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Polysaccharide Production by *Pseudomonas elodea* ATCC 31461

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Abstract

Cultural conditions for the polysaccharide production by *Psedomonas elodea* ATCC 31461, a thermogellable polysaccharide producer, were investigated. Optimum condition for polysaccharide production were: temperature; 30°C, pH; 6.5, glucose; 25 *g/L*, nitrogen source; peptone, C/N ratio; 5.0. Maximum production and yield at this condition were ca. 32 *g/L* and 128%, viscosity of 1% polysaccharide solution was 837 *mPas* at 35/sec.. Consistency index and flow behaviour index were 2570 *mPas* and 0.66

Introduction

Many microorganisms are synthesizing polysaccharides intracellularly or extracellularly.[1-4] These polysaccharides are postulated as doing some function for the organisms such as reserve carbon or energy material, protection against desiccation, amoebic attack, bacteriophage, and as an endotoxin[5] However, some extracellular homopolysaccharides such as levan or dextran which are formed as a by-product of enzyme are reported as not having such function.

These kind of biopolymers have been drawn attention due to increasing world wide consumption and shortage of plant gums[6] and their unique rheological properties such as gelling or viscosifying nature.[7] Therefore, there were lots of effort to search new, novel hydrocolloids which are able to replace the hydrophillic gums of plant origin. Xanthan gum is one of the typical biopolymers of microbial origin under commercial production[8] and has been widely used as stabilizer in food industry and as a drilling mud in oil industry. Harada *et al.*[9,10] discovered curdlan, a kind of beta glucan polymer, which is produced by *Alcaligenes* and *Agrobacterium* sp.[11] and forms a firm, resilient, irreversible gel upon heating in aqueous suspension. Murao *et al.*[12] and Morita and Murao[13] reported on the chemical and physical properties of another thermogellable polysaceharide produced by *Bacillus subtilis.* Hisatsuka *et al.*[14] studied the biopolymer produced by *Arthrobacter carbazolum.* This paper reports on the cultural condition for polysaccharide production by *Pscudomonas elodea* ATCC 31461, a thermogellable polysaccharide producer.[15]

Materials and Methods

Microorganism

Pseudomonas elodea 31461 was obtained from American Type Culture Collection and used throughout this study. The stock culture was grown on Plate Count Agar at 30°C for 24 hours and transferred every one month.

Starter Culture

Starter culture was grown in YM broth at 30°C for 18 hours and inoculum size was 5%.

Medium and Cultivation

The chemical composition of basal medium was as follows per liter of distilled water: Glucose 25 *g*, Bacto peptone 2.06 *g*, K2HP04 0.5 *g*, MgSO4.7H2O 0.2 *g*. Culture was performed in 250 *ml* erlenmyer flask containing 50 *ml* of the sterilized medium on the rotary shaker at 120 *rpm* or in 2.5 liter fermentor (Marubishi L., E. Co. Japan, Model MD 25, Working volume 1.5 liter). Agitation and aeration rate were 200 *rpm* and 1 *v/v/m* respectively. Polysaccharide production was evaluated depending upon carbon and nitrogen sources, their concentration, cultivation temperature, and initial pH.

Polymer Recovery[15]

Alcohol precipitation was used to recover the polysaccharide by adding 2 volumes of 99% isopropanol to the culture broth. Fibrous polymer thus obtained was filtered and washed with 66% isopropanol solution and then freezedried.

Viscometry

Viscosity was measured by Brabender Viscotron (Mode 80241, West Germany, System E-17, Range 10, Shear rate 35/sec) at 25°C.

pH

pH was measured by Fisher Accumet ion analyzer (Model 750, U.S.A)

Results and Discussion

Optimum Nitrogen Source

Organic and inorganic nitrogen sources were tested for their effect on the polysaccharide production on the glucose medium (C/N ratio:30). Peptone and urea were found to be good nitrogen sources for polysaccharide production by *Pseudomonas elodea* ATCC 31461 (Table 1). Polysacharide productions (yields) were 18.66 *g/L*(75.44%) and 12.21 *g/L* (48.86 %) after 18 hours of incubation for the respective nitrogen sources. The rest of nitrogen sources used were poor in polysaccharide production. A sporeformer was reported to prefer peptone for biopolymer production[16] and some workers found that best nitrogen source for *Alcaligenes* sp.[3] and *Bacillus polymyxa*[17] was yeast extract. *Arthrobacter* sp.[1] utilized well the enzymatic digest of casein for its polysaccharide production. Final pH of culture broth was increased to be alkaline except the medium with ammonium dihydrogen phosphate.

