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Characterization and evaluation of a *pta* (phosphotransacetylase) negative mutant of *Escherichia coli* HB101 as production host of foreign lipase

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Abstract In order to evaluate the *pta*(phosphotransacetylase) (–) mutant of *Escherichia coli* as a potential host of foreign lipase expression, the *pta*(–) mutant HB101 was constructed for the purpose of blocking the acetate synthetic pathway. Since acetate is known as a major inhibitory by-product of cell growth and foreign protein production, the growth characteristics and expression kinetics of the microbial lipase of the *pta*(–) *E. coli* mutant were investigated. The growth rate was considerably decreased (about 30%) when grown on M9 minimal media containing glucose, mannose or glycerol. Growth retardation was not observed when a gluconeogenic carbon source (acetate, malate or succinate) was utilized. It should be noted that the growth rate of the mutant was enhanced (about 20%) in modified M9 media including a gluconeogenic carbon source and NZ-amine. Growth inhibition of the *pta*(–) mutant by menadione, a representative redox-cycling drug, was more pronounced than that of the parental type of *E. coli*. Furthermore, the inhibition effect was more pronounced in glucose minimal medium, whereas the menadione sensitivity was not observed when a gluconeogenic carbon source was used as a sole carbon source or the lactate dehydrogenase gene from *Lactobacillus casei* was introduced in the *pta*(–) mutant. Therefore, it is suggested that the growth deficiency of the *pta*(–) mutant is closely related to the intracellular redox balance. When the pseudomonad lipase was expressed in the *pta*(–) mutant, a comparable expression rate and yield to the parental type strain was observed. High-cell-density culture of the mutant was easy to achieve even under the fluctuating conditions of residual glucose concentration.

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Introduction

The production of acetate by aerobically growing *Escherichia coli* occurs when the critical growth rate is excessive in the presence of a high glucose concentration. Because *E. coli* has a limited capacity for aerobic respiration, especially the electron transport chain or ATP-synthesizing machinery, carbon flux into the cells overloads the central metabolic pathways above the critical growth rate and is excreted as acetate (Andersen et al. 1980; El-Mansi and Holms 1989).

For many years, world-wide research has been executed to discover the physiological function of the acidic by-product in *E. coli*. Compared with other fermentative by-products such as lactate, formate and ethanol, three different properties of acetate can be noted. First, acetate production is concomitant with equimolar ATP synthesis. Therefore, a rapidly growing *E. coli* cell is able to utilize the pathway as a secondary energy-yielding source. Second, NADH oxidation does not occur by the acetate synthetic pathway. Because one of the most important functions of the fermentative pathway of *E. coli* is the regeneration of NAD^+, the amount of acetate produced is seriously affected by the intracellular redox state [NAD^+/NADH], especially under anaerobic conditions. Recently, the possibility of acetyl phosphate, an intermediate of the acetate pathway, as an important global signal was proposed (McCleary et al. 1993; Wanner and Wilmes-Riesenberg 1992). In most of the phosphorylation-dependent regulation of cellular signal transduction including Pho, Ntr and the motility regulon, acetyl phosphate could be responsible for the activation of many response regulator proteins because this small molecule could donate its phosphoryl group non-specifically. Therefore, the acetate pathway appears to be absolutely required for survival and optimal growth in a nutritionally poor environment, for example the intestinal tract of animals or in soil. However, there has been no clear explanation as to the physiological significance of the existence of the acetate synthetic pathway in *E. coli*.

The accumulation of acetate in culture medium has been suggested to have deleterious effects on cell growth and the expression of cloned genes by recombinant *E. coli* (Landwall and Holme 1977; Brown et al. 1985; Pan et al. 1987; Rinas et al. 1989; Luli and Strohl 1990). At present, the optimization strategy applied for the production of valuable materials in recombinant *E. coli* is focused on high-cell-density culture, that is, recombinant cells should be cultured in as high a density as possible in a fermentor in which the volume is restricted for secure process control, and the foreign gene should be efficiently expressed in such a state (Riesenberg 1991). Because most of the culture media for high-cell-density culture of *E. coli* include a substantial amount of glucose and acetate production is a major factor in the limitation of high-density growth of *E. coli* cells, many biochemical engineers have developed optimal feeding strategies for fed-batch operation to prevent the accumulation of acetate for both wild-type and recombinant *E. coli* (Konstantinov et al. 1990; Riesenberg et al. 1991).

