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Metabolic Flux Analysis of *Beijerinckia indica* for PS-7 Production

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Abstract In order to investigate central metabolic changes in *Beijerinckia indica,* cells were grown on different carbon sources and intracellular flux distributions were studied under varying concentrations of nitrogen. Metabolic fluxes were estimated by combining material balances with extracellular substrate uptake rate, biomass formation rate, and exopolysaccharide (EPS) accumulation rate. Thirty-one metabolic reactions and 30 intracellular metabolites were considered for the flux analysis. The results revealed that most of the carbon source was directed into the Entner-Doudoroff pathway, followed by the recycling of triose-3-phosphate back to Hexose-6-phosphate. The pentose phosphate pathway was operated at a minimal level to supply the precursors for biomass formation. The different metabolic behaviors under varying nitrogen concentrations were observed with flux analysis.

Keywords: metabolic flux analysis, flux distribution, *Beijerinckia indica*, PS-7, exopolysaccharide

INTRODUCTION

The heteropolysaccharide-7 (S-7 or PS-7) from *Azotobacter indicus var.* myxogens was identified and examined as a potential candidate for the production of a bacterial polysaccharide with desirable rheological properties [1]. The bacterial strain was later renamed to *Beijerinckia indica* [2], or *Sphingonomas S7* [3]. A structural analysis of the PS-7 polysaccharide [4] found in *Beijerinckia indica* showed that it has a similar backbone with members of the gellan family of polysaccharides. PS-7 is a water soluble exopolymer that can generate a highly viscous solution with twice the viscosity of xanthan gum. The viscosity of PS-7 solution is stable at a temperature range of 4 to 93°C and at a pH range of 3.0 to 12.0 [5]. PS-7 has good pseudoplasticity and shows excellent suspending ability.

Metabolic flux analysis (MFA) is a powerful technique used to characterize intercellular fluxes of microorganisms, which aid in the identification of potential genetic modifications that may improve the behavior and productivity of microorganisms [6]. The metabolic fluxes distributed within microorganisms are estimated through a combination of biochemical, microbiological, and mathematical approaches, as well as data from experimental measurements. The flux distributions are correlated to the strength of each individual enzyme, which represents the *in vivo* metabolic state of the cell. The flux information is then used to examine the influence of process parameters or cultivation conditions on the physiological states of microorganisms and to identify rate-limiting steps in pathways. Once such steps are identified, molecular biological techniques can be applied to modify the corresponding gene or enzyme to bring about significant shifts in the yield of desired end products.

Beijerinckia indica is a strictly aerobic, gram-negative bacterium. When grown on a carbohydrate-containing medium, the colonies glisten due to capsular slime exopolysaccharide formation [1]. Like other important polysaccharides such as xanthan, gellan, and curdlan, PS-7 production favors high carbon/nitrogen ratio conditions in the substrate [7,8].

In this paper, *Beijerinckia indica* was cultivated in media with different carbon sources and a high or low nitrogen content for EPS fermentation. Metabolic flux was calculated to investigate the flux distribution in the central metabolism of the strain and metabolic changes under different nitrogen conditions. The Pentose Phospate pathway (PP), the semi- Embden-Meyerhof-Parnas (EMP) pathway, the Entner Doudoroff (ED) pathways, the Tricarboxylic acid (TCA) cycle, and EPS synthesis were used in the proposed metabolic network for metabolic flux analysis.

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MATERIALS AND METHODS

Bacterial Strain and Culture Conditions

Beijerinckia indica ATCC 21423 was obtained from the American Type Culture Collection and maintained on agar plates of mineral salts medium. The mineral salts medium (MSM) used for cell growth and the production of PS-7 was supplemented with the following components (g/L): KH_2PO_4, 5.0; $MgSO_4 \cdot 7H_2O$, 0.1; NH_4NO_3, 0.6; yeast extract, 0.5; bacto peptone (Dofico Lab., Detroit, USA), 0.1; glucose, 20; 1 mL trace minerals solution. The pH of the medium was adjusted to 6.8. The trace minerals solution was composed of 1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnSO_4 \cdot 5H_2O$, 0.25% $ZnSO_4 \cdot 6H_2O$, 0.2% $CuSO_4 \cdot 6H_2O$, and 0.25% $Na_2MoO_4 \cdot H_2O$. The carbon sources of glucose, glucosamine, glycerol and gluconate each had a concentration of 20 g/L. Carbon sources were autoclaved separately for 15 min at 120°C for analysis of carbon metabolism of *Beijerinckia indica*. In order to investigate various nitrogen levels, the cells were grown in media supplemented with 0.6, 1.3 and 1.5 g/L ammonium nitrate (corresponding to 7.5, 16.25 and 18.75 mM).

