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Effect of different parameters on the production of Exopolysaccharides with Antioxidant activity from marine Streptomycetes

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Abstract
Marine bacteria have amazing ways of adapting to harsh environments. One way they do this is by making a wide range of bioactive compounds that have different metabolic and physiological properties. These adaptations ensure their resilience in severe conditions and facilitate their significant contributions to the oceanic ecosystem. Hence, it is anticipated that the bacteria obtained from marine environments will exhibit enhanced efficacy in the synthesis of novel bioactive chemicals. A total of twenty-two streptomycetes isolates were collected from diverse marine samples sourced from Hurghada, Safaga, and Marsa Alam. The isolates were assessed for their capacity to generate exopolysaccharides (EPSs). As a result, isolate (S10) was chosen due to its exceptional productivity, reaching a maximum of 7.6 g/l. So, the antioxidant potential of EPSs was measured by how well they got rid of DPPH free radicals. The highest score achieved at the 120-minute mark was EPSS10, with a mean value of 88.74% ± 1.1%. Conversely, the synthesis and production of EPSs are subject to several circumstances. Hence, the impact of these variables on the formation of exopolysaccharides (EPSs) by the isolate (S10) was observed. The findings indicated that the highest EPS yield recorded was 8.5 g/l, achieved under the following conditions: incubation temperature of 30°C for a duration of six days, rotational speed of 150 rpm, pH level of 7, utilization of peptone as the nitrogen source, and utilization of starch as the carbon source.

Keywords: exopolysaccharides, production, antioxidant, marine, streptomycetes

1. Introduction

Polysaccharides, an essential class of organic molecules derived from plants, microbes, and animals, exhibit a diverse array of biological features. Notably, these compounds include immunostimulant capabilities, as well as demonstrate antioxidant, anti-inflammatory, and anticancer activities [1]. Exopolysaccharides (EPSs) refer to large molecular structures that are secreted by bacteria and can be found either as a firmly attached capsule or a loosely adhered slime layer in the extracellular environment. Microbial exopolysaccharides possess diverse composition, structure, and physical and chemical properties, rendering them valuable in a wide range of disciplines such as food industries, farming, and pharmacy [2]. In recent decades, there has been significant interest in microbial polysaccharides due to their diverse range of biological and pharmacological properties. Microbial EPSs possess many biological features that have been effectively harnessed in numerous industrial sectors, including food, agriculture, cosmetics, and pharmaceuticals [3]. Various studies have demonstrated the utilization of these substances in medicinal contexts, specifically as antioxidants [4], anticancer agents [5], antiviral compounds [6], anticoagulants [7], as well as possessing anti-inflammatory and immune-stimulatory properties [8].

Marine microorganisms frequently produce secondary metabolites that exhibit unique structural characteristics and diverse biological properties [9]. Gram positive bacteria of the genus *Streptomyces* exhibit a complicated morphological cell cycle and are classified under the phylum Actinobacteria. These bacteria possess notable hydrolytic enzymes, antibiotics, and secondary metabolites that hold medical significance. Approximately 70% of the total number of medications currently known to humanity are derived from actinobacteria, with 75% of these compounds having been utilized in the field of medicine [10]. *Streptomyces* is widely recognized as a prevalent host organism for applications in industrial and pharmaceutical sectors. The identification of the anticancer capabilities of exopolysaccharides derived from various prokaryotes and microalgae is considered a significant breakthrough in the field of bioactivity research [11, 12]. Additionally, certain strains of *Streptomyces* bacteria have the capability to produce exopolysaccharides that have antioxidant properties [13].

Scientists often face the challenge of oxidative stress caused by the buildup of reactive oxygen species (ROS) within cellular environments. ROS play a significant role in cellular signaling, maintenance of homeostasis, and the elimination of pathogens. Nevertheless, the build-up of reactive oxygen species (ROS) within cellular structures has been linked to numerous severe ailments, including cancer, autoimmune diseases like arthritis, neurological disorders, and cardiovascular diseases. As a consequence of the pronounced reactivity exhibited by reactive oxygen species (ROSs), they possess the capability to engage in chemical reactions with macromolecules,

including nucleotides, proteins, and lipids. This interaction has the potential to induce alterations in gene sequences, impair protein functionality, and disturb the integrity of cellular or organelle membranes [14, 15].

