Carbon source-dependent modulation of NADP-glutamate dehydrogenases in isophthalate-degrading Pseudomonas aeruginosa strain PP4, Pseudomonas strain PPD and Acinetobacter lwofii strain ISP4

C. Vamsee-Krishna and Prashant S. Phale

Biotechnology Group, School of Biosciences and Bioengineering, Indian Institute of Technology – Bombay, Powai, Mumbai 400 076, India

Acinetobacter lwofii strain ISP4 metabolizes isophthalate rapidly compared with Pseudomonas aeruginosa strain PP4 and Pseudomonas strain PPD. Isophthalate has been reported to be a potent competitive inhibitor of glutamate dehydrogenase (GDH). Exogenous supplementation of isophthalate with glutamate or α-ketoglutarate at 1 mM concentration caused strains PP4 and PPD to grow faster than in the presence of isophthalate alone; however, no such effect was observed in strain ISP4. When grown on isophthalate, all strains showed activity of NADP-dependent GDH (NADP-GDH), while cells grown on glucose, 2× yeast extract-tryptone broth (2YT) or glutamate showed activities of both NAD-dependent GDH (NAD-GDH) and NADP-GDH. Activity staining, inhibition and thermal stability studies indicated the carbon source-dependent presence of two (GDH\textsubscript{I} and GDH\textsubscript{II}), three (GDH\textsubscript{A}, GDH\textsubscript{B} and GDH\textsubscript{C}) and one (GDH\textsubscript{P}) forms of NADP-GDH in strains PP4, PPD and ISP4, respectively. The results demonstrate the carbon source-dependent modulation of different forms of NADP-GDH in these bacterial strains. This modulation may help the efficient utilization of isophthalate as a carbon source by overcoming the inhibitory effect on GDH.

INTRODUCTION

Phthalate isomers (phthalate, isophthalate and terephthalate) and their esters are heavily used as additives in various industries, including the medical, plastic, pesticide and cosmetic industries. They impart flexibility, thermal stability and chemical resistance to the finished product, but can easily be leached out by solvents (Jaeger & Rubin, 1973; Keyser et al., 1976; Tepper, 1973). They have been reported to have toxic, mutagenic, carcinogenic, teratogenic and endocrine-disrupting activities in several organisms (Dillingham & Autian, 1973; Gesler, 1973; Koch et al., 2003; Krauskopf, 1973; Phale et al., 2007; Quan et al., 2005; Rubin & Jaeger, 1973).

Micro-organisms present in the environment have evolved and adapted to degrade these compounds as the sole source of carbon and energy. With respect to phthalate isomers, the metabolic pathways and enzymes involved in the degradation of phthalate and terephthalate have been studied in greater detail than those of isophthalate (Chatterjee & Dutta, 2003; Eaton, 2001; Eaton & Ribbons, 1982; Keyser et al., 1976; Phale et al., 2007; Quan et al., 2005; Shigematsu et al., 2003; Vamsee-Krishna et al., 2006; Vamsee-Krishna & Phale, 2008; Wang et al., 1995, 2003). There are very few reports that describe the utilization of all three phthalate isomers as the carbon source by a single microbial strain (Vamsee-Krishna et al., 2006; Vamsee-Krishna & Phale, 2008; Wang et al., 1995). The first key step involved in the bacterial degradation of phthalate isomers is the double hydroxylation of the aromatic ring by the respective phthalate dioxygenase to yield a cis-dihydrodiol intermediate, which is further metabolized to aliphatic intermediates of the central carbon cycle via 3,4-dihydroxybenzoate (3,4-DHB) (Ballou & Batie, 1988; Bacie et al., 1987; Schlafl et al., 1994; Tarasev & Ballou, 2005; Vamsee-Krishna et al., 2006).

