Effect of nitrogen sources on production of exopolymer by *Pseudomonas elodea* ATCC 31461

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Abstract

Extracellular polysaccharide was produced by *Pseudomonas elodea* ATCC 31461 under aerobic condition. Nitrogen sources in medium affected cell growth and production of exopolymer. Ammonium nitrate limitation was found to be essential for higher production of exopolymer. Conversion rate of exopolymer from glucose under ammonium nitrate limitation was about 5 times higher than with ammonium nitrate.

Introduction

The extracellular polysaccharide, commercially known as PS-60 or gellan gum, was produced by *Pseudomonas elodea* ATCC 31461. The gellan gum consists of linear repeating tetrasaccharide [→3)-β-D-Glc-(1→4)-β-D-GluA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→] composed of D-glucose (Glc), D-glucuronic acid (GlcA), and L-rhamnose residues (Rha)[6]. Gellan gum contains *O*-acetyl group that are removed by heating at pH 10. Gellan gum with low acetyl groups products a bittle, firm, and optically clear gel. According to its property to produce a thermoreversible gel, gellan gum can be substituted for agar. Due to diversity of its structure and properties, gellan gum has a wide range of applications in the food, pharmaceutical and other industries as texturizing, stabilizing, thickening, emulsifying and gelling agents (Sutherland 1990). Also, gellan gum has been used for enzyme and cell immobilization and gel electrophoresis.

Materials and Methods

Bacterial strain. *Pseudomonas elodea* ATCC 31461 was obtained from the American Type Culture Collection (ATCC) and monthly transfer to fresh agar medium.

Medium and culture condition. The medium used for cell growth and

exopolymer production contained the following composition (g/l): Glucose, 20; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; NH_4NO_3, 0.9; Bacto peptone (Difco Lab., Chicago, USA), 0.5; and mineral salt solution, 0.1ml. The mineral salt solution contained the following composition (mg/l): $MnCl_2 \cdot 4H_2O$, 1.8g; $FeSO_4 \cdot 7H_2O$, 2.487g; H_3BO_3, 0.285g; $CuCl_2$, 27; $ZnCl_2$, 21; $CoCl_2 \cdot 6H_2O$, 74; $MgMoO_4$, 23 and sodium tartrate (dihydrate), 2.1g. The pH of medium was adjusted to 6.5 - 6.8 before sterilization. The carbon source was autoclaved separately for 20 min at 120℃ and added to the medium under aseptic conditions.

Production of exopolymer. Starter cultures were prepared by transferring cells agar slants to 50 ml of the medium with 2% (w/v) glucose in 250 ml Erlenmeyer flasks. These culture were incubated for 4 days at 30℃ and 180 rpm. This starter culture was used as a 5% (v/v) inoculum for 100 ml of the medium with carbon source 2% (w/v) in 500 ml Erlenmeyer flasks. Cultures were incubated for 5 days under the same conditions used in preparing the starter cultures. Samples were periodically withdrawn from the cultures to determine cell growth and exopolymer production.

Fermentor condition. Starter cultures was used for an inoculum for 5 L of medium in 7.5 L fermentor. Inoculum size was 5% (v/v). Culture were incubated for 3 days at 30℃, air rate of 1 vvm and agitation rate of 400-600rpm.

Recovery of PS-60. Deacetylated PS-60 was prepared by heating 95℃ for 10 min. The pH was adjusted to 10 by 1N NaOH and then neutralized with 1N HCl. The pretreated broth was centrifuged at 10000 x g for 10 min to separate the cells[5]. The supernatant was mixed with 2 volumes of isopropanol. The pellet was dissolved in deionized water and dialyzed against deionized water with dialysis tubing with a molecular weight cut off of 12,000-14,000.

Analytical methods. The concentration of exopolymer was determined colorimetrically by the phenol-sulfuric acid method[7]. Dry cell weigh was determined by directly weighing biomass after drying to constant weight at 100-105℃. The optical density (OD) was measured by spectrophotometer at 600nm.

