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Original Article

Production and characterisation of exopolysaccharide from *Streptomyces carpaticus* isolated from marine sediments in Egypt and its effect on breast and colon cell lines

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ABSTRACT

Twenty streptomycete strains were isolated from marine sediment samples collected from Nabq area, Sharm El-Sheikh, Red Sea Coast, Egypt. Four of them produce exopolysaccharides (EPS) showing marked in vitro antitumor activities. Morphological and cultural characteristics of the most significant strain (No. 3) were shown. Moreover, the sequence of this strain showed similarity with *Streptomyces carpaticus*. The results reveal that EPS produced by *Streptomyces carpaticus* No. 3 had high cytotoxicity reaching 51.7% and 59.1% against human tumor cells of breast and colon lines respectively. A chemical analysis of EPS indicated that the composing monosaccharides were galactouronic acid, glucose, xylose, galactose, mannose, and fructose with relative ratio of 3:1:1:2:2:1 respectively, with an average molecular weight (*Mw*) 1.180×10^5 g/mol and of a number average molecular weight (*Mn*) 1.052×10^5 g/mol. Also the EPS contained uronic acid (0.5072%) and monosaccharide sulphates (21.753%).

1. Introduction

The worldwide attention to cancer as second biggest cause for death in humans has prompted research that thinks about the battle against it. Breast cancer accounts 12% of all new cancer causes and 25% of all cancers in women. Also, colon cancer is the third leading reason for cancer worldwide in human, and fourth in the USA [1]. Most tumor treatments show side effects such as anemia, hair loss, diarrhea, pain, and toxicity. The search for natural anticancer products is needed.

Polysaccharides, which are vital characteristic natural compounds from plants, microorganisms and animal sources are found to show a wide range of biological properties, for instance they are immunostimulant, have anti-inflammatory, antioxidant and antitumor activity [2]. Microbial exopolysaccharides have many uses in numerous fields including food industries, farming and pharmacy because of their different composition, structure, physical and chemical properties [3]. Marine microorganisms regularly deliver secondary metabolites with novel structures and different biological activities [4].

Members of the genus *Streptomyces* are Gram positive bacteria characterized by a complex morphological cell cycle, belonging to the phylum Actinobacteria, which conceal significant hydrolytic enzymes, antibiotics and medicinally important secondary metabolites. About 70% of all known drugs are produced by actinobacteria, of which 75% were used in medicine [5]. In this way, *Streptomyces* are considered as common host organisms for industrial and pharmaceutical purposes.

One of the most important discoveries, with respect to bioactivity, is the anticancer properties of exopolysaccharides isolated from numerous prokaryotes and microalgae [6,7]. Also, some *Streptomyces* synthesize exopolysaccharides with antioxidant activities [8]. The anticancer activity of exopolysaccharides delivered by *Streptomyces* is by all accounts promising, however has not been seriously examined yet.

In the current study, 20 streptomycete strains were isolated from Nabq area and checked for exopolysaccharide production. Among these strains, the most promising one, which produced

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significant amount of exopolysaccharides was subjected to identification. In addition, the radical scavenging activity, the cytotoxic activity against breast and colon cancer cell lines had been investigated.

2. Materials and methods

2.1. Collection of samples and sampling site

The present study is a part of a scientific project concerning the production, characterisation and bioactivity assay of polysaccharides isolated from different marine microbes originating from areas along the Red Sea Coast, Egypt. Sediment samples from the seashore (5 cm depth) of Nabq Nature Reserve area at Sharm El Sheikh were collected in sterile jars and kept in refrigerator till laboratory investigation.

2.2. Isolation of streptomycetes

The serial dilution method of Hayakawa and Nonomura [9] was applied for isolation of streptomycetes. Three agar media were prepared for isolation as follows: starch-nitrate [10], malt yeast extract [11] and brain-heart infusion [12] using 50% sea water. 0.1 ml inoculum of the appropriate dilution was plated on each plate. The plates were incubated at 28 °C for 7–14 days to allow the slow growing forms to develop. Streptomycetes were isolated based on their specific morphological characteristics and then subjected to purification.