It is known that two enzymatic pathways for acetate synthesis under aerobic conditions exist in *E. coli.* In the minor one, acetate is derived directly and irreversibly from pyruvate by pyruvate oxidase. The major one, which is reversible, is the phosphotransacetylase(*pta*)-acetate kinase(*ackA*) pathway starting from acetyl-coenzyme A (CoA). Therefore, it could be argued that an *E. coli* mutant defective in either or both *ackA* and *pta* could be utilized as a more preferable host for recombinant protein production. Recently, it has been reported that when human interleukin-2 (IL-2) was expressed in an acetate-negative mutant of *E. coli*, improved accumulation of IL-2 resulted and growth deficiency was not observed (Bauer et al. 1990). In contrast, Diaz-Ricci et al. (1991) reported that an *E. coli* mutant without both *pta* and *ackA* activities showed some problems of growth and glucose uptake relative to the parental strain. To our knowledge, there have been no reports on the characterization of an acetate-negative mutant as an expression host. We have constructed an insertion mutant of the phosphotransacetylase gene of *E. coli* HB101 by blocking the acetate synthetic pathway. By using the mutant, we have tried to suggest the potential of an acetate-negative mutant of *E. coli* as an excellent host for recombinant protein production. First of all, the growth characteristics of the mutant was investigated under various culture conditions. Then the expression profile was observed when the microbial lipase was induced into the mutant. Finally, the possibility of high-cell-density culture of the *pta* negative mutant without any fine strategy of glucose feeding was examined.

Materials and methods

Microorganisms and plasmids

The microorganisms used in this study were *E. coli* HB101 (F-*hsd20*(r_B–m_B–) *leu supE44 ara14 galK2 lacY1 proA2 rpsL20(Str^r) xyl-5 mtl-1 recA13 mcrB*) and its acetate-negative mutant PN101 (HB101 *pta*: :*TnphoA*'$^{-3}$(Km^R)). The acetate-negative mutant PN101 lacked phosphotransacetylase (*pta*) [acetyl-CoA: orthophosphate acetyl transferase ; EC 2. 3. 1. 8] activity and was constructed by P1 transduction with selection for growth deficiency in LB (Luria Bertani)-kanamycin agar plates. P1 lysates used for construction of the mutant were obtained from Professor Park's laboratory at KAIST. Plasmid pTTY2 contained the *tac*–promoter-controlled lipase gene (about 1.6 kb), which originated from *Pseudomonas fluorescens* SIK W1(Chung et al. 1991b). Lipase expression from pTTY2 was carried out by the addition of 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG) to the medium. The L-lactate dehydrogenase (LDH) gene (about 1.6 kb) from *Lactobacillus casei* was consistently expressed in *E. coli* at the same level as that of natural production in *L. casei* (about 12.6 units/mg protein), irrespective of the growing phase (Kim et al. 1991).

Table 1 The medium composition for high-cell-density culture of the *pta*(–) mutant PN101 (g/l)

Component	Initial concentration	Feeding medium
Glucose	10	600
NH_4Cl	10	
KH_2PO_4	10	
$NaH_2PO_4 \cdot 2H_2O$	10	
$Na_2HPO_4 \cdot 2H_2O$	5.0	
$MgSO_4 \cdot 7H_2O$	1.0	25
Trace elements[a]	2.0 ml	
Trisodium citrate	5.0	
Proline	3.0	
Thiamine	5 mg	
Ampicillin	100 mg	

[a] Composition of trace elements (g/l): $CaCl_2 \cdot 2H_2O$, 13.2; $FeSO_4 \cdot 7H_2O$, 8.4; $MnSO_4 \cdot 4H_2O$, 2.4; $ZnSO_4 \cdot 7H_2O$, 2.4; $CuSO_4 \cdot 5H_2O$, 0.48; $CoCl_2 \cdot 6H_2O$. 0.48; $Na_2MoO_4 \cdot 2H_2O$, 0.24; $K_2B_4O_7 \cdot xH_2O$, 0.06

Media

The medium for the present study was M9ZB: 6.0 g/l of Na_2HPO_4, 3.0 g/l of KH_2PO_4, 0.5 g/l of NaCl, 1.0 g/l of NH_4Cl, 0.1 mM $CaCl_2$, 2.0 mM $MgSO_4$, 4 g/l of glucose and 2 g/l of NZ-amine (Scheffield). For the study of growth characteristics of the *pta* mutant, the medium was M9ZB, of which the sole carbon source was glucose, glycerol, mannose, acetate, malate or succinate. The medium composition for high-cell-density culture of the *pta* negative mutant is shown in Table 1.

Culture conditions

Most of cultivations were done in a 500-ml baffled flask or a 5-l fermentor (Korea Fermentor Co., Korea), which were operated at an aeration rate of 0.5–1.0 vvm and agitation speed of 500–1000 rpm. The working volume of the fermentor was 3.5 l. Temperature and pH were controlled at 37° C and 7.0, respectively. When necessary, ampicillin (50 μ g/ml) or kanamycin (50 μ g/ml) was added to the culture broth as an antibiotic marker. Menadione, a representative redox cycling drug, was purchased from Sigma (St. Louis, Mo., USA). In high-cell-density culture of the *pta* negative mutant, feeding of the medium was manually controlled and induction was carried out at dry cell weights (DCW) of 40 and 75 g/l.

