MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Production of exo-polysaccharides by different microorganisms

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Received:20/10/2021
Accepted:5/3/2021

Abstract: Microorganisms are used in several industrial processes, such as the production of exopolysaccharides. The importance of exo-polysaccharides is increasing especially microbial enzymes which are of indefinite industrial purposes. Liquid state fermentation is an economical alternative for the large scale production of enzymes which produced by microorganisms. Six fungal species and eight bacterial genera were screened for the ability to produce exo-polysccarides. Maximum production was recorded by *B. amyloliquefacien*. (98.44%) followed by *Escherichia coli* (35.88%) and *Aspergillus flavus* (30.11%). However, low values for exo-polysaccharides production were recorded by *Trichoderma viridi* (1.77%) and *Cladosporium* sp. (1.08 %). The results also showed that the production of exo-polysaccharides of each individual was always correlated with the density on the isolation plates and their frequencies. Based on the above results, *B. amyloliquefacien* was selected as the highest producer of exo-polysaccharides.

keywords: Exo-polysaccharides, Fungi, Enzyme production, Culture optimization of production,.

1.Introduction

Microorganisms can produce both intracellular in addition to extracellular enzymes in addition to exo-polysaccharides; the extracellular polysaccharides are easier to be extracted [1].

Exo-polysaccharide (EPS) biopolymers produced by microorganisms play a crucial role in the environment such as health and bio-nanotechnology sectors, gelling agents in food and cosmetic industries in addition to bio-flocculants in the environmental sector as they are degradable, nontoxic. Exo- polysaccharides are high molecular weight polymers composed of saccharides subunits and are secreted by a microorganism into the surrounding environment [2].

It is now recognized that the microorganisms living in extreme environments have different genetic background and metabolic pathway when compare to general microbiology and their second-day metabolites have special function [3]. Among the secondary metabolites from extreme environments, polysaccharide for biotechnological appli-cations has been reported in various literature [4]. Exopolysaccharides (EPS) produced by microorganisms in BSCs are barely reported in recent research. The present study aims to isolate and identify some microorganisms from different soil samples producing exopolysaccharides and the study was prolonged to select the most active isolate produced exopolysaccharides.

2.Materials and methods

Sampling

Ten soil samples were used for the isolation of exopolysaccharides producing microorganism. The soil samples were collected from different crop fields at different locations of El-Dakahlia governorate in Egypt.

Chemicals

Nutrient agar medium for isolation of Bacteria.

Potato Dextrose Agar medium for isolation of fungi with supplemented by an antibacterial agent. All chemical used were obtained from EL NASER Company Pharmaceutical and Chemicals Company, Egypt.

Isolation of lipase producing fungi

By using the soil dilution method [5], fungi and bacteria were isolated from soil samples collected from different localities of El-

Dakahlia governorate. One gram of each air dried samples was suspended in a sterilized test tube containing 9 ml of sterile water. The mixture was mechanically shaken for 10 min. Then, one ml of the soil solution was aseptically transferred into another test tube containing 9 ml sterilized water until the desired final dilution was reached. Also, one ml of each the desired dilution was transferred carefully to clean and sterilized petri-dishes (three plates for each dilution). Nutrient agar for bacteria and potato dextrose agar medium for fungi was poured through each dish. All dishes were rotated by hand to ensure the equal dispersion of the soil.

After the solidification of the medium, the plates were incubated at 30C for 10 days (for fungi) and 37C for 48 h. for bacteria and examined daily. All colonies of fungi and bacteria on each plate of the suitable dilution were counted and the percentage frequency was calculated. The isolated were purified by streaking several times on other plates containing suitable medium. The purified isolated strain were stored in medium until identification

Extraction of EPS

The bacterial metabolites in culture media was centrifuged at 5000 rpm for 10 min to remove bacterial cells. Trichloroacetic acid (5%) was added and left overnight at 4ºC and centrifuged at 5000 rpm again. The pH of the clear supernatant was adjusted at 7.0 with NaOH solution and dialyzed three times against flowing distilled water using a dialysis tube. The supernatant was completed up to four volumes with ethanol 95% and left overnight at 4°C. The participated exopolysaccharides were separated by centrifugation at 5000 rpm, washed with acetone and dried at 50°C to get the crude EPS according to the method of El-Newary [6].

