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Bioconversion of Pretreated Molasses into Xanthan Gum

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PRODUCTION of Xanthan gum and the growth of *Xanthomonas campestris* NRRL-B-1459 were studied by using pretreated sugar beet molasses and corn steep liquor (CSL) as cheap carbon and nitrogen source respectively. The gum production is highly affected by initial concentrations of beet molasses and CSL. Maximum gum production was observed by using beet molasses (40g/l) and corn steep liquor (l0g/l).

Keywords: *Xanthomonas campestris* NRRL-B-1459, Xanthan gum, Pretreated beet molasses, Corn steep liquor.

Xanthan gum was first discribed by Rogovine *et al.* (196l) as a product of fermentation by *Xanthomonas campestris* NRRL-B- 1459. This gum has special rheological properties, so it is used in food, cosmetics, pharmaceutical, paper, textiles, adhesives and oil recovery (Linda *et al.*, 1987). Many researchers have studied the fermentation conditions required for optimal gum production (Unashanakar *et al.*, l996, a,b; Amanullah *et al.*, l996, 199; Lo *et al.*, l997; Janna and Ghosh, 1997., Bruggeman *et al.*, 1999, and Pollock *et al.*, 999). In addition, this polymer is readiy produced by fermentation of glucose (Agricultural Research Service, 1976), but it can also produced by the fermentation of some industrial wastes such as citrus waste (Michael *et al.*, 1994), whey permeate (Yang and Silva, 1995). This work aims to production of xanthan gum by a local cheap by products such as sugar beet molasses and corn steep liquor.

Material and Methods

Microorganism and culture conditions

Xanthomonas campestris -NRRL-B-1459 was obtained from the Northern Regional Research Laboratory of the U.S. Department of Agriculture Peoria. The strain was maintained on slants with medium composed of (g/l): glucose, 10, peptone, 5, yeast extract, 3, malt extract, 3.0 and agar 15. pH was adjusted to 7.0 before autoclaving at 121°C, 20 mim. The culture was incubated at 30°C, 250 rpm for 48hr before use.

Production medium

Production medium composed of (g/l) glucose, 20; $MgSO_4$ $7H_2O$. 0.1 K_2HPO_4, 0.5 and NH_4 Cl 8.0 (pH 7.0). The meduim was autoclaved in 250 ml Erlenmeyer Flasks (50 ml of each) and cultivation with fresh slant and incubated shaken at 30°, 250 rpm for 48hr.

Corn steep liquor (CSL)

CSL 40% solids was obtained from Starch and Glucose Company, Torra, Cairo, Egypt.

Pretretment of sugar beet molasses

Curde sugar beet molasses used as cheap carbon source was kindly supplied by the Delta beet sugar factory, Egypt. This molasses was pretreated before used as follows:

Removal of suspended matter (centrifugation)

The crude molasses solution was repeatedly (3 times) centrifuged at 2000 rpm for 20 min for each. The muddy precipitate was then discarded, while the supernatant called centrifuged molasses.

Potassium ferrocyanide treatment

The crude molasses was centrifuged as mentioned above and treated with potassium ferrocyanide (0.7 g/l) while hot. The mixture was left overnight at room temperature, centrifuged, and the clear supernatant was then used in the fermentation process (Hamissa, 1970).

Sulphuric acid treatment

The crude molasses solution was acidified with 1.0N H_2SO_4 to pH 4 heated to 90°C for 30 min and finally centrifuged (Forage and Righelato, 1 978).

Calcium phosphate treatment

To molasses solution, calcium super phosphate (10 g/l) was added. The mixture was boiled, allowed to stand for one hour, then centrifuged. The supernatant solution was then treated with CaO to pH 7.0 and after a further 8hr standing, the sediment of calcium salts and organic matter settled at the bottom and the clarified solution was decanted for use (Allam, 1990)

Cell dry weight (CDW)

The fermentation broth was diluted two folds with distilled H_2O, and centrifuged at 2000 rpm for 20 min. The precipitate biomass was washed two times with distilled H_2O and transferred to a preweighed pyrex cup, placed in an oven (80°C) overnight, and cooled in a desiccator for 1 hr before weighing for dry weight.

Xanthan dry weight and viscosity measurement

Two volumes of ethanol were added slowly to one volume of cell free supernatant (prepared as mentioned above) containing 1 % KCl, and allowed to stand for one hour before centrifugation at 2000 rpm, for 20 min to precipitate xanthan gum which was separated and transferred to a preweighed pyrex cup, then placed in an oven (100°C) to constant weight (Osato *et al.*, 1996). The dried xanthan was dissolved in 50 ml distilled H_2O before using for viscosity measurements in a BROOKFIELD DV-I + VISCOMETER at 20 rpm and spindle No 4.