Measurement of cell growth

Cell mass was determined by measuring the optical density (OD) at 600 nm (Bausch & Lomb Spectronic 21 and Beckman DU-6 spectrophotometer). For exact measurement of maximum specific growth rate of cells, Bio-SCR (Lab System, Sweden), an automated OD measuring instrument, was used in the experiments of growth effects of menadione. The loading volume was 0.3 ml and the interval of OD measurement was 20 min.

Analyses of glucose, acetate, lactate, pyruvate and CO_2

Concentrations of residual glucose and L-lactate were determined by using a Glucose and L-Lactate Analyzer (YSI Model 2000, YSI, Yellow Springs, Ohio, USA). Concentrations of acetate, D-lactate and pyruvate were determined using an enzymatic test kit from Boehringer-Mannheim (Germany). The CO_2 evolution rate was measured by a TOA O_2–CO_2 analyser (FOCA-1, TOA Electronics, Japan).

Isolation of plasmid pTTY2

For rapid isolation of the plasmids from bacterial culture, the alkaline lysis method described by Birnboim and Doly was employed (Maniatis et al. 1982).

Transformation of *E. coli* by plasmid pTTY2

Competent cells of *E. coli* HB101 and PN101 for transformation were prepared using the method described by Hanahan (1985). Transformation by plasmid DNA was carried out as follows: a DNA sample was added to 100 μl of *E. coli* competent cells and mixed gently. After standing in ice for 30 min, a heat shock was applied to the cell suspension at 42° C for 90 s and the mixture was diluted into an appropriate volume of SOC medium and incubated at 37° C for 1 h with shaking. In order to isolate the transformants exhibiting lipase activity, the incubated suspension was spread on a turbid agar plate containing 100 μg/ml of ampicillin and tributyrin (0.5%). After incubation overnight at 37° C, colonies that showed clear areas were selected.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of lipase and analysis of the expression level of the cloned lipase gene in *E. coli*

Discontinuous SDS-PAGE employed 2-mm-thick slab gels that contained 9% (w/v) polyacrylamide for lipase expression (Laemmli 1970). For analysis of total protein at different times after induction, 1 ml culture broth was centrifuged for 1 min in an Eppendorf microcentrifuge and bacterial cells were resuspended in 100 μl Laemmli sample buffer (2X). The samples were boiled for 5 min and then 30 μl of each sample was loaded for SDS-PAGE analysis. The electrophoresis was run at a constant current of 20 mA. Gel was stained in the mixed solution of methanol, acetic acid and water (400:70:530, v/v/v) with 0.25% Coomassie brilliant blue G-50 and was destained by soaking in the same solution without Coomassie brilliant blue G-50. After destaining, the gel was dried inside a gel drying film and then was scanned by a laser densitometer (LKB, USA).

Results

Batch cultivation

Among the several *E. coli* mutants tested for expression of the lipase gene, strain HB101 was found to be the most stable as a host strain for the overexpression of foreign lipase by IPTG. Therefore, we constructed the *pta*(–) and *ackA*(–) mutants of strain HB101, compared the growth and acetate production between both mutants using a baffled flask and confirmed that the *pta*(–) mutant was more effective than the *ackA*(–) mutant of HB101 for blocking the acetate pathway (Fig. 1). Moreover, it was previously reported that acetyl phosphate, an intermediate that accumulated in the *ackA*(–) mutant, was spontaneously hydrolysed to acetate under the physiological conditions used even in the absence of acetate kinase (Brown et al. 1977). Therefore, we finally adopted the *pta*(–) mutant of HB101 as a non-acetate-producing microorganism.

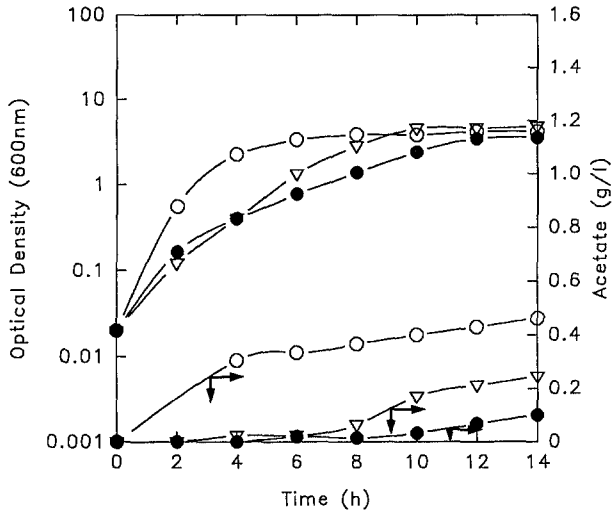

Fig. 1 Time course of cell growth and acetate production of the parental type (○), the phosphotransacetylase-negative mutant [*pta*(–)] (●) and the acetate kinase negative mutant [*ackA*(–)] ▽ of *Escherichia coli* HB101 (baffled flask experiment)