Optimum Nitrogen Concentration

Effect of C/N ratio on the polysaccharide production

Table 1. Effect of nitrogen sources on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Nitrogen source	Crude polymer [*g/L*]	Yield [%]	pH
Peptone	18.66	75.44	7.6
Urea	12.21	48.87	7.9
NaNO3	2.33	9.33	7.9
NH4H2P04	2.70	10.80	6.4
NH4Cl	1.34	5.36	7.9
KN03	2.54	10.16	8.0
(NH4)2S04	1.44	5.74	7.7
NH4N03	1.23	4.93	7.8
(NH4)2HP04	3.10	12.39	8.0

C/N ratio:30, Basal medium: Glucose 25*g*, MgS04.7H20 0.1 *g* K2HP04 0.5 *g/L*, Initial pH 6.5

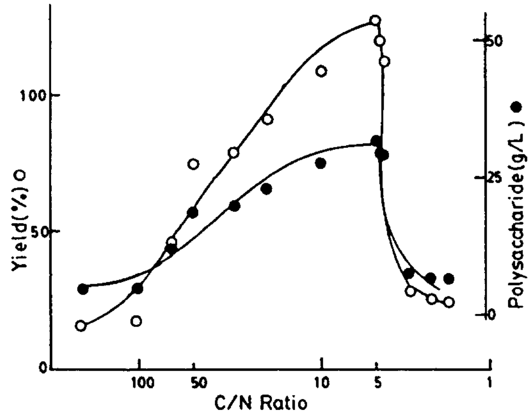

Fig. 1. Effect of C/N ratio on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C (Nitrogen source: peptone)

was studied by adding different quantity of nitrogen source on the medium (Fig. 1 and Fig. 2). When using peptone, optimum C/N ratio for maximum production was 5.0, where 31.98 *g/L* (yield: 127.92 %) of polysaccharide could be produced. However, C/N ratio 100 was found to be most suitable when using urea as nitrogen source where 14.66 *g/L*(yield: 58.64%) of polysaccharide was produced. Biopolymer production was decreased on the medium with lower or higher C/N ratio than optimum condition. Kang and Cottrell[18] reported that excess nitrogen in medium reduced conversion of carbon source to extracellular polysaccharide although they were necessary for cell growth and polysaccharide synthetase. Congregado *et al.*[19] reported that high C/N ratio promoted extracellular polysaccharide production and optimum C/N ratio for *Pseudomonas* sp. EPS-5028 was 4.0. Ninomiya and Kizaki[16] tried to partially substitute organic nitrogen

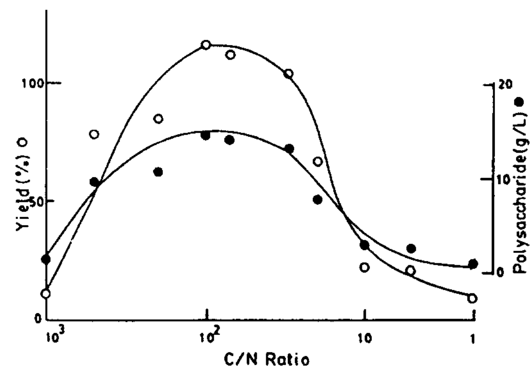

Fig. 2. Effect of C/N ratio on the polysaccharide production by *pseudomonas elodea* ATCC 31461 at 30°C (Nitrogen source: urea)

Table 2. Effect of combination of nitrogen source on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Nitrogen source		Crude polymer [g/L]	Yield [%]
Peptone	Urea		
100	0	31.96	121.84
70	30	24.46	97.84
50	50	22.32	89.28
30	70	18.35	73.40
10	90	17.35	69.40
0	100	2.63	10.52

Basal medium: Glucose 25 *g* K2HP04 0.5 *g* MgS04.7H20 0.1 *g*, Initial pH 6.5, Nitrogen source: Peptone and Urea, C/N ratio:5.0

with synthetic nitrogen and could obtain similar polysaccharide production. However, in this study (Table 2), substitution of peptone with urea nagatively affected the production.