Culture Methods

Seed cultures of 20 mL were incubated for 24 h at 30°C in a shake incubator and were used to inoculate 50 mL medium in a 250-mL Erlenmeyer flask. The subsequent culture was incubated for 72 h under the same conditions stated above. The cultures were also used to inoculate 3 L medium in a 5-L fermentor (KF-5, Korea Fermentor Co., Inchon, Korea) installed with DO and pH sensor. One vessel volume per minute (vvm) of air was supplied through a sparger, and the speed of agitation was controlled at 500 rpm.

Analytical Methods

Culture broths were centrifuged at 15,000 × g for 30 min at 4°C to remove cells with a dilution. Two volumes of isopropanol was added to the supernatant to precipitate the exopolysaccharide and then centrifuged at 10,000 × g for 30 min to separate the precipitate. The precipitate was washed with isopropanol and weighed after drying at 105°C. To determine biomass, culture broths were centrifuged, the resultant pellet was washed with distilled water, and dry cell weight was measured after drying at 105°C. The residual glucose concentration was determined by the phenol sulfuric acid method [9]. The ammonia was determined spectrophotometrically by the indophenol blue method [10]. The nitrate was determined spectrophotometrically by the method by Cataldo et al. [11]. The broth viscosity was measured at 30°C with a Brookfield viscometer DV III using SC4-34 spindle (Brookfield engineering laboratories, Stoughton, Mass.).

Evaluation of Metabolic Flux Distribution

The biochemical reaction network of *Beijerinckia indica* was constructed based on previous reports of Klinke *et al.* [12], Ketusse *et al.* [13] and Narredra *et al.* [14]. The results of sequence analysis data of the *Pseudomonas* and *Azotobacter* genome were also examined [15,16]. Serial reaction steps were considered as a "lumped" reaction (several reactions combined in one equation). The stoichiometric matrix was formulated according to the biochemical reactions (see Appendix) present in *Beijerinckia indica*. The metabolite requirements for biomass synthesis were obtained from Neidhardt *et al.* [17] with the modifications proposed by Ampe *et al.* [18]. The principles of stoichiometric flux analysis, based on the metabolite balance, biochemical constraints and pseudo-steady-state assumption for intracellular metabolites, have been extensively described by Valino *et al.* [19]. Specific metabolite concentrations can be balanced according to the dynamic flux balance. Mass balance equations were formulated from the biochemical network of *Beijerinckia indica* (Appendix) by MetaFluxNet® [20]. Boundaries were imposed so that irreversible reactions had nonnegative fluxes. The carbon dioxide and oxygen balance were excluded. The dimension of the stoichiometric matrix was 31 × 30 and the-linear equation system was solved by using the *linprog* function from the MATLAB Optimization Toolbox (The Mathworks Inc., MI, USA). The biomass molecular weight was assumed to be 100 g/M with 3% ash. Three fluxes (r_1, r_{29}, r_{30}) were directly calculated from the experimental data and all the fluxes (mM/(g DCW.h)) were normalized with respect to glucose uptake rate.

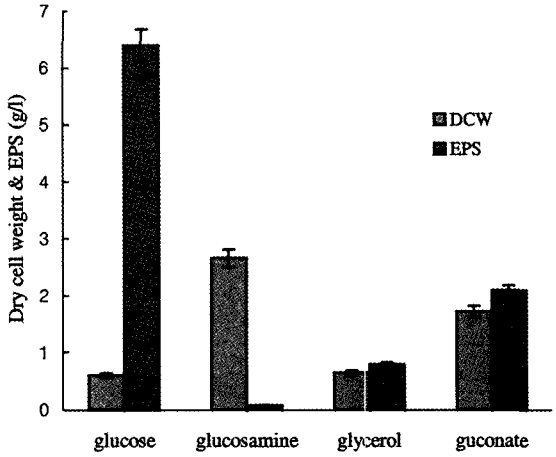

Fig. 1. The effects of different carbon sources on cell metabolism.