The primary objective of this work was to generate a bioactive EPSs derived from a strain of Streptomycetes, while also establishing a systematic timetable and identifying said strain. The aims of this study were to analyze the antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and investigate the impact of various factors on the generation of EPS.

2. Methods

1. Sampling and isolation of Streptomycetes

The obtained samples from multiple sites within the Red Sea, specifically including Hurghada, Safaga, and Marsa Alam. The media composition employed for the isolation of streptomycetes comprised the subsequent constituents per liter: 10.0 g starch, 1.0 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 2.0 g KNO_3, 2.0 g $CaCO_3$, 0.01 g $FeSO_4.7H_2O$, and 20.0 g agar. The aforementioned constituents were dissolved in a total volume of 750 mL of seawater, which was subsequently augmented by the addition of 250 mL of distilled water, resulting in a final volume of 1 liter. The pH of the medium was adjusted to a value of 7.0. [16]. The process of selecting and isolating the *streptomyces* isolates was carried out by considering the morphological traits displayed by the colonies.

2. Screening of EPSs production from liquid culture

The *Streptomyces* isolate was inoculated into a production medium at a specified concentration of grams per liter. The experimental medium was prepared by dissolving glucose (10.0 g), tryptone (5.0 g), yeast extract (5.0 g), K_2HPO_4 (3.0 g), NaCl (3.0 g), KH_2PO_4 (1.0 g), $MgSO_4.7H_2O$ (0.5 g), and $CaCO_3$ (0.5 g) in 750 mL of sea water. Subsequently, the capacity was modified to 1 liter by means of distilled water. The pH of the medium was adjusted to a value of 7 [13]**.** The medium was thereafter placed in an incubator set at a temperature of 28°C for a period of 5 days, during which it was agitated at a speed of 120 revolutions per minute. After the completion of the incubation period, the medium underwent centrifugation at a rate of 5000 revolutions per minute for a period of 30 minutes. The supernatant obtained was subsequently mixed with Trichloroacetic acid (TCA) at a concentration of 10% and subjected to overnight incubation at a temperature of 4 degrees Celsius. Following that, the specimen underwent centrifugation at a velocity of 5000 revolutions per minute (rpm) for a period of 20 minutes with the intention of removing protein content. The pH of the supernatant was neutralized to a value of 7 by the addition of a sodium hydroxide (NaOH) solution. The solution containing EPS was recovered and later mixed with four liters of ethanol with a concentration of 95%. Subsequently, the mixture was permitted to incubate for a duration of one night, maintaining a temperature of 4°C. The precipitated EPSs underwent centrifugation at a speed of 5000 revolutions per minute for a period of 20 minutes. Following this, the specimens were once again dissolved in deionized water and subjected to a triple dialysis process, with each dialysis utilizing a 1-liter volume. The dialysis procedure was conducted over a duration of 48 hours utilizing dialysis tubing with a molecular weight cut-off (MWCO) of 2000. In order to assist the purification process and identify the primary fraction, a stepwise addition of 100% cold ethanol was carried out in incremental quantities of 1, 2, 3, and 4. The precipitated EPS obtained was then collected. The majority of the collected EPS, obtained from a single volume, underwent dialysis against distilled water for a period of 72 hours, and was subsequently treated to two rounds of acetone washing. Following this, the sample underwent dehydration with the application of ether and later underwent drying at a temperature of 40°C [17].