2.3. Production of exopolysaccharide (EPS)

Streptomycete strains were grown aerobically for four days in a production medium (containing [g/l]: glucose 30.0, $NaNO_3$ 3.0, yeast extract 5.0, NaCl 4.0, $MgSO_4$ 0.5, K_2HPO_4 1.0, and $CaCO_3$ 1.0) at pH 7, 28 °C and 150 rpm on a rotary shaker [13]. After incubation the cells were harvested by centrifugation at 5000 rpm for 30 min and the soluble exopolysaccharide was precipitated by adding to the supernatant 4 volumes of absolute ethanol and then agitated vigorously and kept at 4 °C overnight [14]. The precipitate was collected by centrifugation at 5000 rpm for 15 min. The resulting precipitate was re-dissolved in distilled water and dialyzed by dialysis tube (MWCO 3000 Da) using running tap water for 48 h and distilled water for another 48 h [15]. The dialyzed solution was lyophilized to obtain the dry exopolysaccharide.

2.4. Analysis of monosaccharide composition

Twenty milligrams of EPS were hydrolysed with 6N HCl at 100 °C in a sealed tube for 5 h and excess acid was evaporated on water bath at 40 °C and co-distilled with water [16]. The content of monosaccharides was quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm × 30 cm); deionized water was used as the mobile phase (flow rate 0.5 ml/min), as described by Kwon and Kim [17]. Uronic acid content was determined by the m-hydroxydiphenyl method using glucuronic acid as standard [18]. The sulphate content of the exopolysaccharide, originating from monosaccharide (galactouronic acid, glucose, xylose, galactose, mannose and fructose) sulphates was measured using the turbidimetric method [19] together with sodium sulphate as standard. N-acetyl glucose amine was estimated by the Elson and Morgan reaction [20] and protein was determined using the Bradford method [21].

2.5. Determination of the EPS molecular weight

Polysaccharide molecular weight was determined on an Agilent 1100 HPLC system equipped with a refractive index detector and FPl gel particle (5 μm) columns. Three columns were used of pore type (100, 104, and 105 Å) in series, (1000, 5,000,000 mwt) for *N,N*-dimethyl formamide (DMF) solvent styrogel high resonance DMF, 3 μm (7.8 × 300 mm) (Waters, Milford, MA, USA). One column (5000–600,000 mwt) was used for water as solvent (polyethylene oxide/glycol standard), OH 7.5 mm and 30 μm pore, 8 um particle size respectively. Sample (0.01 g) was dissolved in 2 ml of solvent and filtrated with 0.45 mm Teflone syringe filter and transferred to a gel-permeation chromatography (GPC) device [22]. The weight average molecular weight (*Mw*) and number average molecular weight (*Mn*) were directly calculated according to the definition of *Mn* and *Mw* using molecular weight and refractive index signal values at each elution volume [23].

2.6. Infrared spectroscopy

The infrared spectrum of EPS was measured on a Bruker Scientific 500-IR Spectrophotometer. The polysaccharide was mixed with KBr powder, ground and pressed into 1 mm pellets for FTIR measurements in the range of 400–4000 cm^{-1} [24].

2.7. Radical scavenging activity of EPS toward DPPH radical

The free radical scavenging activity of EPS was measured against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals using the method of Yang et al. [25]. Five ml of DPPH ethanolic solution (freshly prepared at a concentration of 0.1 mM) was added to 1 ml of EPS solution of different concentrations (50–250 μg/ml) in water. After 30 min incubation under ambient temperature in the dark, absorbance was measured at 517 nm using an UV–Vis Spectrophotometer 2401PC (Shimadzu, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The experiment was carried out in triplicate and averaged. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging ability}(\%) = [(\text{A517of control} - \text{A517of sample})/\text{A517of control}] \times 100.$$

The EC_{50} value is the effective concentration (μg) of EPS at which the DPPH radicals were scavenged by 50%.

2.8. Biological activity of EPS

2.8.1. Cell lines

Cell lines of human breast carcinoma (MCF-7) and colon carcinoma (HCT-116) were obtained from Vacsera (Holding Company for Biological Products and Vaccines) at Giza Governorate, Egypt. Skin normal human cell line (BJ-1) "A telomerase immortalized normal foreskin fibroblast cell line" were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

2.8.2. Cell culture

Culture was maintained in culture medium RPMI with 1% antibiotic-antimycotic mixture (10,000 U/ml potassium penicillin, 10,000 μg/ml streptomycin sulphate and 25 μg/ml amphotericin B), 1% L-glutamine, and supplemented with 10% heat inactivated fetal bovine serum. Culturing and subculturing were carried out according to Thabrew [26]. Doxorubicin was used as a positive

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