KOR. JOUR. MICROBIOL. March, 1987 p. 28-33 Vol. 25, No. 1

Carbohydrate Catabolism in Cellulolytic Strains of *Cellulomonas, Pseudomonas* and *Nocardia*

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Cellulose 분해호기성 세균의 당 대사 경로

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ABSTRACT: Celluloytic bacteria, -Gram positive, Gram negative and actinomycetes-were used to study their catabolic pathways of carbohydrate. It was found that Embden-Meyerhof -Parnas(EMP) pathway and hexose monophosphate(MHP) shunt were operated in *Cellulomonase* sp. CS1-1, *C. flavigena,* and *Pseudomonas fluorescens* subsp. *cellulosa* when they were cultured in a glucose containing medium, whilst gluconate was catabolised mainly via Entner-Doudoroff(ED) pathway, and to some extend through HMP shunt. Enzymes of ED pathway in the organisms were induced by gluconate. On the other hand *Nocardia cellulans* catabolised glucose and gluconate via EMP pathway and HMP shunt. The growth rate of *N. Cellulans* on gluconate were much slower than that on glucose.

KEY WORDS □ Glycolysis, Entner Doudoroff pathway, Cellulolytic bacteria, *Cellulomonas, Pseudomonas, Nocardia.*

Cellulolytic activity is found in wide varieties of bacteria including Gram positive and Gram negative eubacteria as well as actinomycetes. Few attempts have been made to clarify their biochemistry or physiology except for their celluloytic enzyme systems.

Strains of *Cellulomonas* have been used in a radiorespirometric study using labelled glucose(Stackebrandt and Kandler 1980). It was reported that glucose is catabolised mainly via EMP pathway and, to some extent, through HMP shunt.

In the present study attempts were made to find out the catabolic pathway of glucose and gluconate in cellulolytic strains of *Cellulomonas, Pseudomonas* and *Nocardia.*

MATERIALS AND METHODS

Bacterial Strains

Two strains of *Cellulomonas* used were *Cellulomonas* sp. CS 1-1(Choi *et al.*, 1978). (Kindly supplied by Prof. W.Y. Choi, Chungnam Univ.) and *C. flavigena* NCIB 8077. Gram negative bacterium used was *Pseudomonas fluorescens* subsp. cellulosa NCIB 10462. *Nocardia cellulans* NCIB 8868 was also used. The organisms were maintained on potato-cellulose agar slopes(Kim and Wimpenmy, 1981).

Growth of Organisms

The inoculum was grown in a 250 m*l*Erlenmeyer flask containing a mineral salt medium(Kim and Wimpenmy, 1981) added by 0.1%(W/V) yeast extract and carbon source. Carbon sources used were 2%(W/V) glucose, 2.4%(W/V) sodium gluconate and 3.0%(W/V) sodium acetate. The main culture was made in a 2 *l* fermenter(Bench-top fermenter C-32, New Brunswick Scientific Co., New Jersey, U.S.A.). The growth was initiated by a 5% inoculum. The fermenter was operated at pH 6.8-7.2 and volumetric oxygen transfer coefficient of about 100 hr^{-1}. Temperature was kept at 30℃ except *Ps. fluorescens*, which was grown at 37℃. The cell concentration was determined by measuring the absorbance at 660 nm.

Preparation of Cell-free Extract

Cultures at the late exponential growth phase were centrifuged(7,500×g for 10 min) and the cells were washed 3 times with 20 mM(hydroxymethylaminomethane)-acetate buffer(pH 7.5) containing 0.02 M β-mercaptoethanol. The cell paste was resuspended in the same buffer of one twentieth volume of the original culture, and ruptured using a French press(Hughes *et al.*, 1971). The disrupted cells were centrifuged at 22,000×g for 30 min. The supernatant was used for enzyme assay. When the enzyme assay was not performed immediately after the rupture, the superatant was stored at −40℃.