RESULTS AND DISCUSSION

Characterization of Carbon Metabolic Pathway in *Beijerinckia indica*

Beijerinckia indica was grown on minimal salt media with glucose, glucosamine, gluconate or glycerol as carbon sources. As shown in Fig. 1, when *B. indica* was

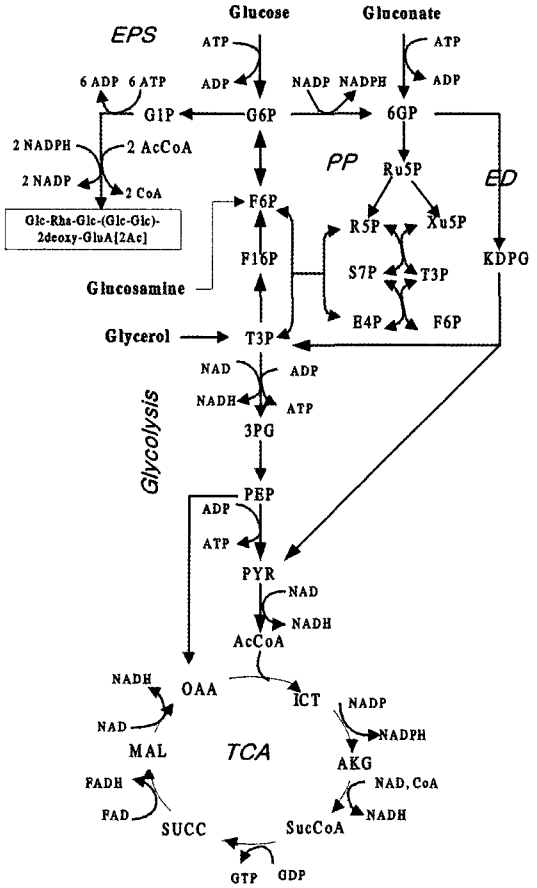

Fig. 2. Proposed metabolic pathway network in *Beijerinckia indica* with different carbon sources.

grown with glucose, a significant amount of carbon source had accumulated as exopolysaccharide (6.4 g/L), and final biomass production (0.6 g/L) was low due to limited nitrogen content. Glucosamine was a favorable carbon source for cell growth, with a final dry cell weight of 2.66 g/L but no production of polysaccharide. In media containing glycerol, the cells grew poorly and produced a small amount of exopolysaccharide with low viscosity. When gluconate was used, a biomass of 1.72 g/L and polysaccharide concentration of 2.1 g/L were produced.

As with the majority of *Pseudomonas sp.* [12], *Beijierinckia indica* [3] employs the ED pathway, PP pathway, and TCA cycle as central metabolic pathways (Fig. 2). This was confirmed by the observation that *B. indica* was able to grow on gluconate. Glycerol was metabolized through triose-3-phosphate [21] and the PP pathway and gluconeogenesis were active to supply precursors for cell growth and polysaccharide biosynthesis. A likely reason for the increased cell mass production and inhibited polysaccharide production with glucosamine is that glucosamine degrades through the fructose-6-biophosphate pathway [22]. Thus, the resulting ammonia produced from this pathway was released and the ammonia concentration in the medium had increased. The high available ammonia concentration was favorable for cell growth and might have inhibited polysaccharide synthesis. This was similar to the result in our previous study in which cell metabolic behavior had changed significantly to inhibit polysaccharide production in a flask culture with high concentrations of nitrogen (18.75 mM) (data not shown). In Fig. 2, we propose a central carbon metabolic pathway network and polysaccharide biosynthesis in *Beijerinckia indica* according to the finding detailed above and previous reports [3,13,14].

Growth of *Beijerinckia indica* under Different Nitrogen Concentrations

As previously mentioned, the findings suggest that ammonia might inhibit polysaccharide synthesis. Thus, the metabolic behaviors of *B. indica* were analyzed with various concentrations of nitrogen sources. Batch cultures of *B. indica* in a 5-L fermenter were carried out aerobically under various ammonium nitrate concentrations (7.5, 16.25, 18.75 mM). As shown in Fig. 3, when 7.5 mM NH_4NO_3 was used, the cells reached the exponential phase rapidly, dry cell weight increased slowly from 9 h to 20 h, and then cell density decreased gradually (Fig. 3A). At the cell growth phase at 9 h, approximately 50% of glucose and nearly all ammonia were consumed. Nitrate concentration was constant when ammonia was present in the broth and was only utilized after ammonia was consumed, demonstrating preference of ammonia to nitrate as a nitrogen source by *B. indica*. The polysaccharide concentration continued to increase and more than 70% of the total polysaccharide was produced after 20 h, when the net cell growth was negative. Viscosity showed a parallel increase to the PS-7 polysaccharide profile.