Results

Effect of different concentrations of pretreated molasses

In this experiment, glucose in the basal medium was replaced by different concentrations of pretreated molasses. Data recorded in Table 1 indicated that all chemical treatments of molasses (*i.e.* H_2SO_4, $K_4Fe(CN)_6$ or $Ca_3(PO_4)_2$ gave negative effect on xanthan production compared with physical treatment (centrifugation), but all these treatments (at 40 g/l) gave positive effect on xanthan production compared with crude molasses or even with the medium without molasses (basal medium). High yield of the gum was obtained with 40 g/l of molasses pretreated with only centrifugation. On the other hand, cell dry weight ranged from 3.1-3.4 g/l in all cases.

TABLE 1. Effect of different concentrations of pretreated molasses on the production of xanthan gum by *Xanthomonas campestris* NRRL-B-1459.

Molasses concentrations (g/l)		Xanthan dry weight (g/l)	Viscosity (CP)	Cell dry weight (g/l)
1.Basal medium *		8.7	97.1	3.192
2.Crude molasses	20	8.3	95.2	3.411
	40	15.1	158.3	3.390
	60	15.1	158.3	3.401
3. Molasses treated by centrifugation	20	9.1	100.2	3.403
	40	20.2	212.1	3.390
	60	20.1	211.8	3.410
4. Molasses treated by H_2SO_4	20	10.3	106.3	3.410
	40	18.7	190.0	3.402
	60	18.8	190.7	3.421
5.Molasses treated by $K_4Fe(CN)_6$	20	9.7	101.3	3.431
	40	15.8	162.3	3.418
	60	15.8	162.3	3.392
6.Molasses treated by $Ca_3(PO_4)_2$	20	10.5	111.8	3.218
	40	15.7	160.7	3.189
	60	15.8	162.3	3.330

*Basal medium = (g/l): glucose, 20, K_2HPO_4, 0.5, Mg $SO_4.7H_2O$, 0.1 and NH_4Cl, 8.0.

Effect of different concentrations of corn steep liquor (CSL)

Different concentrations of CSL as a complex organic and cheap nitrogen source (2-20 g/l) was added to molasses medium without NH_4Cl. Data presented in Table 2 showed that xanthan gum reached its maximum yield at 10 g/l of CSL, above this concentration, the gum decreased gradually, while, cell dry weight increased by increasing CSL concentrations.

TABLE 2. Effect of different concentrations of corn steep liquor (CSL) on Xanthan production by *Xanthomonas campestris* NRRL-B-1459.

CSL concentrations (g/l)	Xanthan dry weight (g/l)	Viscosity (CP)	Cell dry weight (g/l)
Control *	18.9	187.8	3.201
2	19.3	192.2	3.461
4	19.9	193.6	3.700
6	20.5	218.6	4.011
8	22.6	239.8	4.203
10	25.7	274.7	4.511
12	23.7	241.8	4.703
14	19.5	192.8	4.911
16	16.3	166.2	5.231
18	12.7	122.4	5.681
20	8.3	91.5	5.913

*Control = (g/l):centrifuged Molasses, 40.0, K_2HPO_4, 0.5, Mg $SO_4.7H_2O$, 0.1 and different concentrations of corn steep liquor.

Effect of different concentrations of K_2HPO_4

Table 3 shows the role of K_2HPO_4 on the biosynthesis of xanthan gum by *Xanthomonas campestris* NRRL-B-1459. Data clearly indicated that the gum was completely inhibited in the basal medium without K_2HPO_4, while the growth is very weak. On the other hand, the growth and the gum biosynthesis were not affected by the presence or absence of the added K_2HPO_4 in case of molasses or even in molasses + corn steep liquor medium.

Effect of different concentrations of Mg SO_4. $7H_2O$

The effect of Mg SO_4. $7H_2O$ on the production of xanthan and the growth of *Xanthomonas campestris* NRRL-B-1459 are shown in Table 4. Data indicated that $MgSO_4$. $7H_2O$ was essential for gum production, since, when the basal medium was free from $MgSO_4.7H_2O$, a weak growth and gum inhibition are

observed. On the other hand, the growth and the gum biosynthesis were unaffected by the absence or presence of the added $MgSO_4$. $7H_2O$ in case of molasses medium or even in molasses + CSL medium. However the maximum gum production was obtained by using centrifuged beet molasses (40g/l) + CSL (10g/l) medium; pH, 7.0.

TABLE 3. Effect of different concentrations of K_2HPO_4 on the production of Xanthan gum by *Xanthomonas campestris* NRRL-B-1459.

Concentrations of K_2HPO_4 (g/l)		Xanthan duy weight (g/l)	Viscosity (CP)	Cell dry weight (g/l)
Basal medium[1]	0.0	0.00	0.00	0.105
	0.5	8.90	95.6	3.157
	1.0	8.90	95.6	3.178
Molasses medium [2]	0.0	20.30	212.7	3.188
	0.5	20.28	121.7	3.200
	1.0	20.31	212.8	3.199
Molasses +CSL medium[3]	0.0	25.40	274.7	3.210
	0.5	25.41	274.7	3.210
	1.0	25.39	274.7	3.012

1. Basal medium (g/l):glucose, 20. Mg $SO_4.7H_2O$, 0.1, NH_4 Cl, 8.0 and different concentrations of K_2HPO_4.
2. Molasses medium (g/l): centrifuged molasses, 40, $MgSO_4$. $7H_2O$, 0.1, NH_4Cl, 8.0 and different concentrations of K_2HPO_4.
3. Molasses + CSL medium (g/l): centrifuged molasses 40, Mg $SO_4.7H_2O$, 0.1, corn steep liquor 10.0, and different concentrations of K_2HPO_4.