Cell growth, acetate production, the specific glucose uptake rate (Q_s) and the specific CO_2 evolution rate (Q_{CO_2}) by *E. coli* HB101 and its *pta*(–) mutant PN101 are shown in Fig. 2. Growth deficiency of strain PN101 was observed in M9ZB glucose medium supplemented with 0.1% proline. When 4 g/l of glucose was used initially, the acetate was produced at an almost constant level of 0.14 g/l for strain PN101, whereas, for the parental strain HB101, the acetate produced was a maximal 0.33 g/l after the exponential phase and then decreased in the stationary phase due to uptake by the cells. It was observed that the Q_s and the Q_{CO_2} of strain PN101 were lower than those of strain HB101 during the exponential phase (Fig. 2B, C).

Growth characteristics of the *pta*(–) mutant

In order to investigate the effects of carbon source on the growth of the *pta*(–) mutant, *E. coli* strains HB101 and PN101 were cultivated and the maximal specific growth rates in different M9ZB media containing glu-

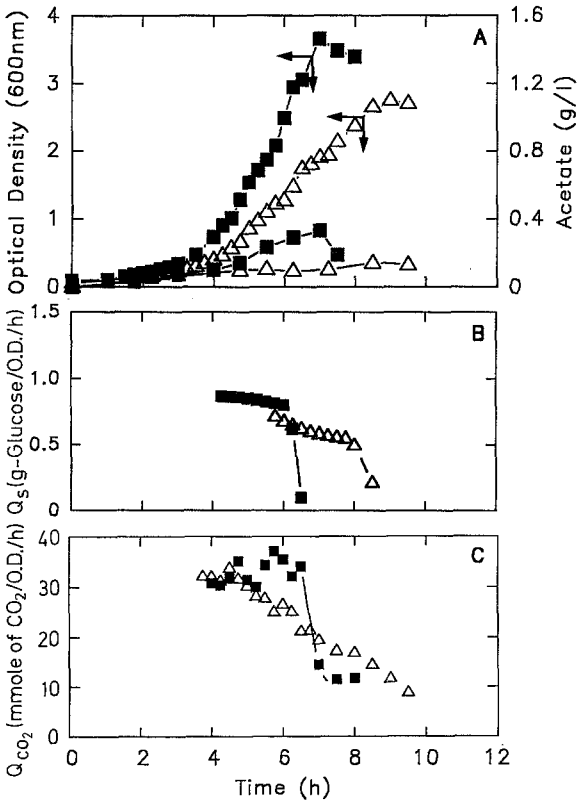

Fig. 2 Time course of cell growth (*OD* optical density), acetate production (**A**), specific glucose uptake rate (Q_s) (**B**) and specific CO_2 evolution rate (Q_{CO_2}) (**C**) of *E. coli* HB101 (■) and its *pta*(–) mutant PN101 △ (fermentor experiment)