Besides being toxic, phthalate, isophthalate and terephthalate are also reported to inhibit glutamate dehydrogenase (GDH) competitively (Caughey et al., 1957). The inter-proton distance between two carboxyl groups of isophthalate and glutamate is 7.45 Å (0.745 nm), which makes isophthalate a potent competitive inhibitor of GDH compared with phthalate and terephthalate (Caughey et al., 1957). Isophthalic acid and its derivatives have been reported to inhibit NAD-dependent GDH (NAD-GDH) from bovine,

Abbreviations: GDH, glutamate dehydrogenase; mHB, m-hydroxybenzoate; pHB, p-hydroxybenzoate; z-KG, α-ketoglutarate; NAD-GDH, NAD-dependent GDH; NADP-GDH, NADP-dependent GDH.
fungal, yeast and pea plant sources (Boots et al., 1976; Caughey et al., 1957; Cunliffe et al., 1983; Rogers et al., 1972; Stevens et al., 1989), as well as NADP-dependent GDH (NADP-GDH) from Aspergillus niger (Noor & Punekar, 2005).

GDH (EC 1.4.1.2-4) plays a crucial role at the crossroads of carbohydrate and amino acid metabolism. It catalyses the reductive amination of α-ketoglutarate (α-KG) to glutamate (anabolic reaction), the oxidative deamination of glutamate to α-KG (catabolic reaction) and shows specificity to NAD or NADP, or both (Hudson & Daniel, 1993). NAD-GDH is involved in the catabolic, and NADP-GDH in the anabolic reaction of glutamate metabolism. NADP- and NAD-GDH are induced by NH₃ and glutamate, respectively (Hudson & Daniel, 1993; Lu & Abdelal, 2001). The induction of different forms of GDH has been shown in many organisms under conditions such as salt, cold, trophic and environmental stress, and they have been found to be either stable at higher salt concentrations and extreme temperatures, or to have varying affinity for substrates (Bonete et al., 2003; Camardella et al., 2002; Moyano et al., 1992).

_Pseudomonas aeruginosa_ strain PP4 and _Pseudomonas_ sp. strain PPD degrade all three isomers of phthalate, while _Acinetobacter lwoffii_ strain ISP4 degrades isophthalate alone as the sole source of carbon and energy (Vamsee-Krishna et al., 2006). The degradative pathways of phthalate isomers have been elucidated by performing various metabolic studies (Vamsee-Krishna et al., 2006). The interesting question is, since it is known that isophthalate acts as a competitive inhibitor of GDH, how are these bacterial strains able to degrade these compounds as the sole source of carbon and energy? We hypothesize that the GDH from these strains is insensitive or less sensitive to inhibition by isophthalate. Therefore, it was interesting to study various properties of GDH from these strains when grown on different carbon sources. The results obtained suggest that in strains PP4 and PPD, external supplementation with α-KG, glutamate or glutamate-family amino acids at millimolar concentrations promotes faster growth on isophthalate, and carbon source-dependent induction of different forms of NADP-GDH was observed. NADP-GDH produced in the presence of isophthalate was less sensitive to isophthalate inhibition than NADP-GDH from glucose-grown cells. However, in strain ISP4, glutamate-family amino acids failed to promote faster growth on isophthalate, and a single form of NADP-GDH with a threefold higher activity than that of 2× yeast extract-tryptone broth (2YT)-grown cells was observed. The carbon source-dependent presence of different forms of GDH or an increase in its levels (activity) might help to overcome the inhibitory effect, thus allowing cells to utilize isophthalate as the carbon source.

**METHODS**

**Chemicals.** HEPES, α-ketoglutarate, glutamate, EDTA, glycine, acrylamide, N,N′-methylenebis-acrylamide, N,N,N′,N′-tetramethylmethylenediamine (TEMED), m-hydroxybenzoate (mHBB), p-hydroxybenzoate (pHBB) and salicylate were purchased from Sigma-Aldrich. All other chemicals used were of analytical grade and purchased locally.

