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OPTIMIZATION OF POLYSACCHARIDES PRODUCTION BY BACTERIA ISOLATED FROM SOIL

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ABSTRACT

Six polysaccharide-producing bacteria, isolated from soil samples and identified as *Pseudomonas* and *Arthrobacter* (Strains 3B, 4B, 7B, 21B, 18E and 21D), were tested for the yield of polysaccharides produced during growth in two culture media: one containing glucose and the other sucrose (1, 2, 3, 4 and 5%). The yield was quantified measuring the viscosity of the broth, using the Poiscuille equation. The effect of temperature and pH of the culture media was investigated. The largest polysaccharide yield was obtained when the concentration of the carbon source was lower than 2%. Glucose and sucrose stimulated the polysaccharide production in a similar way. When the initial pH of the fermentation broth was increased from 5.0 up to 7.0, there was an increase in polysaccharide production. However, higher values (pH=8.0) caused a decrease in polysaccharide production. With regard to temperature, 30°C was shown to be optimum, since higher or lower temperatures had a negative effect on saccharide production.

Key words: bacteria, soil, polysaccharide production, viscosity

INTRODUCTION

In recent years there has been a continuous search for new water-soluble polysaccharides, particularly those produced by microorganisms. The environment contains a great variety of microorganisms, and in consequence it is expected that new productive microorganisms can be isolated. These biopolymers have been attracting interest due to their great application potential in food, cosmetic, pharmaceutical and oil industries, where they are used as thickening, stabilizing and emulsifying agents. Therefore, the identification of new production sources for these materials is of great interest, specially when such biopolymers possess good rheological properties (4).

Microbial exopolysaccharides are traditionally good substitutes of gums from plants and marine algae. Their physical and chemical characteristics show little variability and they are not vulnerable to variations in climatic, cultivation, production or pollution conditions (6). Besides, gums of microbial origin are susceptible to natural biodegradation, promoting little damage to the environment and diminishing pollution. For this reason, some bacterial polysaccharides are produced on an industrial scale and used as raw material for processed foods, in medicine and in industrial preparations (3).

The present work searched for new polysaccharide-producing microorganisms in soil. After isolation and identification of these microorganisms the optimal production conditions were determined. The effect of pH and temperature on growth was also investigated.

MATERIALS AND METHODS

Organism and growth conditions

The bacteria were isolated from soil samples and identified to genus level by means of biochemical tests. The bacteria were

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inoculated in slants of PCA (Plate Count Agar), incubated for 24 h at 30ºC, and then suspended in 10.0 mL sterile distilled water. The cell suspension was transferred to 250 mL Erlenmeyer flasks containing 50 mL of the fermentation medium suggested by Souw and Demain (7), and incubated in a shaker at 30ºC and 210 rpm for 96 h. The basal medium for fermentation consisted of (g/L): KH_2PO_4, 5.0; $MgSO_4.7H_2O$, 0.2; $(NH_4)_2SO_4$, 2.0; Citric acid, 2.0; H_3BO_3, 0.006; ZnO, 0.006; $FeCl_3.6H_2O$, 0.0024; $CaCO_3$, 0.02 and HCl, 0.15. The pH was adjusted to 7.0 with NaOH or HCl (before sterilization). The polysaccharide production medium was obtained by the addition of sterilized solutions of glucose or sucrose, in concentrations of 1.0; 2.0; 3.0; 4.0 and 5.0%. The different formulations of the production medium were inoculated with centrifuged cells (8000rpm for 30 min), previously incubated in Souw and Demain medium (7), to achieve a final optical density of 0.05 at 650 nm. The flasks were incubated at 30ºC, for 72 h at 210 rpm. The polysaccharides produced were separated from the fermentation broth by precipitation with three volumes of ethanol, and removal of the cells by centrifugation at 8000 rpm for 30 min. The solution was concentrated by vacuum evaporation and the gum obtained was vacuum-dried in a oven at 45ºC until constant weight. The yield was calculated as grams of polysaccharide produced per 100 grams carbon source consumed. Growth was determined measuring the dry weight of the centrifuged cells, placed in Petri plates and vacuum-dried in a oven at 45ºC to constant weight. In order to evaluate the influence of pH, the pH of production medium was adjusted 5, 6, 7 and 8, using NaOH 1N. The effect of the temperature was observed at 25, 30 and 35ºC. The cellular growth kinetics was determined using a spectrophotometric method at 650 nm.