As shown in Fig. 3B with 16.25 mM of ammonium nitrate, the profiles of ammonia and nitrate are similar to those in Fig. 3A, with the exception that the time for total consumption of ammonia increased. The cell density continued to increase from 6 h to 16 h. The maximum cell density was 2.2 g/L, with higher cell density than that obtained with 7.5 mM NH_4NO_3 (1.78 g/L). The polysaccharide concentration continued to increase until 40 h. The maximum PS-7 concentration was 5.9 g/L, having lower concentration compared to the 7.42 g/L obtained with 7.5 mM NH_4NO_3.

In Fig. 3C, the metabolism profile was significantly different from the former two experiments, even though the NH_4NO_3 concentration was slightly higher than the experiment shown in Fig. 3B. The ammonia was consumed at 22 h when the cell density reached stationary phase. However, the nitrate was not utilized. Most of the polysaccharide was produced at the cell growth phase and polysaccharide concentration was lower compared to those of the former two experiments. Viscosity was also low. The residual glucose concentration was constant after 22 h and 60% of glucose was not consumed.

In Fig. 3D, the pH pattern of the culture with a high nitrogen level (18.75 mM) was different than those of low nitrogen levels (7.5, 16.25 mM). The pH decreased

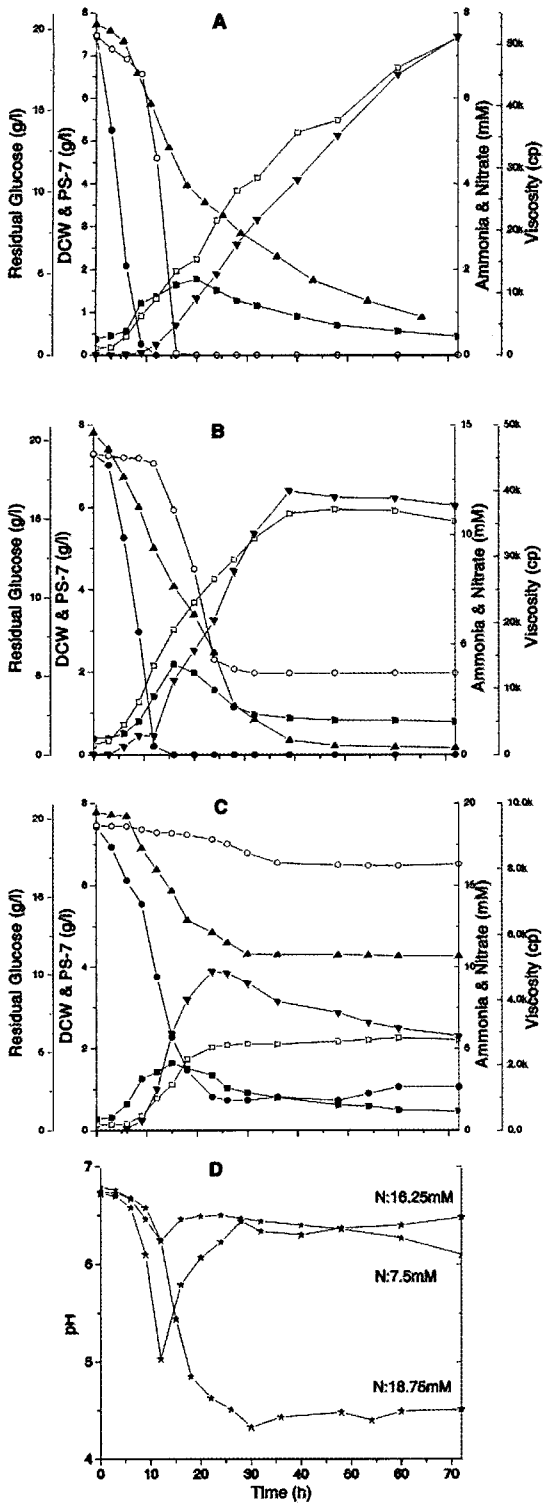

Fig. 3. PS-7 polysaccharide fermentation using *B. indica* under various concentrations of nitrogen source in a 5-L fermenter. A: culture with 7.5 mM NH_4NO_3, B: culture with 16.25 mM NH_4NO_3, C: culture with 18.75 mM NH_4NO_3, D: pH profiles of the cultures with 7.5 mM, 16.25 mM and 18.75 mM NH_4NO_3. Symbols: ■ DCW; □ PS-7; ● Ammonia; ○ Nitrate; ▲ Glucose; ▼ Viscosity; ★ pH.

to 4.3, demonstrating the consumption of glucose to form acids. At the relatively low ammonia concentration (7.5, 16.25 mM), pH profiles show the formation of acids at an early stage; however, the acids were utilized again after the depletion of ammonia. The pH of the cultures was also affected when ammonia was consumed. The pH of the culture decreased to 5.0 with 16.25 mM NH_4NO_3, and 6.2 with 7.5 mM NH_4NO_3.