Assays

Enzyme activities were determine at 25℃ by monitoring the changes in absorbance of the reaction mixture at 340 nm due to the reduction of NAD(P)H or oxidation of NAD(P). Fructose-1,6-diphosphate(FDP) aldolase(E.C. 4.1.2.13) activity was measured by the method of Rutter and Hunsley(1966). The method of Pontremoli and Grazi(1966) was followed for the measurement of 6-phosphogluconate(PG) dehydrogenase(E.C. 1.1.1.44) activity. PG dehydrase(E.C.4.2.1.12) activity was assayed by coupling its activity with endogeneous 2-Keto-3-deoxy-6-phosphogluconate aldolase(E.C.4.1.2.14) as described by Wood(1972) using PG as the substrate. The value for the molar extinction coefficient of NAD(P)H used was 6.22×10^6. Specific activity was expressed as μ mol of product formed in one minute by 1 mg of protein. Protein was determined by the method of Lowry *et al*(1951).

Chemicals used

Chemicals used in the enzyme assay were FDP, PG, NADH, NADP, L-Cysteine, imidazol, glutathione, β-mercaptoethanol, lactate dehydrogenase(E.C.1.1.1.27) (Sigma Chem. Co.), and α-glycerol phosphate dehydrogenase-triosephosphate isomerase mixed crystal(Boeringer Mannheim). All other chemicals used were extra pure grade.

RESULTS AND DISCUSSION

Growth on Glucose and Gluconate

Inocula were grown in the media containing glucose or gluconate. Each inoculum was transferred to the main culture media containing glucose or gluconate. The growth of each organism was monitored. Fig.1a shows the

Table 1. *Growth kinetics of cellulolytic bacteria on glucose and gluconate.*

	Substrate used		Lag phase (hr)	Specific growth rate (hr^{-1})
	Inoculum cultture	Main culture		
Cellulomonas sp. CS1-1	Glucose	Glucose	1.0	0.32
		Gluconate	6.3	0.33
	Gluconate	Glucose	1.0	0.30
		Gluconate	1.0	0.30
C. flavigena NCIB 8077	Glucose	Glucose	1.5	0.28
		Gluconate	2.6	0.27
	Gluconate	Glucose	1.5	0.29
		Gluconate	1.0	0.27
Ps. fluorescens subsp. cellulosa NCIB 10462	Glucose	Glucose	0.5	0.39
		Gluconate	3.0	0.36
	Gluconate	Glucose	0.5	0.35
		Gluconate	0.5	0.39
N. cellulans NCIB 8868	Glucose	Glucose	1.0	0.43
		Gluconate	2.4	0.26
	Gluconate	Glucose	1.0	0.43
		Gluconate	3.0	0.26