Discussion

The above data clearly show that xanthan production is influenced by the type of molasses treatment as carbon source and initial concentration of corn steep liquor (CSL) as nitrogen source as well as the presence of K_2HPO_4 and $MgSO_4.7H_2O$ in the medium. Physical treatment of molasses (centrifugation) with a concentration of 40g/l is the most concentration for high xanthan dry

TABLE 4. Effect of different concentrations of $MgSO_4.7H_2O$ on the production of Xanthan gum by *Xanthomonas campestris* NRRL-B-1459.

Mg $SO_4.7H_2O$ (g/l)		Xanthan dry weight (g/l)	Viscosity (CP)	Cell dry weight (g/l)
Basal medium[1]	0.00	0.00	0.0	0.120
	0.05	5.20	56.7	3.111
	0.10	8.80	97.6	3.105
	0.20	8.10	95.8	3.152
Molasses medium[2]	0.00	18.30	188.9	3.201
	0.05	18.30	188.9	3.181
	0.10	18.30	188.9	3.161
	0.20	18.21	188.5	3.120
Molasses +CSL medium[3]	0.00	26.05	276.2	3.131
	0.05	25.93	275.9	3.193
	0.10	25.92	275.8	3.211
	0.20	25.93	275.8	3.195

1. Basal medium (g/l): glucose, 20, K_2HPO_4, 0.5, NH_4 Cl, 8.0 and different concentrations of Mg $SO_4.7H_2O$.
2. Molasses medium (g/l): centrifuged molasses, 40, NH_4 Cl, 8.0 and different concentrations of Mg $SO_4.7H_2O$.
3. Molasses + CSL medium (g/l): centrifuged molasses 40, CSL, 10 and different concentrations of Mg $SO_4.7H_2O$.

weight and viscosity than other chemical treatments *i.e.* (sulphuric acid, calcium phosphate or potassium ferrocyanide). It is well known that the chemical treatment of molasses causes some reduction in metal ions which seems to be necessary for gum production. CSL (l0g/l) is the most convenient nitrogen source for xanthan gum production. Thonart *et al.* (1985) and Roseiro *et al.* (1992) reported on an inhibitory effect of high CSL level on xanthan production

and this effect may due to the drop in the C/N ratio, while Abd El-Salam *et al.* (1994) found that CSL can be used efficiently for xanthan gum production. At high concentrations of CSL (> 10 g/l), the carbon and nitrogen source seem to preferentially be consumed for growth, resulting in lower xanthan formation.

Though the necessity of inorganic phosphate and magnesium sulfate for xanthan production (Cadmus *et al.*, 1978 and Souw and Demain, 1979), using molasses as carbon source and CSL as nitrogen source, the gum titter was always high even in a medium without additional phosphate and/or Mg $SO_4.7H_2O$. This can be explained by the fact that the K-phosphate and Mg-sulphate contents of the complex CSL and molasses is high enough to favor xanthan production.

From this study CSL (l0g/l), beet molasses pretreated by centrifugation (40g/l) can be used as a combined nitrogen, carbon and mineral sources for high xanthan gum production by *Xanthomonas campestris* NRRL-B- 1459.

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التحول البيولوجى لمولاس بنجر السكر المعامل الى الزانثان

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قسم كيمياء المنتجات الطبيعية والميكروبية - المركز القومى للبحوث - القاهرة - مصر.

استهدف هذا البحث استخدام المولاس المعامل لانتاج صمغ الزانثان بواسطة السلالة الميكروبية *Xanthomonas campestris NRRL-B-1459* حيث أوضحت التجارب ما يلى:

١- تفوق المعاملة الميكانيكية للمولاس (الطرد المركزى) عن مثيلاتها الكيميائية باستخدام حامض الكبريتيك أو فوسفات الكالسيوم أو حديدو سيانور البوتاسيوم لانتاج صمغ الزانثان.

٢- استخدام محلول نقيع الذرة (المخلف من صناعة النشا والجلوكوز) بتركيز ١٠ جم/لتر كمصدر نتروجين بالإضافة الى المولاس (٤٠ جم/لتر) يؤدى الى زيادة انتاج الصمغ.

وأمكن استخدام هذة البيئة لانتاج الصمغ بدون اية اضافات أخرى نظرا لما تحتوية من مصادر للكربون والنيتروجين والاملاح المعدنية الضرورية لانتاج صمغ الزانثان.