Glucose consumption in culture with 16.25 mM NH_4NO_3 (Fig. 4B) was drastic and nearly 90% glucose was exhausted before 32 h, leading to less polysaccharide accumulation and even cease of polysaccharide synthesis after 40 h. In culture with 7.5 mM NH_4NO_3, glucose was consumed at a moderate rate, corresponding to a gradual accumulation of polysaccharide (Fig. 4A). When *B. indica* were cultured in 18.75 mM NH_4NO_3, glucose was consumed slowly before 6 h, indicating that cells would have to adapt to the condition of high nitrogen concentration (Fig. 4C). The long lag phase created by the high nitrogen concentration also resulted in lower biomass production, compared to maximum dry cell weight in culture with 16.25 mM NH_4NO_3 (Fig. 4B).

Most of the bacteria used for polysaccharide production from glucose or glucose derivatives cannot employ the EMP pathway since the phosphofructokinase activity was absent [13,14,23]. Therefore, glucose was metabolized through the Entner-Doudoroff pathway, as described for *X. campestris* [13], *Sphingomonas* [3] and *Sinorhizobium* [24]. The ED pathway produces less ATP and reducing potential than the EMP pathway. However, triose-3-phosphate can be rerouted to glucose-6-phophate, thus glucose-6-phosphate can be channeled to polysaccharide formation or again into the ED pathway [25].

Metabolic Flux Analysis Between High and Low C/N Ratio

From the findings obtained from the three cultures, it is clear that *B. indica* exhibited different metabolic behavior with changes in NH_4NO_3 concentration. Fig. 4 shows the calculated metabolite fluxes through the PP pathway, the ED pathway, and the TCA cycle under different nitrogen concentrations. Cells harvested from cultures with various nitrogen concentrations clearly showed different fluxome pattern.

The ED pathway had operated dominantly while the PP pathway was maintained to a lesser degree for anabolic precursor synthesis. When nitrogen concentration was slightly increased in the culture from 16.25 to 18.75 mM NH_4NO_3, the metabolism of bacteria was significantly changed. The gluconate-6-phosphate flux into the pentose phosphate pathway was 5 times higher than that of low nitrogen level conditions, though the flux to biomass was not greater than that of low nitrogen level condition. The PP pathway operated in an oxidative model and the carbon flux was recycled to fructose-6-phosphate and triose-3-phospahte, and approximately 29% increase of r_2 resulted from fructose-6-phosphate recycled from the PP pathway. The triose-3-phosphate directed to glycolysis (r_5) was also 2 times that in culture with 16.25