Determination of the fermentation broth viscosity

The volumetric flow was calculated measuring the time that 4mL of the fermentation broth took to flow through a glass capillary tube (0.8 cm diameter and 25 cm length) at 25ºC (equation 1). The viscosity was calculated by means of the Poiseuille equation (equation 2) (9).

$$\forall = \frac{v}{t} \quad \text{(equation 1)}$$

$\forall$ = volumetric flow (cm^3/s)
v = volume (cm^3)
t = time (s)

$$\forall = \frac{\pi \Delta P r^4}{8 L \mu} \quad \text{(equation 2)}$$

L = length (cm)
r = radius (cm)
P = pressure (Pa)
$\forall$ = volumetric flow(cm^3/s)
μ = specific viscosity (Pa.s)

Statistical design

Results were compared by means of Tukey´s test, at a level of significance of 1%, using the Estat program, version 2.0.

RESULTS AND DISCUSSION

Several polysaccharide producing bacteria were isolated from soil of Northwest São Paulo Ecological Station, in the State of São Paulo, Brazil. Six strains (3B, 4B, 7B, 21B, 18E and 21D), belonging to *Pseudomonas* and *Arthrobacter* genera, were selected for this study. The steps for their identification are shown in Fig. 1.

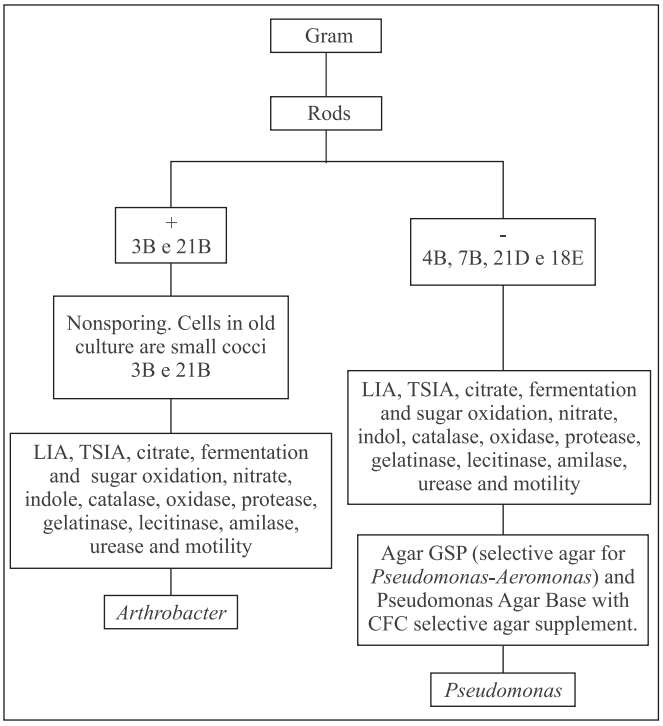

Figure 1. Steps of identification of bacteria isolated from soil.

The biochemical tests employed were LIA (Lysin-Iron-Agar), TSIA (Triple-Sugar-Iron-Agar), citrate, fermentation and oxidation of carbon sources (sucrose, lactose, glucose, maltose and manitol), nitrate, indole, catalase, protease, lecithinase, amylase, gelatinase, oxidase, urease and motility. The specific tests for *Pseudomonas* were growth in Agar GSP (Merck) and *Pseudomonas* Agar Base in the presence of CFC selective agar supplement (Oxoid).

As shown in Fig. 2, broths containing strains 3B, 4B, 7B and 18E presented maximum viscosity after 72 h cultivation. Strains 21B and 18E achieved maximum viscosity after 48 h. The high viscosity presented by strain 4B remained practically constant up to 72 h. In general, after 72 h incubation the viscosity decreased for all studied bacteria (Fig. 2).

The growth kinetics (Fig. 3), carried out to determine the relationship between cellular growth and polysaccharide

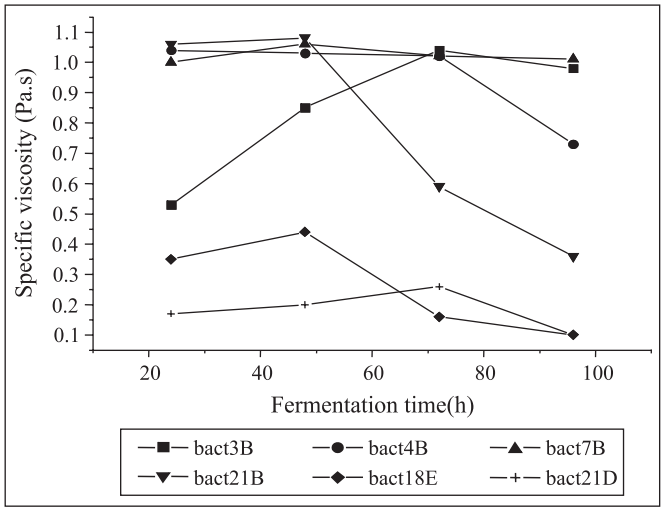

Figure 2. Viscosity of the culture media during growth of polysaccharide-producing bacteria, up to 96 h at 30ºC and 210 rpm.