3. The evaluation of antioxidant activity

The experimental procedure described by **Brand-Williams *et al*.** [18] was employed to assess the radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Following this, the specimens underwent a duration of light deprivation lasting 10, 30, 60, and 90 minutes at a specific wavelength of 517 nm, utilizing a UV-Visible spectrophotometer. The determination of DPPH scavenging activity was conducted using the subsequent equation:

Scavenging ability (%) =

$$(A_{517\ of\ control} - A_{517\ of\ sample} / A_{517\ of\ control}) \times 100$$

4. EPSs production medium optimization

The Streptomyces isolate exhibits a notable ability to produce EPS in liquid cultures, displaying considerable effectiveness. The solution is comprised of glucose (10.0 g), tryptone (5.0 g), yeast extract (5.0 g), K2HPO4 (3.0 g), NaCl (3.0 g), KH2PO4 (1.0 g), MgSO4.7H2O (0.5 g), and CaCO3 (0.5 g), which have been dissolved in 750 ml of sea water. The ultimate volume is modified to 1 liter by the utilization of distilled water.

Consequently, the study examined the effects of different incubation durations (4,5,6,7, and 8 days), pH levels (5, 6, 7, 8, and 9), temperatures (20, 25, 30, 35, 40, and 45 degrees Celsius), rotational speeds (static, 50, 100, 120, 150, and 200 revolutions per minute), and various carbon sources (glucose, xylose, arabinose, mannose, galactose, fructose, sucrose, lactose, maltose, and sorbose). The carbon sources utilized in the experiment encompassed yeast extract, malt

extract, peptone, beef extract, potassium nitrate, ammonium oxalate, ammonium molybdate, and ammonium sulfate.

3. Results and discussion

1. Screening of Streptomycetes for production of EPSs

Marine microorganisms often synthesize bioactive chemicals that exhibit a wide range of activities and possess unique structural characteristics, which can be attributed to the challenging environmental circumstances they inhabit [19]. The exploration of EPSs derived from marine microbes holds significant importance in the discovery of novel medications [8, 13, 20]. A total of 22 streptomycetes isolates were obtained from samples collected in the Red Sea. Specifically, there were 12 isolates from Hurghada, 6 isolates from Safaga, and 4 isolates from Marsa Alam. In light of this rationale, the aforementioned isolates were subjected to a cultivation period spanning seven consecutive days, with the primary objective of assessing their capacity for exopolysaccharide synthesis. After undergoing separation and drying at a temperature of 80 °C, the cells were subsequently collected into a pellet. The crude (EPSs) were precipitated using ethanol, followed by further washing with acetone and dehydration using ether. **Table 1** displays the dry weight of cells and extracellular polymeric substances (EPS) for a total of 22 marine *streptomyces* isolates. According to the results, the most potent ten isolates were screened for its activity as antioxidant. The DPPH radical-scavenging activity was employed to assess the antioxidant activity of an extracellular polysaccharide (EPS) that was obtained from a marine *Streptomyces* violaceus MM72 [13]. therefore, exopolysaccharide (EPS) was extracted from *Streptomyces virginia* H03. The EPS exhibited inhibitory effects against bacteria responsible for food deterioration and food poisoning. Additionally, the EPS demonstrated antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) [21]. While, *Streptomyces globisporus* BU2018 was successfully identified as a producer of extracellular polymeric substances (EPS) from the region of Sharm El-Sheikh. The isolated strain exhibited a notable EPS production capability, yielding a concentration of 8.5 g/L [22].

Table (1) Cell dry weight, EPS of marine streptomycetes isolates

Isolate no.		cell dry wt. (g/L)	EPS dry wt. (g/L)
Hurghada	S1	2.3	3.8
	S2	2.6	0.9
	S3	4.1	8.3
	S4	6.2	3.7
	S5	5.3	5.8
	S6	3.8	2.0
	S7	2.9	3.1
	S8	7.3	4.7
	S9	6.7	5.9
	S10	6.1	7.6
	S11	4.8	6.5
	S12	5.2	3.0
Safaga	S13	7.5	6.8
	S14	5.4	5.7
	S15	3.8	2.5
	S16	4.3	5.4
	S17	5.8	3.6
	S18	2.8	3.1
Marsa alam	S19	5.9	2.1
	S20	3.8	1.9
	S21	7.0	5.4
	S22	6.9	9.2

2. Analysis of free radical-scavenging properties of DPPH

The significance of polysaccharides in mitigating oxidative damage in living organisms is of paramount importance, as they function as scavengers of free radicals and exhibit antioxidant properties [23]. The antioxidant characteristics of several very effective EPSs were evaluated. The EPSS10 sample exhibited the maximum antioxidant activity (88.74±1.1) after a duration of 90 minutes, as seen in **Table 2**. In contrast, three EPS (EPSS 3, EPSS 11, and EPSS 16) exhibited negligible antioxidant-scavenging capabilities.