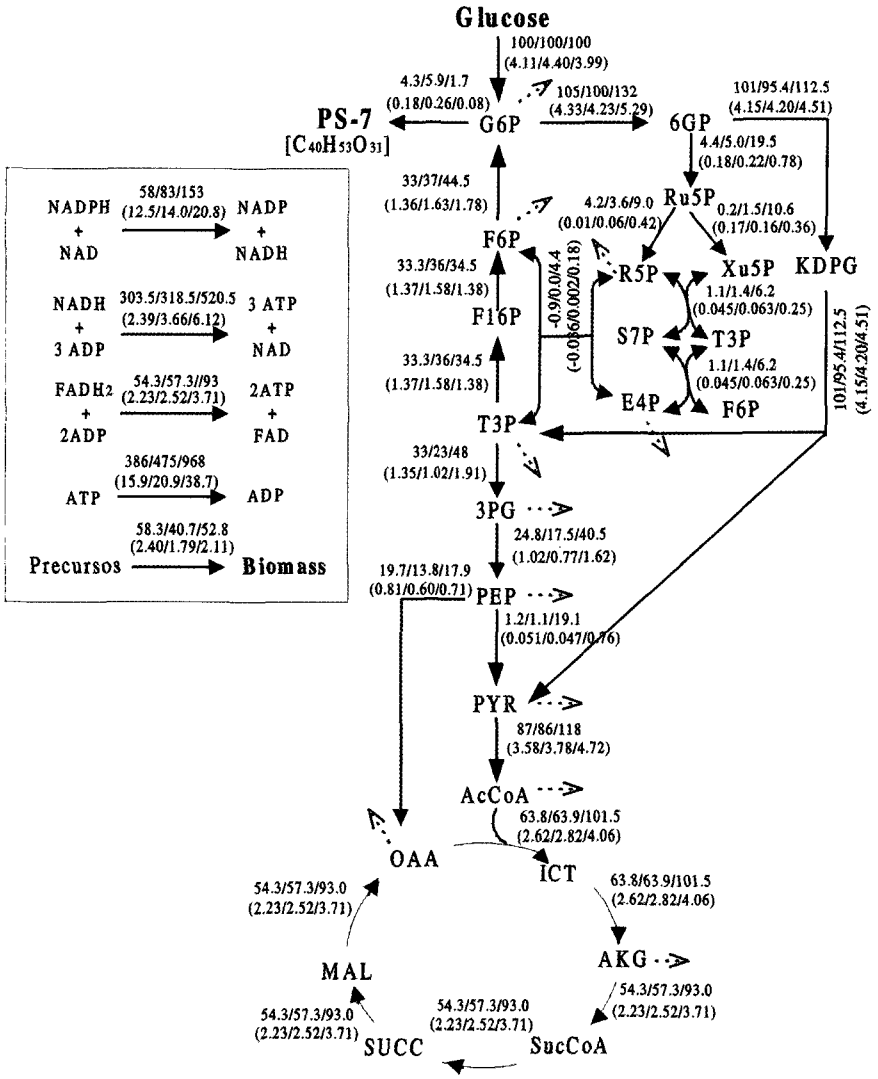

Fig. 4. Flux distributions under different levels of ammonium nitrate (7.5, 16.25, 18.75 mM). The fluxes (mM/(g DCW.h)) include normalized fluxes and actual fluxes (in parentheses). The symbol ⋯➢ indicates the precursors for biomass formation.

mM NH_4NO_3, resulting in more ATP and NADH productions.

The flux into gluconeogenesis pathway (T3P to F6P) was maintained stably at a rate of 1/3 of substrate uptake rate (r_1) and this increased the flux r_{10} (G6P to 6GP) higher than the substrate uptake rate. This was similar to the result (Fig. 3) that much more glucose was consumed at the growth phase. The reaction of PEP to PYR was carried out at a slower rate at low nitrogen levels and most of the produced PEP was used for anaplerotic reaction (OAA). At high nitrogen levels, this flux was about 16 times higher than those with low nitrogen levels. TCA cycle flux under high nitrogen concentration was about 1.64 times that at low nitrogen levels. As a result, excessive energy was formed and dissipated. With regard to glycolysis (r_5), the flux from the culture with 16.25 mM NH_4NO_3 was about 70% that with 7.5 mM NH_4NO_3 condition. This corresponded to low biomass flux and anaplerotic flux r_9 (PEP to OAA).