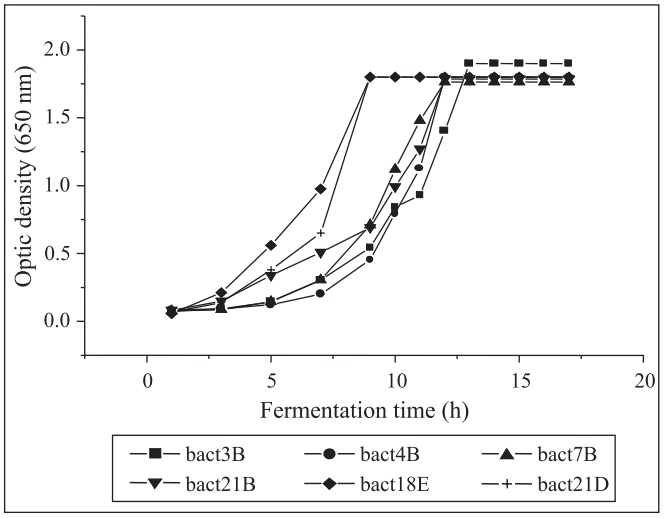

Figure 3. Growth of polysaccharide-producing bacteria determined by spectrophotometric readings (Cultivation conditions: 30ºC and 210 rpm).

production, shows that strains 3B, 4B, 7B and 21B achieved maximum biomass concentration in 12 h, while strains 18E and 21D reached the stationary growth phase in 8-9 h. Previous studies with xanthan gum have shown that the production of the gum occurs after the logarithmic growth phase, i.e. in the stationary phase (5).

Many bacterial polysaccharides are produced in batch fermentation processes using different sugars as carbon sources (1,8), but glucose has been used as the traditional carbon source (2,5,6,10). The productivity can be affected by culture conditions, such as composition of the culture medium, temperature, pH and aeration efficiency (3).

In this work we also studied sucrose, because it is cheaper and more abundant in the national market. The results are shown in Figs. 4, 5 and 6. In Fig. 4, it can be observed that polysaccharide yield decreases when the concentration of carbon sources increases. This behavior has been observed by several researchers (5,7,10) during polysaccharide production using the most varied types of bacteria.

The glucose concentrations that resulted in the largest polysaccharide varied between 1% and 2%. For strains 3B, 18E and 21D, the larger yields were achieved at 1% glucose, and decreased drastically beyond this value.

The sucrose concentration that led to the largest yield was 2%, except for strains 21B and 18E, which produced the largest yield at 1%. The strain 18E presented the same behavior for the two carbon sources (Fig. 4). The variance analysis by Tukey´s test indicated significant differences at a level of 1% for the carbon source concentrations studied.

With regards to the carbon source tested, the strains provided similar results for glucose and sucrose. The same behavior was observed by Souw and Demain (7) during the production of xanthan gum by *Xanthomonas campestris*. Therefore, for polysaccharide production, glucose can be substituted by sucrose, presenting the advantage of being cheaper.

Table 1 shows the effect of glucose and sucrose concentrations on cellular growth. It can be seen that results varied according to the strain and that growth was affected by the type and concentration of the carbon source. In general, as glucose or sucrose concentration increased, growth decreased.

Table 1. Effect of concentration of glucose and sucrose on growth of polysaccharide producing bacteria.