**Bacterial strains and growth conditions.** _P. aeruginosa_ strain PP4, _Pseudomonas_ strain PPD and _A. lwoffii_ strain ISP4, capable of degrading phthalate isomers as the carbon source and isolated from soils by enrichment culture, were used in the present study (Vamsee-Krishna et al., 2006). Cultures were grown in 150 ml mineral salt medium (MSM) (Basu et al., 2003) in 500 ml baffled Erlenmeyer flasks at 30°C on a rotary shaker (200 r.p.m.). The medium was supplemented with either aromatic compounds (0.1%) such as phthalate isomers (α-, m-, and p-) hydroxybenzoates and benzoate, or aromatics (0.1%) plus α-KG, glutamate- or non-glutamate-family amino acids (1 mM). Ammonium nitrate (1 g l⁻¹, 0.1%) was used as the nitrogen source. Alternatively, strains PP4 and PPD were grown on MSM supplemented with either glucose (0.25%) or glutamate (10 mM), while strain ISP4 was grown on 2× yeast extract-tryptone broth (2YT); composition per litre of distilled water: yeast extract, 10 g; tryptone, 16 g; NaCl, 5 g) (Vamsee-Krishna et al., 2006). Cells grown on the respective carbon sources were used as an inoculum (2 ml per 150 ml MSM). Growth was monitored spectrophotometrically (Perkin Elmer Lambda 35) at 540 nm.

**Preparation of cell-free extracts.** Cells grown to late-exponential phase were harvested (12000 g for 15 min at 4°C), washed twice and suspended (1 g wet weight in 4 ml) in ice-cold HEPES-NaOH buffer (20 mM, pH 8.5) containing EDTA (1 mM) and NaCl (100 mM) (Lu & Abdelal, 2001). Cells were disrupted on ice with five cycles of 20 pulses each (1 s pulse, 1 s interval, cycle duration 40 s, output 11–12 W with a 5 min interval between two cycles) using an Ultrasonic processor (model GE130). The cell lysate was centrifuged at 40000 g for 30 min, and the clear supernatant obtained was referred to as cell-free extract and used for monitoring GDH activity. Protein was estimated by the method of Bradford (1976) using BSA as standard.

**Activity staining of GDH.** Activity staining was performed to detect the different forms of GDH from cells grown on various carbon sources (Bellion & Tan, 1984). In brief, native PAGE (7.5%) was performed at a constant current of 4 mA at 4°C with an appropriate amount of protein (Laemmli, 1970). After electrophoresis, the gel was washed and equilibrated for 15 min with ice-cold HEPES-NaOH buffer (100 mM, pH 7.5) followed by incubation in activity staining solution (10 ml) containing glutamate (100 mM), NADP or NAD (0.2 mM), nitroblue tetrazolium (2.5 mg), phenazine methosulphate (2.5 mg) and HEPES-NaOH (100 mM, pH 7.5) at 25°C until bands were distinctly visible. The staining reaction was terminated by soaking the gel in acetic acid (7.5%) for a few minutes followed by extensive washing with distilled water.

**Enzyme assay, inhibition and thermal stability of GDH.** The activity of GDH was monitored as the reductive amination of glutamate or non-glutamate-family amino acids at millimolar concentrations and expressed as nanomoles of NADPH or NADH consumed per minute.

To study inhibition, activity was monitored in the presence of different concentrations of inhibitors such as phthalate isomers (α-, m-, p-) hydroxybenzoates and benzoate. The residual activity (%) was calculated by taking NAD- or NADP-GDH activity in the absence of inhibitor as 100%.

To monitor the thermal stability of NADP-GDH, the cell-free extracts prepared from cells grown on either aromatics (0.1%) or glucose (0.25%) were incubated in the presence or absence of known GDH stabilizers such as α-KG (1 mM) or glutamate (10 mM) at 60°C for different times (5, 10, 20 and 30 min). The mixtures were centrifuged
(40,000 g for 30 min) and the residual activity from the supernatant was measured. Activity at 0 min was taken as 100%, and cell-free extracts without heat treatment were used as controls.

All experiments described were performed at least three times with readings in triplicate using appropriate controls. The SD observed was in the range of 3–8%. The trends observed in activity-staining profiles were identical and the best profiles are shown.