There was no increase in biomass production or polysaccharide production in the culture with high nitrogen level, and even the produced polysaccharide showed low molecular weight, which was estimated from the low viscosity. The bacteria utilized the nitrate as a nitrogen source after the consumption of ammonia when NH_4NO_3 was added to the culture. A large amount of residual nitrate was found in the culture with 18.75 mM NH_4NO_3. But the exopolysaccharide with high viscosity could be only accumulated under a high carbon to nitrogen ratio, signifying that the glucose was used for polysaccharide formation according to the flux distribution. The flux distribution of the cultures with relatively low nitrogen levels (7.5, and 16.25 mM) had similar flux distributions. In the culture with 16.25 mM NH_4NO_3, the flux to biomass was less than that of 7.5 mM nitrogen level and more glucose (r_{30}) was channeled to polysac-

charide biosynthesis at the exponential phase, which were similar to the results shown in Fig. 3A and B. However, polysaccharide continued to accumulate until 72 h in the culture with 7.5 mM NH_4NO_3. In the case of the culture with 16.25 mM NH_4NO_3, it reached its maximal level, 5.9 g/L at 40 h. The reason might be that the metabolic activity was higher in the culture with 16.25 mM NH_4NO_3 and more energy was dissipated (r_{31}). In addition, the nitrate in the culture with 7.5 mM NH_4NO_3 was depleted early, at 16 h, and this created a high C/N ratio that was favorable for polysaccharide biosynthesis. Most of the nitrate in the medium with 16.25 mM NH_4NO_3 was consumed before 40 h and after the consumption of ammonium. Utilization of nitrate required more energy compared to ammonium utilization.

In the culture with 18.75 mM NH_4NO_3, the flux in the TCA cycle and the dissipation (r_{31}) had nearly doubled. After cell growth came to a stop, there was still a small quantity of ammonium and much nitrate in the medium. Under a low carbon/nitrogen ratio, the cells could not direct the substrate flux to polysaccharide synthesis. Thus, the polysaccharide reached a maximal 2 g/L at 24 h and then ceased to increase (Fig. 3). Industrial strain *Xanthanmonas camrestris* can convert 70% of glucose to xanthan production. In this study, *Beijerinckia indica* can only convert about 40% of glucose to PS-7 in batch culture. In the recombinant strain with augmentation of EPS biosynthetic gene [3], it can convert 60% glucose to biopolymer formation. The reason might be that triose-3-phosphate recycling into glucose-6-phosphate (gluconeogenesis pathway) is energetically more inefficient than glycolysis (T3P to PYR). However, in a ^{13}C-labelled metabolic flux analysis with *Azotobacter vinelanii*, bacteria metabolized glucose through the Entner-Dodoroff pathway and synthesis of alginate occurred completely from the gluconeogenesis of triose-3-phosphate generated from the ED pathway [23]. In this study, there was approximately 33% triose-3-phosphate recycling to hexose-6-phosphate under all conditions of nitrogen concentrations. This cyclic organization had negative effects on cell metabolism. If no ATP was directly consumed throughout the gluconeogenic part of the cycle, the conversion of two triose-3-phosphates into one glucose-6-phosphate occurs at the expense of a phosphoester bond. Thus, the cyclic organization of carbohydrate metabolism in *Beijerinckia.indica* appears to be dissipative at the level of both carbon and energy. The repeat unit of PS-7 polysaccharide includes 2-deoxy-glucuronate and formation of these sugar nucleotides requires 2 M NADPH per repeat unit. Therefore, biosynthesis of PS-7 is more energy demanding than xanthan.

CONCLUSION

Beijerinckia indica metabolized carbon sources through ED and PP pathways. Cell growth and EPS biosynthesis were significantly influenced by environmental nitrogen concentration. Under low nitrogen conditions, a greater amount of polysaccharide was converted from glucose than was converted with glucosamine, glycerol and gluconate. Growing *B. indica* on glucosamine increased cell biomass production, but produced little polysaccharide because of the accumulation of ammonia by glucosamine metabolism. In order to investigate the effects of nitrogen in the culture, a metabolic pathway network under varying concentrations of nitrogen was studied by using metabolic flux analysis. The intracellular flux distributions in *Beijerinckia indica* showed that the ED pathway was operated dominantly and the PP pathway was maintained to a lesser degree. Triose-3-phosphate cycling was present in the gluconeogenesis pathway, which increased the intracellular pool of hexose-phosphate for polysaccharide synthesis. The culture containing high levels of nitrogen showed a drastic change in metabolic flux. The fluxes in the ED pathway, PP pathway, glycolysis, and TCA cycle were increased. Thus, more carbon source and energy were dissipated and the production of PS-7 polysaccharide decreased. It appears that the high metabolic activity was due to the depletion of nitrogen sources in the medium; subsequently, carbon sources were directed for polysaccharide accumulation.