Strain	3B	4B	7B	21B	21D	18E
%	Dry Cellular Weight (g)					
Glucose						
1.0	0.7658	0.5275	0.5015	0.5935	0.6342	0.6234
2.0	0.7439	0.5291	0.4919	0.5853	0.6221	0.6220
3.0	0.6850	0.5255	0.4912	0.5681	0.6204	0.6215
4.0	0.6758	0.5284	0.4900	0.5666	0.6015	0.6210
5.0	0.6248	0.5192	0.4860	0.5565	0.6045	0.6205
Sucrose						
1.0	0.6072	0.5331	0.4956	0.5893	0.5362	0.6435
2.0	0.5284	0.5321	0.4990	0.5653	0.5284	0.6390
3.0	0.5261	0.5364	0.4982	0.5546	0.5261	0.6394
4.0	0.5204	0.5320	0.4972	0.5607	0.5104	0.6253
5.0	0.5255	0.5307	0.4575	0.5660	0.5155	0.6224

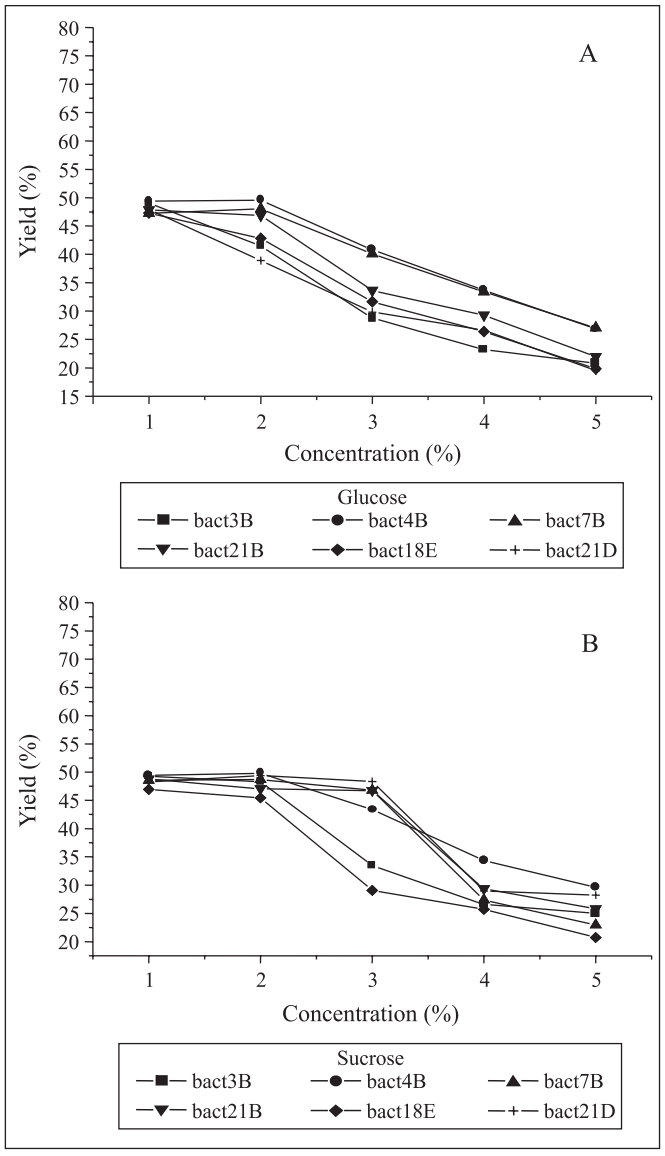

Figure 4. Polysaccharide yields of bacteria isolated from soil, as a function of the carbon source concentration (A=Glucose, B= Sucrose).