Identification of fungi

The developed fungal colonies were examined daily and, according to the following references, the purified fungi were morphologically classified at the species level whenever possible:

3. Results and Discussion

Isolation of microorganisms producing exopolysaccharides

As indicated in Table (1), the highest record of total fungal and bacterial populations (550 and 33930 colonies respectively per g dry soil) was found in sample No. 10 (Mit-anter site, Dakahlia district), followed by sample No. 4 (350 and 24500 colonies per g dry soil) and the smallest count was observed in sample No. 2 (30 and 730 colonies per g dry soil). This was due to deep fluctuations in response to alternation in condition of the atmosphere, chemical and physical analysis of the soil)

Table (1). Total count of bacterial and fungal colonies (T.C) and Locality of 10 soil sample tested

Polluted soil sample No.	Total count (Colony / g dry soil)		locality
	bacterial	Fungi	
1	9660	150	Sherbien
2	750	30	Gamassa
3	2100	80	Elsenblawein
4	24500	350	El-tawila
5	12080	254	Aga
6	12310	395	Duast
7	11580	410	Mansoura
8	2985	80	Talkha
9	21900	370	Kafr- Elhatba
10	33930	550	Mit-anter

Physiochemical properties of the soil samples collected from Dakahlia governorate

The collected soils had different Physicochemical properties; the pH values ranged between 7.32 and 8.49, soil texture varied between sandy and clay and the organic matter (OM) content ranged between 6.27 (Mit-anter) and 0.17 % (Mansoura). The soil salinity appeared to be a limiting factor for the occurrence of isolated fungi (Table 2). Therefore, Gamassa site was found inhabiting a low number of the fungal and bacterial total count when compared with other samples

Identification Exo-polysaccharides producing fungi

Fungal isolates were identified and arranged according to their taxonomical positions as shown in Table (3). All fungal species isolated during this study were belonging to two classes Ascomycetes and Hyphomycetes. Genus *Fusarium* was represented by three species namely: F. oxysporium, F. solani and F. *equiseta*. Furthermore, the genus Aspergillus was represented by two genera likely *A. flavus*

and *A. terreus*. On the other hand, the genus *Penicillium*, *Cladosporium* and *Trichoderma* were represented only by one species.

Table (2): Physiochemical properties of the soil samples collected from different Egyptian sites

Sample No.	Locality	Soil Texture	pH	EC(µS.cm)	OM (%)	Total count (colony g^{-1} dry soil)	
						Bacteria	Fungi
1	Sherbien	Clay	8.25	320	3.20	9660	150
2	Gamassa	Sandy	7.78	149	4.58	750	30
3	Elsenblawein	Sandy	7.88	110	2.99	2100	80
4	El-tawila	Clay	7.90	320	2.24	24500	350
5	Aga	Clay	7.95	350	5.11	12080	254
6	Duasat	Clay	7.33	658	2.24	12310	395
7	Mansoura	Sandy	7.32	1870	0.17	11580	410
8	Talkha	Sandy	8.49	2530	6.27	2985	80
9	Kafr- Elhatba	Sandy	7.11	167	0.59	21900	370
10	Mit-anter	Clay	7.54	2820	0.27	33930	550

Table (3) List of fungal species isolated producing exo- polysaccharides

I. **AscomyceteGenus: *Aspergillus***
1- *A. flavus* Teigh
2- *A. terreus* Thom Genus *Penicellium*
3- *P.notatum* Thom
II. **Hyphomycetes (Duetromycetes)**
Genus: *Trichoderma*
4- *T. viridi* PersGenus: *Fusarium*
5- *F. oxysporium* Schledht
6- *F. solani* Mart
7- *F. equiseti* CordaGenus: *Cladosporium*
8- *C. arta* Thom

Table (4) List of bacterial species producing exo- polysaccharidesGenus Bacillus

1. ***Bacillus* sp**
2. ***B. amyloliquefacien***
3. ***Pseudomanes* sp.**
4. ***Escherichia coli***
5. ***Proteus* sp.**
6. ***Salmonela* sp.**

Identification of exo-polysaccharides bacteria

All bacterial isolates isolated during this study were recorded in **Table (4).** Five genera were identified as *Bacillus*, *Pseudomonas*, *Escherichia*, *Proteus* and *Salmonela*. In this connection, genus *Bacillus* was represented by two species namely *B. amyloliquefacien* and *Bacillus* sp. *B. amyloliquefacien* was the highest count during this survey as compared to other species.