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NOMENCLATURE

T3P	Triose-phosphate
SUCC	Succinate
AcCoA	Acetyl-coenzyme A
OAA	Oxaloacetate
F6P	Fructose-6-phosphate
NADH	Nicotinamide adenine dinucleotide, reduced
E4P	Erythrose-4-phosphate
3PG	3-Phosphoglycerate
G6P	Glucose-6-phosphate
6GP	Gluconate-6-phosphate
F16P	Fructose-1, 6-biphosphate
GLC	Glucose
MAL	Malate
NAD	Nicotinamide adenine dinucleotide
S7P	Sedohepulose-7-phosphate
X5P	xylulose-5-phosphate
PYR	Pyruvate
R5P	Ribose-5-phosphate

Biomass

SucCoA	Succinyl-coenzyme A
PEP	phosphoenolpyruvate
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced
ADP	Adenosine 5'-diphosphate

ATP	Adenosine 5'-triphosphate	FADH	Flavine adenine dinucleotide, reduced
CIT	Citrate	FAD	Flavine adenine dinucleotide
ICIT	Isocitrate	KDPG	2-keto-3-deoxy-6-phosphate-gluconate
Ru5P	Ribulose-5-phosphate	AKG	2-keto-glutarate
PS-7	Exopolysaccharide	NADP	Nicotinamide adenine dinucleotide phosphate

Appendix

NO.	Name	Reaction
r_1	Trans1	GLC + [ATP] -> G6P + [ADP]
r_2	Gly01	F6P <-> G6P
r_3	Gly02	F16P -> F6P
r_4	Gly03	2 T3P <-> F16P
r_5	Gly04	T3P + [NAD] + [ADP] <-> [NADH] + [ATP] + 3PG
r_6	Gly05	3PG <-> PEP
r_7	Gly06	PEP + [ADP] -> PYR + [ATP]
r_8	Gly07	PYR + [COA] + [NAD] -> [NADH] + ACCOA
r_9	Gly08	PEP -> OAA
r_{10}	PPP01	G6P + [NADP] -> [NADPH] + 6GP
r_{11}	PPP02	6GP + [NADP] -> [NADPH] + RL5P
r_{12}	PPP03	Ru5P <-> R5P
r_{13}	PPP04	Ru5P <-> X5P
r_{14}	PPP05	R5P + X5P <-> T3P + S7P
r_{15}	PPP06	T3P + S7P <-> E4P + F6P
r_{16}	PPP07	X5P + E4P <-> F6P + T3P
r_{17}	ED01	D6PGC -> KDPG
r_{18}	ED02	KDPG -> T3P + PYR
r_{19}	TCA01	ACCOA + OAA -> [COA] + CIT
r_{20}	TCA02	CIT <-> ICIT
r_{21}	TCA03	ICIT + [NADP] -> [NADPH] + AKG
r_{22}	TCA04	AKG + [NAD] + [COA] -> [NADH] + SUCCOA
r_{23}	TCA05	SUCCOA + [ADP] -> [ATP] + [COA] + SUCC
r_{24}	TCA06	SUCC + [FAD] -> [FADH] + MAL
r_{25}	TCA09	MAL + [NAD] -> [NADH] + OAA
r_{26}	Energy01	[NADH] + 2 [ADP] -> [NAD] + 2 [ATP]
r_{27}	Energy02	[NADPH] + [NAD] <-> [NADP] + [NADH]
r_{28}	Energy03	[FADH] + [ADP] -> [FAD] + [ATP]
r_{29}	Growth	4.11 [ATP] + 0.3547 [NAD] + 1.8225 [NADPH] + 0.0205 [G6P] + 0.0071 [F6P] + 0.0898 [R5P] + 0.0361 [E4P] + 0.0129 [T3P] + 0.0719 [PEP] + 0.2833 [PYR] + 0.2928 [ACCOA] + 0.1787 [OAA] + 0.1079 [AKG] + 0.1496 [3PG] -> 4.11 [ADP] + 0.3547 [NADH] + 1.8225 [NADP] + [BIOMASS]
r_{30}	EPS synthesis	6 [ATP] + 6 G6P + 2 [NADPH] + 2 [ACCOA] -> PS-7 + 2 [NADP] + 6 [ADP] + 2 [COA]
r_{31}	Energy04	[ATP] -> [ADP]

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