Table 3. Effect of sugar source on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Sugar source	Crude polymer [g/L]	Yield [%]
Galactose	3.80	15.20
Raffinose	11.14	47.75
Rhamnose	4.73	20.76
Glucose	32.26	129.04
Sucrose	24.51	103.20
Mannose	29.13	116.52
Arabinose	3.82	15.28
Maltose	3.19	13.43
Lactose	29.13	122.65
Fructose	4.32	17.28
Mannitol	3.36	13.29
Glycerol	2.54	9.94
Xylose	1.47	5.88
Ethylene glycol	2.11	8.17

Basal medium: Peptone 12.4 *g*, K2HP04 0.5 *g*, MgS04.7H20 0.1 *g/L*, Initial pH 6.5, Sugar: Galactose Fructose, Mannose, Arabinose, Xylose, Glucose; 25 *g* Sucrose, Maltose, Lactose: 23.75 *g*, Mannitol: 25.28 *g*, Rhamnose: 22.28, Raffinose: 23.33 *g* Ethyleneglycol: 25.84 *g*, Glycerol: 25.56

Optimum Carbon Source and Concentration

To investigate the effect of different carbon sources, glucose was replaced by several carbon sources. Nitrogen sources were peptone (Table 3) or urea (Table 4) and C/N ratio was 5.0. Suitable carbon sources were glucose, lactose, mannose and sucrose in decreasing order, where polysaccharide productions (yields) were 32.26 *g/L* (129.04%), 29.13 *g/L* (122.65%), 29.13 *g/L* (116.52%) and 24.51 *g/L* (103.20%). The rest of carbon sources were found to be poor, whose productions were below 5 *g/L* Similar trend was also recognized when using urea as nitrogen source, so that glucose, mannose, and lactose were high in production. The fact that glucose was suitable carbon source for polysaccharide production was reported elsewhere[12,16,20], however, sucrose was best sugar for *Alcaligenes faecalis*. Yim *et al.*[21] reported starch to be suitable for pullulan production. Carbon concentration can affect polysaccharide production[3,22,23]. To examine the effect of glucose concentration, different quantity of glucose was added to the medium (Table 5). It was found that maximum production of polysaccharide was obtained when 25 *g/L* of glucose was used.

Optimum Temperature and Initial pH

Polysaccharide production at various temperatures (20 ~40°C) was investigated (Table 6). Optimum temperature was 30°C. Williams and Wimpenny[24] reported that maximum polysaccharide production by *Pseudomonas* sp. was obtained at 30°C. Optimum temperature for pullulan[21] and curdlan[3] was reported to be 28°C. Initial pH is also an influencing factor for polysaccharide produc-

Table 4. Effect of sugar sources on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Sugar source	Crude polymer [g/L]	Yield [%]
Mannose	14.13	58.12
Raffinose	4.54	19.45
Glucose	14.66	58.64
Sucrose	11.22	47.24
Maltose	1.50	6.31
Lactose	0.92	3.82

Basal medium:Urea 4.29 *g*, K2HP04 0.5 *g*, MgS04.7H20 0.1 *g/L* Initial pH 6.5, Sugar; Glucose, Mannose 25 *g*, Sucrose, Maltose Lactose 23.75 *g*, Raffinose 23.33 *g/L*

Table 5. Effect of glucose concentration on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Concentration [g/L]	Crude polymer [g/L]	Yield [%]
10	10.61	106.10
20	26.33	131.65
25	31.44	124.56
30	20.47	68.23
40	5.15	12.88

Basal medium:K2HP04 0.5 *g*, MgS04. 7H2O 0.1 *g/L* , Initial pH 6.5 C/N ratio: 5.0, Nitrogen source: Peptone

tion[17,23,25,26]. Optimum pH for polymer production by *Pseudomonas elodea* ATCC 31461 was 6.5 (Table 7). Sometimes optimum pH for growth and polymer production could be different. Therefore, Lacroix et al.[27] recommended bi-staged pH fermentation process for pullulan production.