Frequency of occurrence of microbial isolates

Total counts and frequency of occurrence of identified fungi and bacteria are shown in **Table (5)**. *Bacillus amyloliqrefacien* was the most frequent species recorded during this survey as compared with other isolated species. While *Salmonela* was the lowest frequent species recorded through this survey and it represented by one occurrence. H= high is isolated more than 6 times out of 10 samples

M=Moderate, from 5 to 6 times out of 10 samples

L= Low, from 3 to 4 times out of 10 samples

R= Rare less than 3 times out of 10 samples

Screening of microorganisms for Producing exo-poysaccharides

Eight fungal species and six bacterial genera were screened for the ability to producing exo-polysaccharides as shown in **Table (5)**. Maximum production of exo polysaccharides recorded by *B. amyloliquefacien*. (98.88%) followed by *Escherichia coli* (35.88%) and *Aspergillus flavus* (25.11%). However, low values for production of exo polysaccharides were recorded by *Trichoderma viridi* (1.77%) and *Cladosporium arta* (1.08 %). The results also showed that the phenol degradation of each individual was always correlated with the density on the isolation plates and their frequencies. Based on the above results, *B. amyloliquefacien* was selected the highest producer of exo=polysaccharides

Table (5). Frequency of occurrence of microbial isolates from 10 sources of soil samples.

Microbial Species	No. of isolates out of 10 samples	Occurrence(%)	Occurrenceremarks
Fungal isolates			
Aspergillus flavus	2	20	R
Aspergillus terreus	5	50	M
Penicillium notatum	4	40	M
Trichoderma viridi	8	80	H
Fusarium oxysporum	2	20	L
Fusarium solani	5	50	M
Fusarium equiseti	6	60	M
Cladosporium arta	8	80	H
Bacterial isolates			
Bacillus sp**.**	5	50	M
B. amyloliquefacien	10	100	H
Pseudomanes sp	5	50	H
Escherichia coli	4	40	M
Proteus sp.	4	40	L
Salomenla sp.	1	10	R

Table 6 Percentage of exopolysaccharides production by isolated fungal and bacterial isolates

Fungal Species	Exopolysaccharides(%)
Aspergillus flavus	30.11
Aspergillus terreus	8.42
Penicillium notatum	7.44
Trichoderma viridi	1.77
Fusarium oxysporum	1.88
Fusarium solani	11.66
Cladosporium arta	1.08
Bacillus sp.	20.41
B. amyloliquefacien	98.44
Pseudomanes sp	75.01
Escherichia coli	35.88
Proteus sp.	15.02
Salmonella sp.	45.4

Discussions

Exo-polysaccharides are great molecular weight polymers composed of saccharides subunits and are secreted by a microorganism into the surrounding location [7]. Microorganisms like fungi and bacteria produce large spectrum multifunctional polysaccharides including intracellular polysaccharides and extracellular polysaccharides [8]. Exo-polysaccharides generally consist of monosaccharides and some non-carbohydrate substituents (such as protein, nucleic acids, lipids, acetate, pyruvate, succinate, and phosphate). Microbial EPS plays an important task in interaction between bacteria and their environment [9].

In this study, eight fungal species and six bacterial genera were screened for the ability to produce exo-polysaccharides. Maximum exo-polysaccharides production was recorded by *B. amyloliquefacien* (98.44%) followed by *Escherichia coli* (32.88%) and *Aspergillus flavus* (30.11%). However, low values for polysaccharides production were recorded by *Fusarium oxysporum* (1.88%) and *Cladosporium arta* (1.08 %). In this connection, the results also showed that the polysaccharides production of each individual was always correlated with the density on the isolation plates and their frequencies. Vijayabaskar et al. [10] reported that the maximum level of exo-polysaccharides production was achieved by *Bacillus subtilis* (MTCC 121) in basal extract medium. The usage stretch of nucleic acid and C-O extending modes of EPS complexes in the food industry has been sugar- phosphate [11].

B. amyloliquefacien evacuated significant maximum quantities of EPS, when cultivation occurred under optimum growth conditions. The greatest quantity of bacterial growth where glucose was utilized as carbon source in the exo-polysaccharides basal medium. These results were confirmed by Khopade et al. [14] who concluded that the production of microbial exo-polysaccharides is highly affected by nutritional and environmental conditions.

Conclusion

Among all fungal and bacterial strains that were isolated during this study, *B. amyloliquefacien* was the most frequent species recorded during this survey. In this connection,

B. amyloliquefaciens showed a potent isolate for exo-polysaccharide production

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