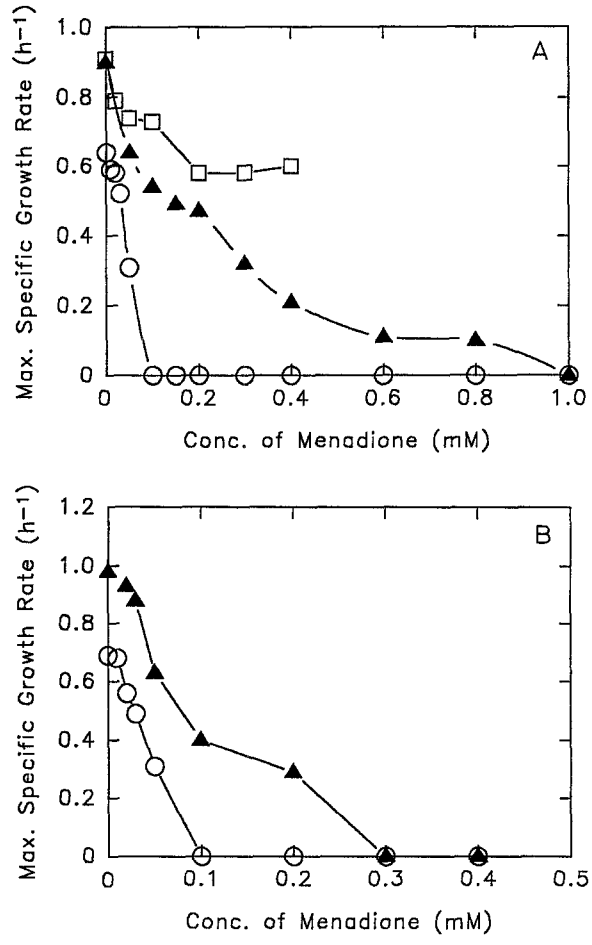

Fig. 3 Effect of menadione on the maximum specific growth rates of *E. coli* HB101 (▲) and its *pta*(–) mutant PN101 (○) when glucose (**A**) or mannose (**B**) was used as a carbon source. Lactate dehydrogenase from *Lactobacillus casei* was introduced into strain PN101 in glucose minimal medium (□)

Table 2 Effect of carbon sources on the specific growth rates of *Escherichia coli* HB101 and its *pta*(–) mutant PN101 harbouring pTTY2 containing the lipase gene

Carbon source	Maximum specific growth rate (h^{-1})	
	HB101/pTTY2	HB101/pta-/pTTY2
Glucose	0.90	0.64
Mannose	0.98	0.69
Glycerol	0.82	0.57
Acetate	0.40	0.52
Succinate	0.38	0.45
Malate	0.46	0.64

cose, mannose, glycerol, acetate, succinate and malate, respectively, were measured. It has been previously reported that little or no acetate was produced when mannose, glycerol or gluconeogenic substrates such as acetate and tricarboxylic acid (TCA) cycle intermediates were used as a carbon source (Andersen and Meyenberg 1980). As shown in Table 2, the growth rate of strain PN101 was considerably decreased to the level of about 30% when glucose, mannose or glycerol was used. The growth deficiency of mutant strain PN101 was not observed, however, when gluconeogenic carbon sources were used. On the contrary, the growth rate of the mutant was enhanced to the level of about 20% as compared with the parental strain HB101.

Menadione sensitivity of the *pta*(–) mutant

Menadione is an artificial redox-cycling drug that is repeatedly reduced and oxidized at the expense of cellular NAD(P)H and molecular O_2 (Greenberg and Demple 1989). Therefore, when this drug was added to culture media at sublethal doses, the cellular redox state [$NAD(P)^+$/NAD(P)H] and energy consumption rate could be easily diminished. It was observed that the specific growth rate of *E. coli* was inversely proportional to the concentration of menadione (Figs. 3, 4).

Figure 3 shows the menadione sensitivities of parental HB101 and its mutant PN101 when glucose (Fig. 3A) and mannose (Fig. 3B) were utilized as carbon sources, respectively. Whereas the growth of parental HB101 was completely inhibited by menadione above 1.0 mM in glucose medium and 0.3 mM in mannose medium, respectively, the *pta*(–) mutant strain did

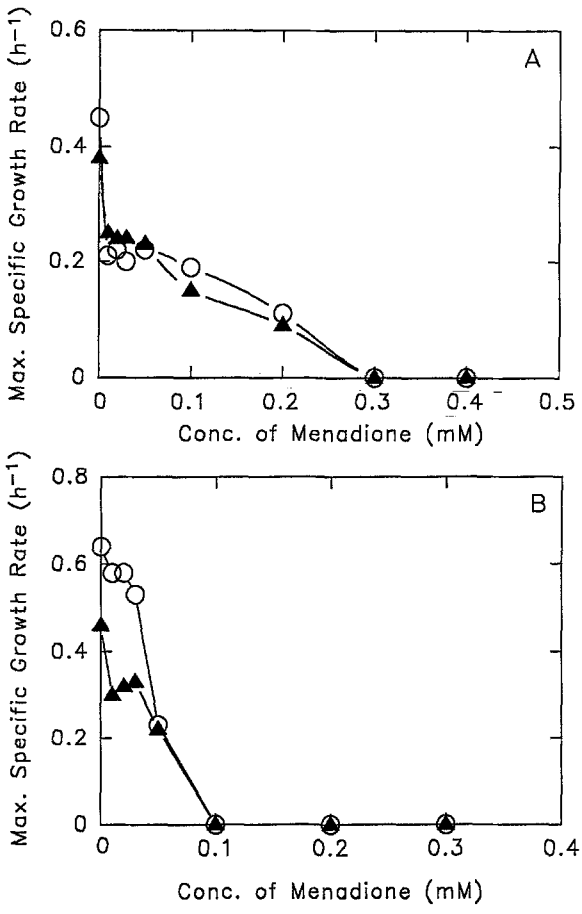

Fig. 4 Effect of menadione on the maximum specificgrowth rates of *E. coli* HB101(▲) and its *pta*(–) mutant PN101 (○) when succinate (**A**) or malate (**B**) was used as a carbon source