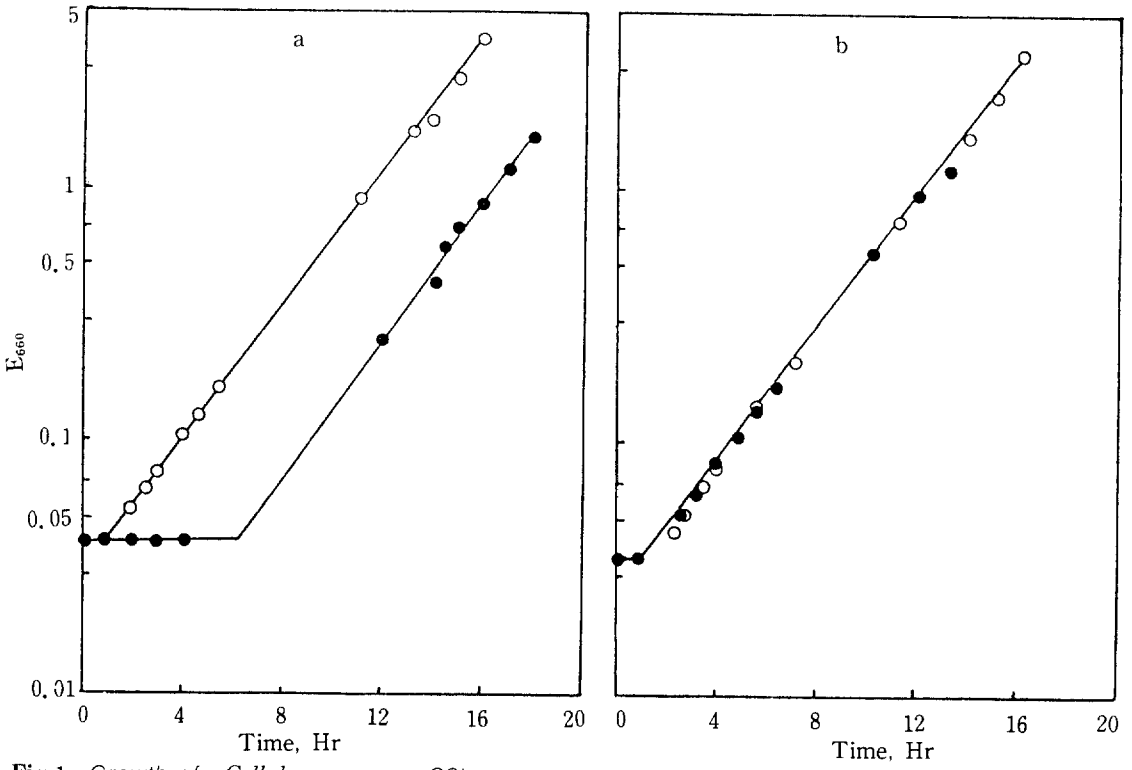

Fig.1. *Growth of Cellulomonas sp CSI-1 on glucose and gluconate*
a; glucose inoculum, b; gluconate inoculum ○—○ glucose ●—● Gluconate

growth of *Cellulomonas* sp. CSl-1 precultured on glucose, and the growth of the organism precultured on gluconate was shown in Fig.1b.

Little difference was observed in the growth rates of the cultures using glucose and gluconate. But the lag phase was longer when inoculm grown on glucose was used in the main culture on gluconate. Lag phase was about 1 hr in the other cultures. Table 1 summerises the results of similar experiments using other organisms.

C. flavigena and *Ps. fluorescens* needed longer time to adopt new substrate in the main culture but their growth rates on gluconate were practically the same as those on glucose. These rusults suggest that the organisms can utilise gluconate as efficiently as glucose in a different pathway. On the other hand cultures of *N. cellulans* on gluconate needed longer lag phase and grew more slowly than those on glucose regardless the carbon sources used in the inoculum culture.

Enzyme activities of *Cellulomonas* and *Ps. fluorescens* Grown on Glucose and Gluconate.

Cells harvested from the previous experiments were extracted to use in enzyme assays of glycolytic pathways. The results are give in Table 2.

When the organisms were cultured on glucose a high activity level of FDP aldolase was observed and PG dehydrogenase activities were low compared to the former. Low PG dehydrase activity was observed in *Ps. fluorescens* cells grown on glucose whilst glucose grown *Cellulomonas* showed no PG dehydrase activity. No difference were observed between inoculum prepared on different carbon sources. These results shows that *Cellulomonas* catabolise glucose mainly via EMP pathway and to some extent through HMP shunt. The same conclusion was made by Stackebrandt and Kandler

Table 2. *Glycolytic enzyme activities in cellulolytic bacteria grown on glucose and gluconate.*

	Substrate used		Activity (Unit/mg protein)		
	Inoculum culture	Main culture	FDP aldolase	PG dehydr ogenase	PG dehydrase
Cellulomonas sp. CS1-1	Glucose	Glucose	0.55	T[a]	–[b]
		Gluconate	0.03	T	0.20
	Gluconate	Glucose	0.29	T	–
		Gluconate	0.02	T	0.17
C. flavigena NCIB 8077	Glucose	Glucose	0.48	T	–
		Gluconate	T	0.01	T
	Gluconate	Glucose	0.46	T	T
		Gluconate	0.01	T	0.17
Ps. fluorescens subsp. cellulosa NCIB 10462	Glucose	Glucose	0.41	0.18	0.16
		Gluconate	0.03	0.19	0.44
	Gluconate	Glucose	0.51	0.17	0.03
		Gluconate	0.02	0.23	0.18
N. cellulans NCIB 8868	Glucose	Glucose	0.40	0.46	–
		Gluconate	0.60	0.63	–
	Gluconate	Glucose	0.49	0.47	–
		Gluconate	0.43	0.31	–

a; T; trace, enzyme activity less than 1×10^{-3} unit/mg protein
b; activity was not detected.