Table (2) DPPH free radical scavenging activity (%) for marine streptomycetes EPS at different time.

Isolate no.	DPPH free radical scavenging activity (%)			
	10 min	30 min	60 min	90 min
EPSS3	0.0	0.0	0.0	0.0
EPSS4	20.90±1.1	38.05±1.1	43.15±0.9	52.05±1.2
EPSS9	13.65±1.3	25.11±0.9	37.42±1.3	48.60±1.1
EPSS10	60.16±1.5	72.81±1.4	81.96±0.9	88.74±1.1
EPSS11	0.0	0.0	0.0	0.0
EPSS13	32.84±1.0	39.62±1.2	48.84±1.2	54.10±1.2
EPSS14	42.53±0.9	53.08±1.1	60.13±1.1	63.51±1.3
EPSS16	0.0	0.0	0.0	0.0
EPSS21	0.0	5.04±0.9	10.95±1.1	13.57±1.3
EPSS22	31.55±1.1	38.52±1.1	46.71±1.2	51.12±1.1

3. Effect of different parameter on the EPSS10 production

The cultivation of a specific bacterial strain in a controlled laboratory setting allows for the investigation of extracellular polymeric substance (EPS) formation in the marine ecosystem. This approach is necessary due to the limited occurrence of these polymers in their native marine habitat, which poses challenges in analyzing the composition of the extracellular matrix [24]**.** Due to significant variations among microorganisms in terms of their capacity to metabolize carbon and nitrogen sources, their mineral necessities, temperature preferences, and optimal pH levels, it is not possible to establish a universally applicable set of growth conditions that ensure consistently high outputs of extracellular polymeric substances (EPS). Utilizing a physiological control, it is also possible to modify the molecular weight, number of residues, and degree of branching in EPS. Indeed, it is accurate to assert that the synthesis and quality of microbial extracellular polymeric substances (EPS) can be influenced by nutritional composition and environmental circumstances, including culture conditions [25].

The strain ESS10 exhibited a peak rate of EPS production at 7.7 g/l when the cell dry weight reached 6.2 g/l, under the condition of incubating the fermentation medium at a temperature of 35 °C, as documented in prior research. The generation of EPS is diminished by any fluctuation in temperature (**Figure 1**). Following a six-day incubation period, the strain EPSS10 achieved its peak production of extracellular polymeric substances (EPS) at a concentration of 7.8 grams per liter, with a corresponding cell dry weight of 6.5 grams per liter. Based on the results obtained, it can be observed that the correlation between the production of extracellular polymeric substances (EPS) and the duration of incubation is subject to the influence of the organism's classification (**Figure 2**). The RPM has an impact on the EPS output, as depicted in **Figure 3**. As a result, the dry weight of the cells reached 6.5 g/l when the rotational speed was set at 150 RPM, and the highest achievable yield was 8.0 g/l. A decrease in exopolysaccharide (EPS) synthesis is observed when there is a variation in the size of the inoculum. The EPSS10 factor exerts an influence on the production of extracellular polymeric substances (EPSs) at the cellular scale. As a result, under pH 7 conditions, the highest yield observed was 7.8 g/l, while the cell dry weight remained at 6.3 g/l, irrespective of any variations in extracellular polymeric substance (EPS) formation in the medium (**Figure 4**). According to the findings of [26], multiple factors were employed to investigate the production of extracellular polymeric substances (EPS) by *Pseudovibrio* strain 4MS 2020. The study revealed that the greatest EPS concentration recorded was 10.8 g/l, accompanied by a cell dry weight of 3.8 g/l. These optimal conditions were achieved at a temperature of 40 °C, pH level of 7, rotational speed of 150 RPM, utilization of yeast extract as a nitrogen source, and the presence of sucrose (20 g/l) as a carbon source. The introduction of both organic and inorganic nitrogen sources into the medium resulted in a substantial increase in the synthesis of extracellular polymeric substances (EPS). Among the nitrogen sources investigated it was observed that peptone facilitated the most substantial formation of extracellular polymeric substances (EPS), up to 8.2 g/l. Additionally, peptone also resulted in the largest cell dry weight, reaching 6.7 g/l. **Figure 5** illustrates the variability in EPS production seen among the various nitrogen sources investigated. The introduction of various carbon sources into the medium at a concentration of 1% led to a notable