RESULTS

Effects of glutamate-family amino acids on growth profiles using various aromatics as the carbon source

*P. aeruginosa* strain PP4 and *Pseudomonas* strain PPD utilize phthalate, isophthalate, terephthalate, mHB, pHB, benzoate, glucose and glutamate (Vamsee-Krishna *et al.*, 2006). *A. lwoffii* strain ISP4 grows on isophthalate, benzoate and 2YT, but fails to grow on glutamate or glucose (Vamsee-Krishna *et al.*, 2006). Growth profiles of these strains on various carbon sources in the presence or absence of Δ-KG or glutamate (1 mM) are shown in Fig. 1. Strain ISP4 utilized isophthalate very rapidly compared with strains PP4 and PPD.

*P. aeruginosa* strain PP4 grown on isophthalate alone entered stationary phase at ~48 h (Fig. 1a). However, on isophthalate plus glutamate or isophthalate plus Δ-KG (a precursor of glutamate), cells entered stationary phase by ~34 and 28 h, respectively. Glutamate alone at 1 mM concentration failed to support growth (Fig. 1a). This observation suggests that Δ-KG or glutamate promotes

![Fig. 1](http://mic.sgmjournals.org)

*Fig. 1.* Growth profiles of *P. aeruginosa* strain PP4 (a–c), *Pseudomonas* strain PPD (d–f) and *A. lwoffii* strain ISP4 (g–i) on different carbon sources. (a, d, g) Growth profiles on isophthalate (●), isophthalate (0.1%) plus glutamate (1 mM, ○), isophthalate (0.1%) plus Δ-KG (1 mM, △) and glutamate alone (1 mM, ◆). (b, e, h) Growth profiles on mHB (0.1%, ●), mHB (0.1%) plus glutamate (1 mM, ○), benzoate (0.1%, △) and benzoate (0.1%) plus glutamate (1 mM, ◆). (c, f) Growth profiles on glucose (0.25%, ●), glucose (0.25%) plus glutamate (1 mM, ○) and glutamate (1 mM, ◆). (i) Growth profiles on 2YT (●) and 2YT plus glutamate (1 mM, ○). For all growth profiles, the medium used was MSM plus carbon source (aromatics or glucose) and supplementation (amino acids) was as indicated above.
faster growth on isophthalate (Fig. 1a). Supplementing isophthalate medium with glutamate-family amino acids such as glutamine, arginine or proline also promoted faster growth. However, such an effect was not observed with non-glutamate-family amino acids such as glycine and lysine (data not shown). A similar effect was observed with cultures grown on phthalate plus glutamate, terephthalate plus glutamate (growth profiles not shown) and mHB plus glutamate (Fig. 1b); however, the effect was less prominent compared with isophthalate plus glutamate. No significant difference in the growth profiles was observed when cells were grown on glucose, benzoate or pHB (Fig. 1b, c and data not shown, respectively) in the presence or absence of glutamate. These results suggest that the effect is significant for isophthalate plus glutamate-family amino acids.

In Pseudomonas strain PPD, supplementing isophthalate- or mHB-containing media with glutamate or α-KG promoted faster growth compared with aromatics alone (Fig. 1d, e). However, the observed difference was less significant compared with strain PP4. No appreciable effect of glutamate supplementation was observed on the growth profiles in the presence of phthalate and terephthalate (growth profiles not shown). Strain PPD failed to show a clear difference between the growth profiles of isophthalate plus glutamate- or non-glutamate-family amino acids (data not shown). The growth profiles on glucose, benzoate and pHB in the presence or absence of glutamate were similar (Fig. 1f, e and data not shown, respectively).

Interestingly, A. lwoffii strain ISP4 did not show any effect on growth in the presence or absence of α-KG, glutamate- or non-glutamate-family amino acids (Fig. 1g–i).