(1980).

When *Ps. fluorescens* were grown on glucose, low activity of PG dehydrase was found in addition to FDP aldolase and PG dehydrogenase activity. Similar results can be found in other fluorescent pseudomonad(Wettermark *et al.*, 1979). When the organisms were grown on gluconate FDP aldolase activity was much lower compared to glucose cultures, and high PG dehydrase activities were observed. These results show that *Cellulomonas* and *Ps. fluorescens* catabolise glucose via EMP pathway and HMP shunt and gluconate through ED pathway and HMP shunt. Similar results were found in fluorescent *pseudomonads* (Wettermark *et al.*, 1979) and *E. Coli*(Eisenberg *et al.*, 1967, Kornber and Soutar, 1973). In these organisms enzymes of ED pathway were reported to be induced by gluconate.

This is the first to show enzyme activity of ED pathway in *Cellulomonas*. ED pathway is rarely found in Gram positive bacteria. Another Gram positive bacterium reported to operate ED pathway is *Arthrobacter*(Zagallo and Wang, 1967) which belongs to the same group, coryneform bacteria as *Cellulomonas* does.

Enzyme Activities of Acetate Grown cells of *Cellulomonas*

Cellulomonas cells grown on acetate were extracted and measured for activities of FDP aldolase, PG dehydrogenase and PG dehydrase(Table 3).

PG dehydrease activities were not found in *Cellulomonas* sp. CS 1-1 and *C. flavigena*.

Table 3. *Glycolytic enzyme activities of Cellulomonas grown on acetate.*

Strain	Acetivity (Unit/mg protein)		
	FDP aldolase	PG dehydrogenase	PG dehydrase
Cellulomonas sp. CS1-1	0.86	0.51	T
C. flavigena NCIB 8077	0.74	0.63	–

These results shown that PG dehydrase is induced by gluconate not depressed by glucose.

Much higher PG dehydrogenase activity was found in *Cellulomonas* cells grown on acetate than those on glucose or gluconate. These explain that active HMP shunt is necessary for the conversion of acetate into hexoses and pentoses, which are needed as the cell components.

Glycolytic Pathway in *N. cellulans*

As shown in table 2 little differences in enzyme activities between cells of *N. cellulans* grown on glucose and gluconate. Relatively high PG dehydrogenase activity were measured compared to the other organisms tested, and no measurable PG dehydrase activities were found.

From the data of growth kinetics and enzyme assays it can be said that glucose and gluconate are catabolised through EMP pathway and HMP shunt by *N. cellulans*. It is believed that part of gluconate was utilised through EMP pathway after it had been reduced to glucose using NAD(P)H obtained via HMP shunt.

적 요

Cellulose 분해호기성 세균 중 Gram 양성균인 *Cellulomonas*, Gram 음성균 *Pseudomonas* 및 actinomycetes에 속하는 *Nocardia*를 포도당 및 gluconate를 탄소원으로 배양할때 그들의 해당과정에 관여하는 효소의 역가를 측정하였다. 포도당을 포함하는 배지에서 배양했을 경우 사용한 모든 세균은 Embden-Meyerhof-Parnas(EMP) 경로와 Hexose Monophosphate(HMP) 경로로 당을 자화하였다. Gluconate를 기질로 이용할때 *Cellulomonas*와 *Pseudomonas*는 Entner-Doudoroff(ED) 경로와 HMP 경로가 해당과정이었으며 *Nocardia*는 EMP 경로로 해당하였다. ED 경로에 관여하는 효소는 gluconate에 의해 유도되는 것으로 나타났다.

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(Received Jan. 19, 1987)