Profile of Production

Polysaccharide production and rheological properties were studied during fermentation in 2.5 L jar fermentor (Table 8). Polysacharide production was maximum after 22 hours of fermentation (20.64 *g/L*). Flow of fermentation broth showed pseudoplastic behaviour, so that flow behaviour index was 0.48 after 22 hours of incubation.

Table 6. Effect of temperature on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 after 18 hours of incubation

Temperature [°C]	20	25	30	35	40
Crude polymer [g/L]	4.39	6.13	31.11	25.95	21.52
Yield [%]	17.56	24.52	124.44	103.80	86.08

Medium: Glucose 25 *g*, Peptone 12.4 *g*, K2HPO4 0.5 *g*,MgSO4. 7H2O 0.1 *g/L* Initial pH 6.5

Table 7. Effect of initial pH on the polysaccharide production by *Pseudomonas elodea* ATCC 31461 at 30°C after 18 hours of incubation

Initial pH	4.5	5.5	6.5	7.5	8.5
Crude polymer [g/L]	4.22	10.6	29.02	6.10	5.08
Yield [%]	16.88	42.40	116.08	24.40	20.32

Medium: Glucose 25 *g*, Peptone 12.4 *g*, K2HPO4 0.5 *g*, MgSO4. 7H2O 0.1 g/L

Table 8. Time course of polysaccharide production by *Pseudomonas elodea* ATTC 31461 at 30°C

Elapsed fermentation time [hrs]	Crude polymer [mPs]	Consistency index[K] index [n]	Flow behaviour
12	2.34	123	0.58
18	6.53	664	0.54
22	20.64	1047	0.48
46	17.60	1314	0.48
63	20.46	1998	0.40

Agitation: 200 rpm, Air: 1 vvm, 1.5*l* batch culture. Medium: Glucose 25*g* Peptone 12.4*g*, $MgSO_4$. 7H2O 0.1*g*,K2HPO4 0.5*g/L*, *p*H 6.5

Table 9. Concentration dependence of polysaccharide produced by *Pseudomonas elodea* ATCC 31461

Concentration [%]	Vicosity [mPas]	Consistncy index [K,mPas]	Flow behaviour index [n]
0.5	167.4	802	0.57
0.75	334.8	1878	0.51
1.00	837.0	2570	0.66
1.25	993.2	1751	0.84
1.50	1562.0	1959	0.96

Viscosity was measured at 35 sec-1

Consistency index of broth increased as the fermentation time elapsed. The consistency index after 63 fours of fermentation was 1998 *mPas*.

Viscosity

Viscosity of polysaccharide solution was compared (Table 9). Solution became thicker as the polysaccharide concentration increased. However, they lost pseudoplastic behaviour. Viscosity, consistency index and flow behaviour index of 1% polysaccharide solution were 837 mPas at 35/sec, 2570 *mPas* and 0.66, respectively.

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Pseudomonas elodea ATCC 31461에 의한 Polysaccharide 생산

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gel형성 다당류 생산균주인 Pseudomonas elodea ATCC 31461의 배양조건을 검토하기 위하여 탄소원, 질소원, 온도, pH의 영향을 조사한 결과 적당한 온도는 30°C, pH는 6.5이고, 배지조성은 포도당 25*g/L*, peptone 12.38g/*L*, K_2HPO_4 0.5g/*L*, $MgSO_4 \cdot 7H_2O$ 0.1*g/L*이 적합하였으며 이때 다당류의 생성량은 32*g/L*이었다.

생산다당류의 1% 용액은 점도가 837*mPas*, 점조성지수는 2570*mPas*, 유동지수는 0.66이었다.