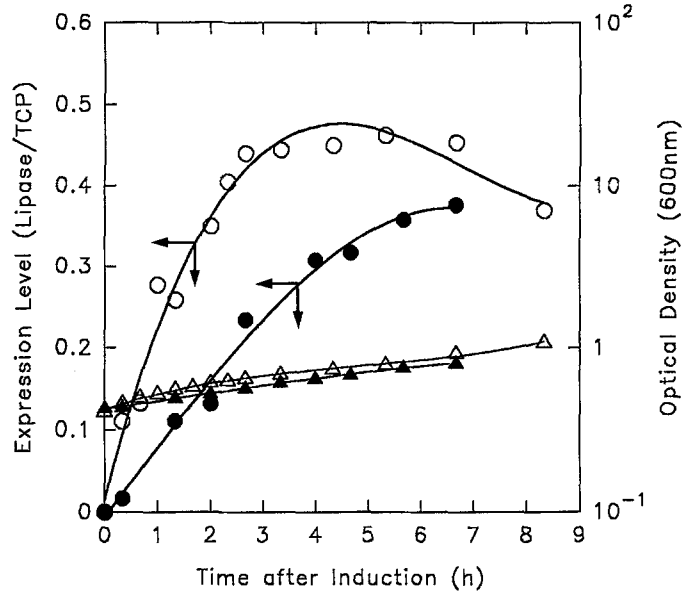

Fig. 5 Time course of cell growth and expression profile of lipase in *E. coli* HB101 (△, ○) and its *pta* mutant PN101 (▲, ●). Time zero indicates the addition of 0.5 mM isopropyl-β-*D*–thiogalactopyranoside. Glucose at 4 g/l was used as a carbon source. The expression level of lipase is the ratio of the amount of foreign lipase to the amount of total cell protein free of the lipase (TCP)

not grow above 0.1 mM menadione in both media. The inhibition of growth of the mutant strain by menadione was not observed with gluconeogenic carbon sources (Fig. 4). It was noticeable that when the LDH gene from *L. casei* was expressed in the *pta*(–) mutant strain, the growth deficiency of the *pta*(–) mutant strain PN101 was recovered and the growth inhibition of the strain by menadione was not observed (Fig. 3A). In expression of the LDH gene, L-lactate was not secreted into the culture broth even though the enzyme activity could be detected in *E. coli*.

Expression of lipase gene in the *pta*(–) mutant

In order to identify the potentials of the *pta*(–) mutant of *E. coli* HB101 as an expression host of foreign gene, the lipase gene from *P. fluorescens* was induced into *pta*(–) strain PN101 (Fig. 5). The expression level was represented by the ratio of the amount of foreign lipase to the amount of total cell protein free of the lipase (TCP). As soon as 0.5 mM IPTG was added to the culture media, the lipase was synthesized and accumulated inside the cell as an inclusion body (Chung et al. 1991a). Lipase synthesis lasted for about 4 h and then decreased. The sudden drop in the expression level at a later phase could be explained by the outgrowing of non-induced cells (data not shown). It was noticeable that the expression rate and yield of *pta*(–) mutant strain PN101 were comparable with those of the parental strain HB101.

In order to examine the fact that menadione also affects lipase gene expression of the *pta*(–) mutant PN101 in addition to the cell growth rate, the lipase induction was executed with menadione. Figure 6 shows the expression profile of the lipase in strains HB101 and PN101 when the cultivation included 0.05 mM menadione. In this case, the final yields and the duration of lipase synthesis were decreased by menadione. It was observed that, compared with the parental strain HB101, the expression rate of lipase in *pta*(–) strain PN101 was considerably reduced by menadione.

High-cell-density culture of *pta*(–) mutant

For examination of the availability of the *pta*(–) mutant as a production host, a high-cell-density culture of this mutant was performed without any fine control of glucose feeding (Fig. 7). Concentrated glucose solution was supplied manually according to the concentration of the residual glucose. Although the residual glucose level fluctuated considerably within the range 0–5 g/l to cause acetate-producing conditions, about 75 g/l of dry cell weight (DCW) was easily achieved and acetate was