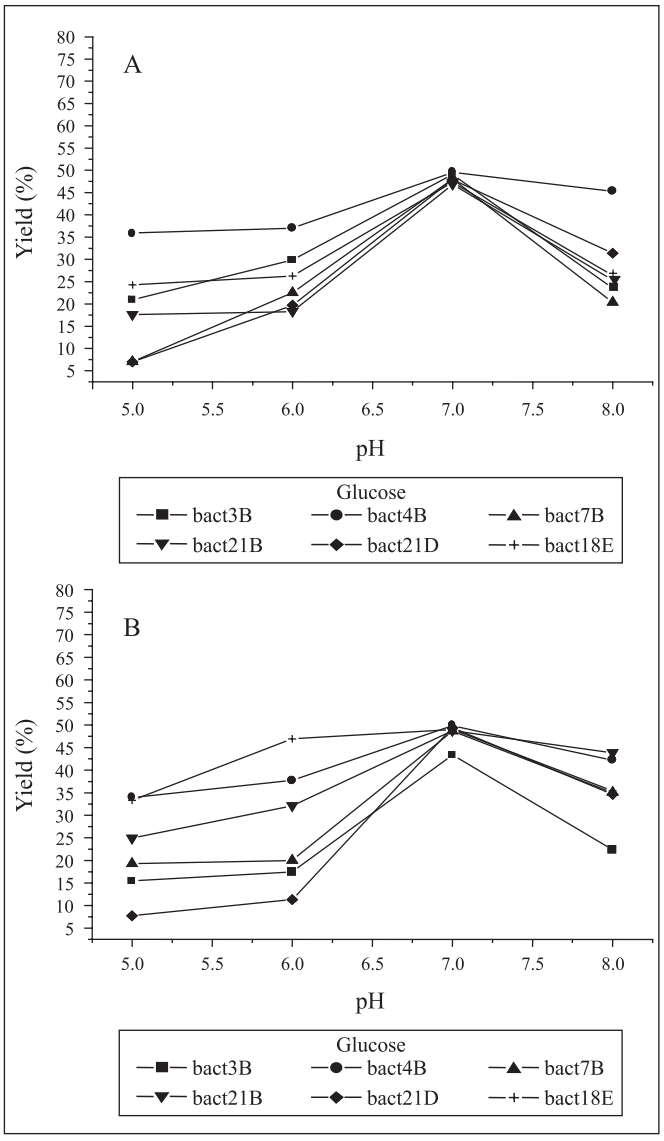

Figure 5. Influence of pH of the fermentation broth on polysaccharide yield.

To study the effect of pH and temperature on polysaccharide production, glucose and sucrose concentration were set at values which had provided the best production, i.e., 1% of glucose for 3B, 18E and 21D, 2% glucose for 4B, 7B and 21B, 1% sucrose for 21B and 18E and 2% sucrose for 3B, 4B, 7B and 21D (Fig. 4).

Fig. 5 and Table 2 show increase in the effect of pH on polysaccharide production and cellular growth respectively. It can be observed that increase of pH of the fermentation broth from 5.0 to 7.0 resulted in increase of polysaccharide yields. However, at pH 8.0 the yield decreased. The variance analysis by Tukey´s test presented significant differences at a level of 1% for the pH values tested.

Fig. 6 and Table 3 show the effects of incubation temperatures on polysaccharide production and the cellular growth, respectively. It may be observed that the optimal temperature was 30ºC. Lower or higher temperatures had a negative effect on the production of the polysaccharide, indicating that temperature of the culture medium is an important factor to be controlled during production of polysaccharides. The variance analysis by Tukey´s test presented significantly differences at a level of 1% for the temperatures tested.

Table 2. Effect of pH of the culture medium containing glucose or sucrose on growth of polysaccharide producing bacteria.

Strain	3B	4B	7B	21B	21D	18E
pH	Dry Cellular Weight (g)					
Glucose	1.0%	2.0%	2.0%	2.0%	1.0%	1.0%
5	0.0299	0.0516	0.0348	0.0212	0.1021	0.1049
6	0.1599	0.1723	0.0443	0.0373	0.1189	0.1334
7	0.6658	0.6291	0.4919	0.5653	0.5842	0.6234
8	0.1825	0.2217	0.0834	0.0566	0.1178	0.1434
Sucrose	2.0%	2.0%	2,0%	1.0%	2.0%	1.0%
5	0.0246	0.0514	0.0308	0.0131	0.0405	0.0839
6	0.1511	0.1470	0.0982	0.0315	0.0656	0.0992
7	0.6658	0.6321	0.4890	0.5393	0.5284	0.6235
8	0.3901	0.1585	0.1135	0.0812	0.0340	0.1526

Table 3. Effect of temperature on growth of polysaccharide producing bacteria.

Strain	3B	4B	7B	21B	21D	18E
Temperature ºC	Dry Cellular Weight (g)					
Glucose	1.0%	2.0%	2.0%	2.0%	1.0%	1.0%
25	0.2360	0.5185	0.0213	0.0358	0.1286	0.1820
30	0.6058	0.6291	0.4819	0.5653	0.5842	0.6234
35	0.1080	0.5010	0.048	0.0238	0.1145	0.1497
Sucrose	2.0%	2.0%	2.0%	1.0%	2.0%	1.0%
25	0.2757	0.5205	0.0372	0.0474	0.0605	0.1593
30	0.6072	0.6321	0.4890	0.5393	0.5284	0.6235
35	0.1185	0.5183	0.0450	0.0283	0.0345	0.1731