augmentation in the production of extracellular polymeric substances (EPSs). In addition to starch, all the remaining carbon sources demonstrated substantial production of EPS, with a recorded quantity of 8.5 g/l, as depicted in **Figure 6**. In a recent study conducted by Abd El Nasser et al. [27], a total of 12 bacterial strains were isolated from various maritime settings. The generation of EPS was assessed using the aforementioned isolates. The maximum EPS yield recorded was 8.2 g/l, while the cell dry weight reached 4.5 g/l after a three-day incubation period at a temperature of 40°C. The RPM was set at 120, the pH was maintained at 7, peptone was utilized as the nitrogen source, and sucrose (20 g/l) served as the carbon source for the bacterial isolate. In a study conducted by **Abdel-Monem *et al.*** [28], a total of 10 bacterial isolates were obtained from diverse maritime samples. The bacterial isolate BS6 was chosen based on its high productivity, which achieved a maximum yield of 5.9 g/l. Hence, the investigation into the impact of different variables on the production of exopolysaccharides (EPSs) by the isolate (BS6) demonstrated that the highest EPS yield recorded was 7.2 g/l, with a corresponding cell dry weight of 3.9 g/l, achieved under the following conditions: incubation temperature of 40 ºC over a duration of three days, rotational speed of 120 RPM, pH level of 7, utilization of peptone as the nitrogen source, and sucrose as the carbon source.