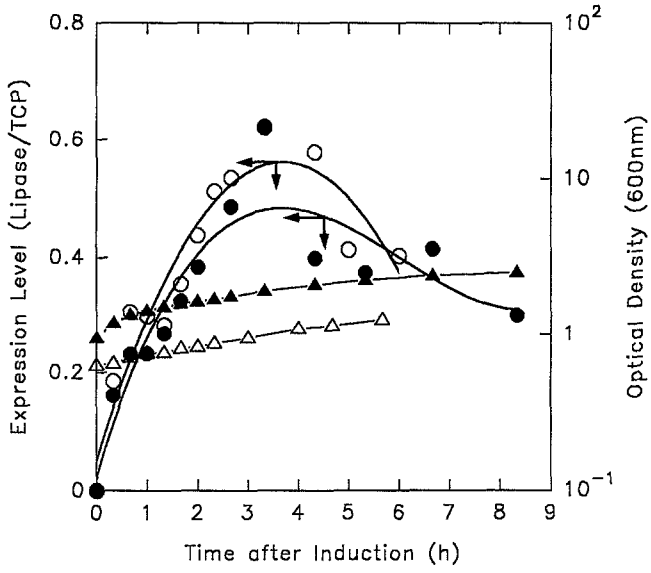

Fig. 6 Time course of cell growth and the expression profile of lipase in *E. coli* HB101 (△, ○) and its *pta*(–) mutant PN101 (▲, ●) when 0.05 mM menadione was added to the culture medium. Glucose at 4 g/l was used as a carbon source

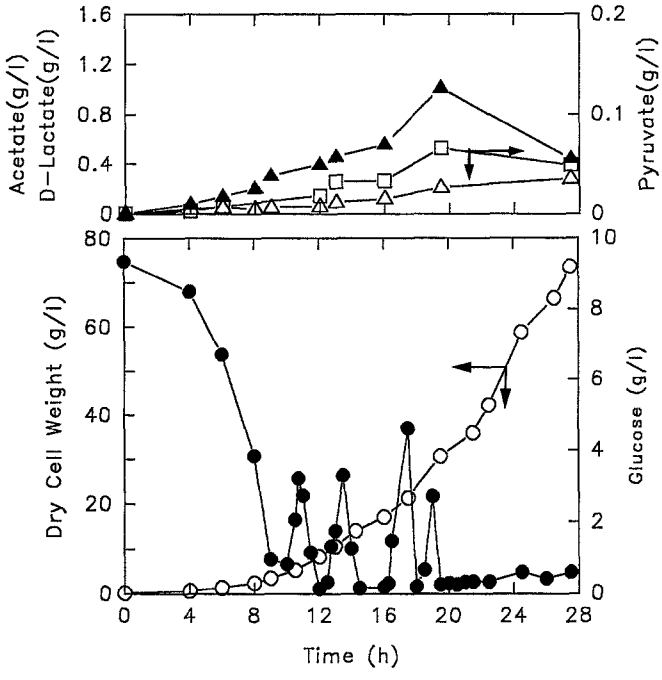

Fig. 7 The profile of a high-cell-density culture of the *pta*(–) mutant PN101 harbouring pTTY2:○ optical density; ● residual glucose; ▲ acetate; △ lactate; □ pyruvate

produced at only about 1 g/l. It was observed that the acetate concentration decreased in the region of glucose-limited growth (above 30 g/l of DCW). D-Lactate and pyruvate were not produced, as shown in Fig. 7.

In order to examine the optimum cell density for lipase expression, IPTG induction was carried out in a high-cell-density culture of the *pta*(–) mutant. When the DCW of the culture reached 40 and 75 g/l, respectively, 100 ml of each culture broth was aseptically drawn out from the fermentor and placed in the small baffled bioreactor, equipped with simple agitation and aeration devices, with 2 or 4 mM IPTG. After cultivation of induced cells for 4 h, we identified an expression level of the lipase gene of about 10–15% by SDS-PAGE (data not shown). Although the maximum induction of the lipase (about 40%) was not achieved, probably due to the increasing viscosity of the culture broth or the physiological changes of cells by carbon starvation stress, a considerable expression was observed.

Discussion

An insertion mutation of the *pta* gene caused a growth deficiency of *E. coli* HB101 in the presence of glucose, mannose or glycerol as sole carbon sources in M9ZB media under aerobic conditions. Because acetate secretion is known to be closely related to the growth rate and glucose uptake rate of *E. coli* (Andersen and Meyenburg 1980; El-Mansi and Holms 1989; Diaz-Ricci and Bailey 1991), we compared the glucose uptake rate between parental HB101 and the *pta*(–) mutant PN101. It was observed that the Q_s of strain PN101 was decreased by blocking acetate synthetic pathway. Moreover, the Q_{CO_2} of the mutant was somewhat lower than that of the parental strain during the exponential phase. These results confirmed that an overall decline in glucose metabolic rate occurred by blocking acetate metabolism.

