentified sugars. This study will open doors for further studies on attaining a greater production of EPS from this newly discovered source and also for clarifying their exact composition, structures, and biological activities. Moreover, the oligosaccharides and low-molecular-weight polysaccharides that come out of the dialysis bag have to be identified.

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Conflicts of interest

There are no conflicts of interest.

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