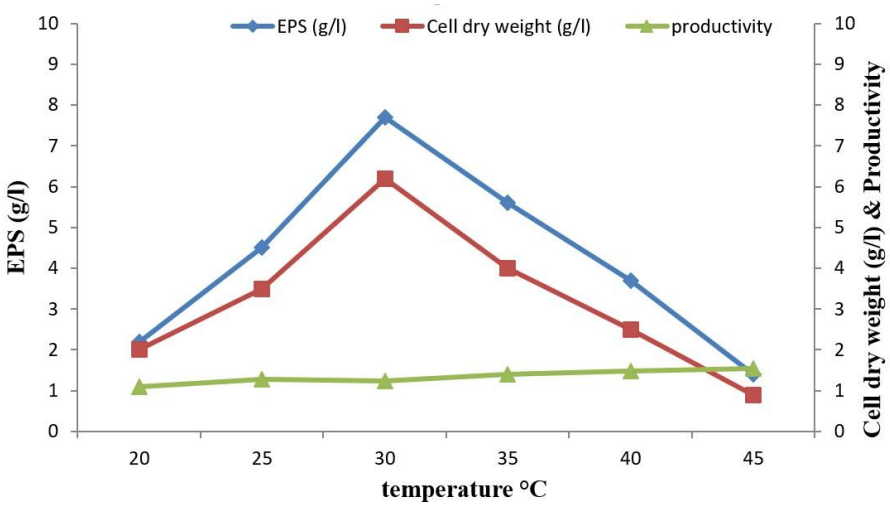

Fig. (1) Effect of different temperature on production of EPSS 10

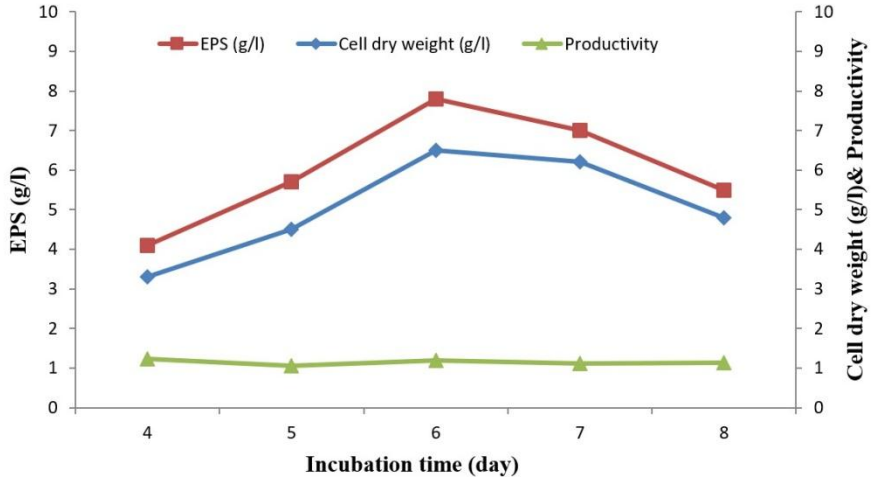

Fig. (2) Effect of different incubation time on production of EPSS 10

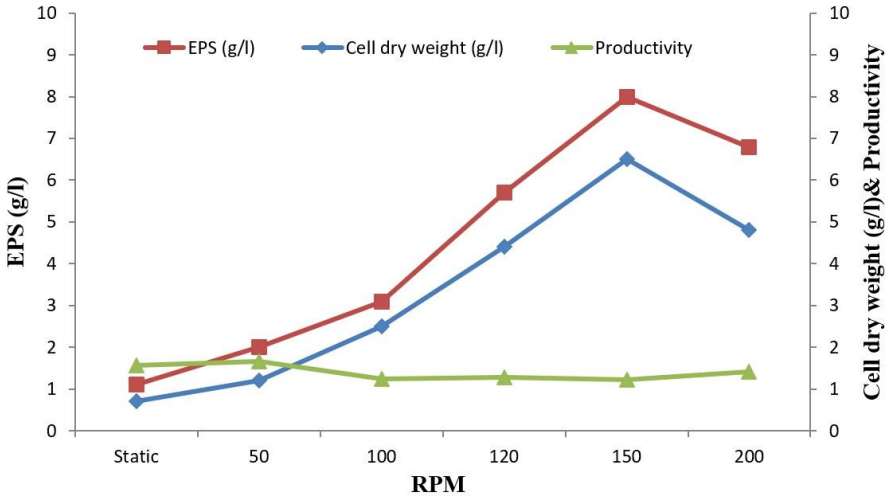

Fig. (3) Effect of different RPM on production of EPSS 10

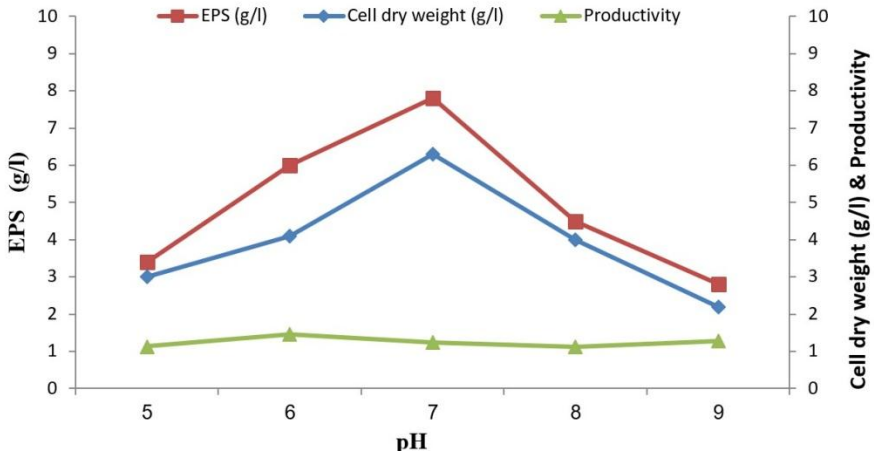

Fig. (4) Effect of different pH on production of EPSS 10

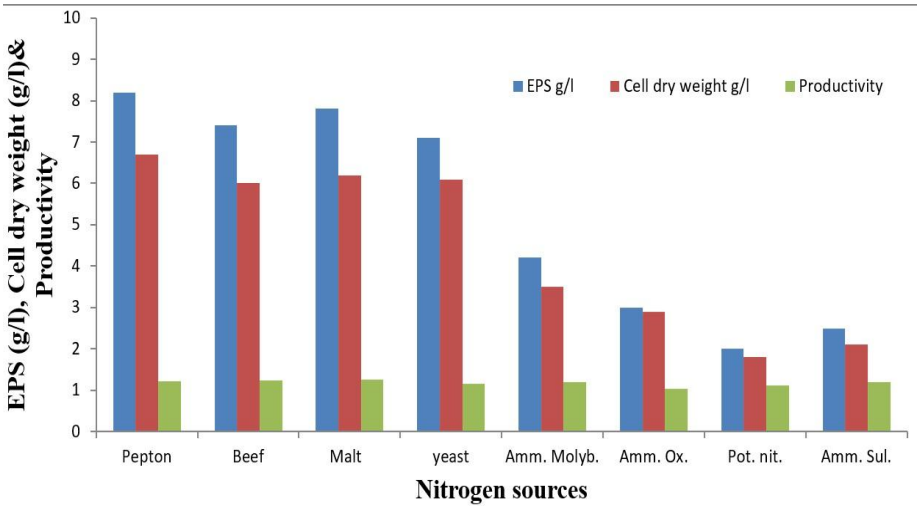

Fig. (5) Effect of different nitrogen sources on production of EPSS 10

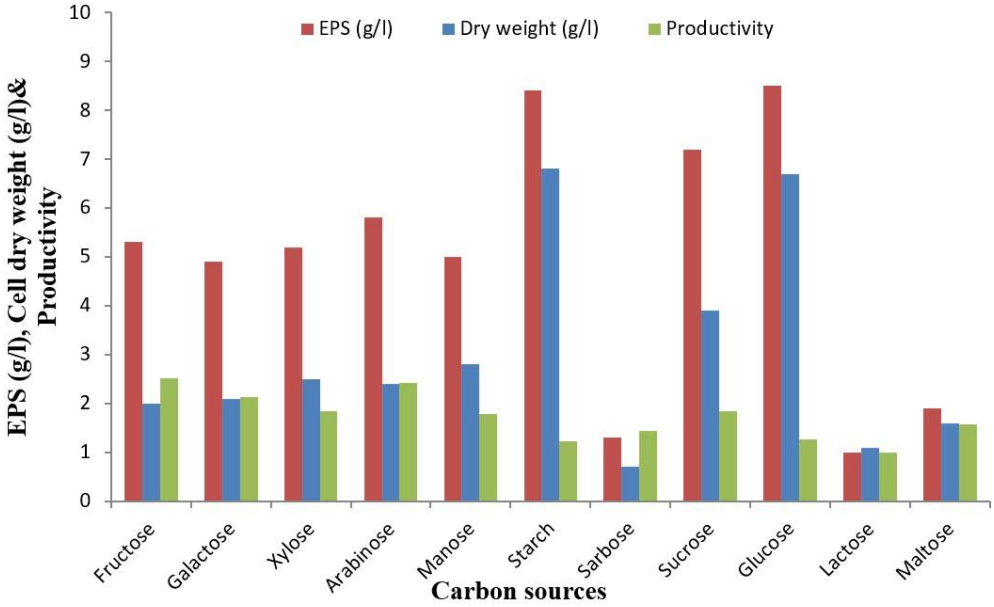

Fig. (6) Effect of different carbon sources on production of EPSS 10

4. Conclusion

Twenty-two streptomycetes isolates were collected from diverse marine samples and assessed for their ability to produce EPS. As a result, isolate (S10) maximum production 7.6 g/l. So, EPSS10 had high the antioxidant activity (88.74% ± 1.1%). Enhancement pf the production of EPS by the isolate (S10) was observed by using different parameter found that the highest EPS yield was 8.5 g/l by incubation temperature of 30°C for a duration of six days, 150 RPM, pH 7, utilization of peptone as the nitrogen source, and utilization of starch as the carbon source.

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