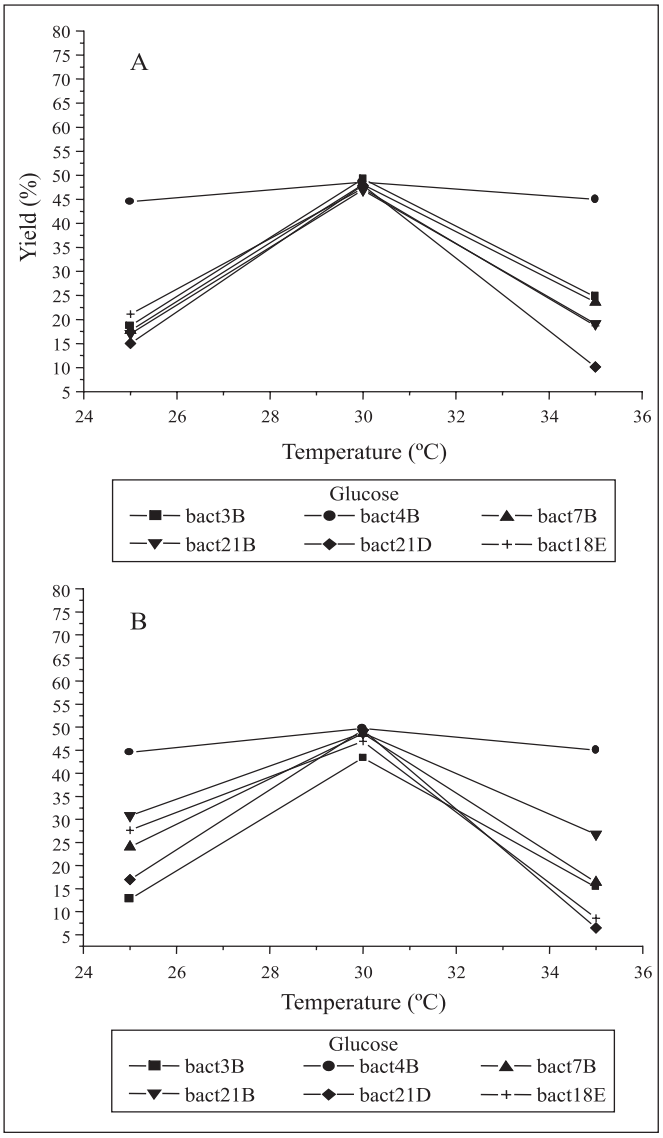

Figure 6. Influence of temperature of incubation on polysaccharide yield.

After optimization of production conditions, the pH during the fermentation was monitored. The final pH values are present in Table 4. These results suggest that the product is probably an acidic polysaccharide or that some organic acids are formed during the synthesis of the polysaccharide by strains 3B, 4B, 7B and 21B, whereas strains 21D and 18E probably produce neutral polysaccharides.

Table 4. Final pH of the fermentation broth containing glucose or sucrose.

	Final pH	
Strain	Glucose	Sucrose
3B	5.45	4.75
4B	5.47	5.52
7B	5.48	5.46
21B	6.90	5.00
21D	7.08	7.53
18E	7.05	6.88

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RESUMO

Otimização das condições de produção de polissacarídeos por bactérias isoladas do solo

Seis bactérias produtoras de polissacarídeos isoladas de amostras do solo (3B, 4B, 7B, 21B, 18E e 21D) pertencentes aos gêneros *Pseudomonas* e *Arthrobacter*, foram testadas quanto ao rendimento da produção de polissacarídeos em dois caldos de cultura: um com glicose e outro com sacarose, nas concentrações de 1, 2, 3, 4 e 5%. O rendimento foi calculado medindo-se a viscosidade dos caldos, através da equação de Poiseuille. Também foi verificado o efeito da temperatura de incubação (25, 30 e 35ºC) e o do pH (5, 6, 7 e 8) dos meios de cultura. Os resultados obtidos mostraram que concentrações da fonte de carbono até 2% apresentaram os maiores rendimentos do polissacarídeo. Glicose e sacarose estimularam a produção de polissacarídeos de forma similar. Quando o pH inicial do caldo de fermentação aumentou de 5,0 para 7,0 ocorreu um aumento da produção do polissacarídeo. Entretanto, valores mais altos (pH=8,0) causaram uma diminuição na formação do produto. Com relação à temperatura, 30ºC foi considerada ótima, pois temperaturas maiores ou menores do que esta exerceram um efeito negativo na produção do polissacarídeo.

Palavras-chave: bactérias, solo, produção de polissacarídeos, viscosidade

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