Result

Effect of bacto peptone with ammonium nitrate. Bacto peptone was known to be one of the best nitrogen source for production of gellan gum. Production of gellan gum increased and reached at 0.61 g/l as concentration of bacto peptone increased up to 0.1% (Table 1).

Table 1. Effect of bacto peptone on cell growth and production of exopolymer.

Bacto Peptone (%)	pH (Initilal / Final)	OD (600nm)	DCW (g/l)	Polymer (g/l)
0	7.69 / 3.70	2.15	0.64	0.17
0.01	7.62 / 3.64	3.26	0.76	0.23
0.03	7.62 / 3.81	4.37	0.96	0.30
0.05	7.61 / 3.84	6.49	1.32	0.50
0.75	7.59 / 3.92	9.24	2.20	0.58
0.10	7.57 / 4.00	9.46	2.04	0.61

Effect of ammonium nitrate with bacto peptone. The highest production of gellan gum was obtained without ammonium nitrate in the medium. Productivity of gellan gum without ammonium nitrate was about 7 times higher than that with 0.18% (w/v) ammonium nitrate (Table 2). It seems that ammonium nitrate limitation is essential for the production of gellan gum.

Effect of bacto peptone without ammonium nitrate. The effect of bacto peptone on production of gellan gum without ammonium nitrate was investigated (Table 3). Bacto peptone enhanced cell growth and increased production of gellan gum. Productivity of gellan gum without ammonium nitrate was about 5 times that with ammonium nitrate.

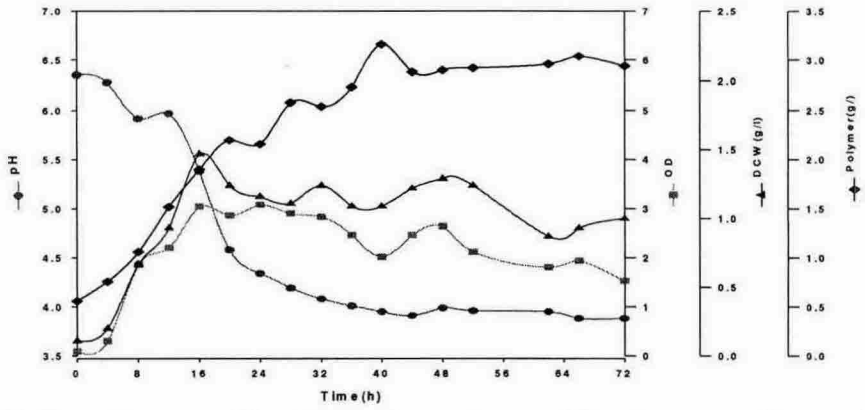
Table 2. Effect of ammonium nitrate(NH_4NO_3) on cell growth and production of exopolymer.

NH_4NO_3 (%)	pH (Initila / Final)	OD (600nm)	DCW (g/l)	Polymer (g/l)
0	7.82 / 3.96	4.17	1.32	2.10
0.03	7.75 / 3.74	3.06	0.80	0.29
0.06	7.62 / 3.75	3.80	0.84	0.29
0.09	7.59 / 3.79	4.30	1.12	0.31
0.12	7.55 / 3.73	4.76	1.08	0.33
0.15	7.52 / 3.78	4.58	1.12	0.33
0.18	7.50 / 3.70	4.47	1.00	0.28

Table 3. Effect of bacto peptone without ammonium nitrate on cell growth and production of exopolymer.

Bacto Peptone (%)	pH (Inital / Final)	OD (600nm)	DCW (g/l)	Polymer (g/l)
0	8.11 / 5.42	0.71	0.45	0.54
0.01	8.03 / 5.29	1.34	0.90	1.99
0.03	7.94 / 4.81	2.64	1.10	2.52
0.05	7.87 / 4.01	5.02	1.60	2.62
0.75	7.80 / 3.87	3.90	1.70	3.43
0.10	7.73 / 3.87	3.98	1.50	3.37

Fig. Fermentation without ammonium nitrate in 7.5 L fermentor.



Cell culture with 7.5L fermentor without ammonium nitrate. The fermentation was started without ammonium nitrate. The highest production of gellan gum was 3.16 g/l on 40 hr (Fig.).

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