Under aerobic conditions, wild-type *E. coli* excretes several fermentative by-product, such as acetate, ethanol and lactate, and the secreted portions of those by-products were determined depending on the carbon source, culture conditions and intracellular redox state. It has been also reported that carbon efflux due to the blocking of acetate synthesis could not be redirected toward other by-products such as ethanol or formate (Diaz-Ricci and Bailey 1991; Dedhia et al. 1992). They argued that the decreases in growth rate and glucose uptake rate were basically due to the accumulation of acetyl-CoA and NADH, which inhibited the metabolic rate of glycolysis and consequently the synthetic rate of phosphoenolpyruvate-phosphate. However, our recent findings showed that the blocked biosynthesis of acetate redirected the carbon flux of central metabolism to make D-lactate under anaerobic conditions (Pan et al., unpublished). It was also observed that the growth deficiency of the *pta*(–) mutant occurred even in glycerol [non-phosphotransferase system (PTS) sugar, no acetate accumulation] medium as well as in glucose or mannose (PTS sugars) medium (Table 2).

As previously mentioned, the acetate pathway has its own peculiarity with respect to the generation of ATP and the absence of NADH oxidation (Majewski and Domach 1990; Han et al. 1992). In order to investigate the portion of the acetate pathway contributing as

a metabolic energy supplier, the metabolic energy (ATP and NADH) that could be obtained from glucose was calculated and compared between the two strains by balancing the carbon precursor metabolites, acetate produced and NADPH required for 1 g dry cells of *E. coli* HB101 and its *pta*(–) mutant (Holms 1986; Neidhardt et al. 1990). Conclusively, it was identified that the blocking of the acetate pathway in *E. coli* HB101 could cause only about 9% energy deficiency (data not shown). Therefore, a 30% decrease in the growth rate of the *pta*(–) mutant could not be explained by the energy loss due only to the blocking of acetate secretion.

In order to investigate the importance of the cellular redox state [$NAD(P)^+$/NAD(P)H] on the physiology of the *pta*(–) mutant, menadione, a redox-cycling drug, was used as a metabolic inhibitor (Figs. 3, 4). Whereas growth inhibition by menadione was intensive in glucose or mannose M9ZB media, the inhibition was not observed when gluconeogenic carbon sources such as succinate and malate were used as a sole carbon source. It was suggested that the growth deficiency related to the acetate synthesis was closely related to the cellular redox balance. Therefore, the growth deficiency and menadione sensitivity were not necessarily detected when the gluconeogenic TCA intermediates, which do not need to utilize the acetate pathway, were utilized as a carbon source. This suggestion was confirmed by the fact that when the metabolic stress due to the blocking of the acetate pathway was relaxed by expression of the *L. casei* LDH gene; that is, the growth deficiency and menadione sensitivity were recovered although L-lactate was not excreted into the culture media (Fig. 3A). We are also convinced of this suggestion from the fact that the growth deficiency of the mutant in glucose media was more pronounced under anaerobic conditions than under aerobic conditions (unpublished).

Based on recent findings revealing that acetyl phosphate extensively affected the overall regulation of intracellular signal transduction as a phosphate-donating agent (McCleary et al. 1993; Wanner and Wilmes-Riesenberg 1992) and that the PTS of *E. coli* was seriously affected by acetyl phosphate or intracellular level of [NAD^+/NADH] (Dannelly and Roseman 1992; McCleary et al. 1993), the deleterious effect of acetate blocking on cell metabolism would be magnified by a far-reaching influence of acetyl phosphate.

It is widely accepted that a large amount of metabolic energy (ATP) as well as carbon precursors and reducing power (NADPH) are required for protein synthesis. Because menadione functions as an accelerating agent of energy consumption, it was assumed that the instantaneous deprivation of metabolic energy could result in a decline of expression yield in both parental and *pta*(–) strains (Figs. 5, 6).

However, as shown in Fig. 6, the expression yield of the lipase gene of the *pta*(–) mutant PN101 was more reduced than that of parental strain HB101 when menadione was added. Although the reason could not be explained exactly, it was suggested that menadione could seriously change the expression pattern of lipase, especially in the *pta*(–) mutant because the mutant previously showed more sensitive growth characteristics by using menadione (Figs. 3, 4).

Through the high-cell-density culture of *pta*(–) strain PN101 without any fine control of glucose feeding, we suggested that the *pta*(–) mutant could be utilized as a convenient and general host for foreign protein production in spite of the small deficiency of growth in glucose minimal media. The small decrease in growth rate would not be a serious problem in a high-cell-density culture of *pta*(–) *E. coli* because the usual practice of high-cell-density culture requires limited feeding of the substrate for restricted growth of *E. coli* (Falk et al. 1989; Riesenberg et al. 1991).

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