**Carbon source-dependent forms of GDH**

Late-exponential-phase cells showed a higher activity of GDH compared with early exponential-phase cells; hence, late-exponential-phase cells were used for this study. The activity staining profiles of NAD- and NADP-GDH from cells grown on various carbon sources are shown in Fig. 2. In strain PP4, GDH preparations from cells grown on isophthalate, phthalate, terephthalate or mHB in the presence or absence of glutamate (1 mM) showed a single NADP-GDH activity band with a lower electrophoretic mobility on native PAGE, referred to as GDH_{II}. However, cells grown on glucose, glutamate plus glutamate (1 mM), glutamate (10 mM) alone, pHB or benzoate showed a higher-mobility NADP-GDH activity band, referred to as GDH_{II} (Fig. 2a). These results indicate that strain PP4 has two different forms of NADP-GDH. In strain PPD, the GDH preparation from cells grown on isophthalate, phthalate, terephthalate and mHB in the presence or absence of glutamate (1 mM) showed a single NADP-GDH activity band with a lower electrophoretic mobility, referred to as GDH_{II}, while the preparation from glucose, glucose plus glutamate (1 mM) or glutamate (10 mM) alone showed a higher mobility NADP-GDH activity band, referred to as GDH_{II} (Fig. 2b). Benzoate-grown PPD cells showed a staining band with the highest mobility (referred to as GDH_{II}). Interestingly, cells grown on pHB showed two activity staining bands that corresponded to GDH_{II} and GDH_{II} (Fig. 2b). These results suggest that strain PPD has three different forms of NADP-GDH. Incubating cell-free extracts of glucose-grown cells of PP4 or PPD with isophthalate, or mixing and incubating cell-free extracts from glucose- and isophthalate-grown cells, did not affect the observed mobility pattern (data not shown), thus ruling out the possibility of enzyme modification. In strain ISP4, irrespective of the carbon source, a single NADP-GDH activity band, referred to as GDH_{II}, was observed. However, the staining intensity was significantly higher for cells grown on aromatics than for those grown on 2YT (Fig. 2c).

On native PAGE, glucose- or 2YT-grown cells of PP4, PPD and ISP4 showed a lower-mobility NAD-GDH band.
compared with the relatively higher-mobility NADP-GDH activity staining band (Fig. 2d), suggesting that NAD- and NADP-GDH are different and do not show dual specificity for the coenzymes.

**Carbon source-dependent activity and inhibition studies of GDH**

Table 1 and Fig. 3 depict the specific activities and inhibition profiles of NAD- and NADP-GDHs measured from the cell-free extracts of strains PP4, PPD and ISP4 grown on different carbon sources. In all three strains (i) irrespective of the aromatic carbon source, cell-free extracts showed the activity of NADP-GDH only, while cells grown on glucose (0.25%), glutamate (10 mM) or 2YT showed activities of both NADP- and NAD-GDH (Table 1); (ii) isophthalate at 1 mM inhibited GDH$_I$, GDH$_A$ and GDH$_P$ (~60–70%), while phthalate and terephthalate did not inhibit these enzymes (Fig. 3a–c). Other compounds such as $o$-, $m$- and $p$-hydroxybenzoates and benzoate also failed to show any inhibition, even at 10 mM concentration (data not shown); (iii) irrespective of the carbon source, NADP-GDH showed inhibition with isophthalate alone (Fig. 3d–f); (iv) isophthalate failed to inhibit NAD-GDH (Table 1); and (v) effectors such as ATP, ADP, AMP and GTP (50 mM) failed to show any effect on NADP-GDH activity (data not shown).

The unique features in strain PP4 and PPD are: (i) cells grown on glucose showed five to sixfold lower specific activities of NAD-GDH compared with NADP-GDH (Table 1); (ii) compared with NADP-GDH, the activity of NAD-GDH from isophthalate plus glutamate (1 mM) - grown cells was either low (25-fold less in PP4) or absent (PPD) (Table 1); and (iii) GDH$_{II}$, GDH$_B$ and GDH$_C$ were more susceptible to inhibition by isophthalate (~50–65% at 1 mM and ~90% at 10 mM) than GDH$_I$ and GDH$_A$ (~25–30% at 1 mM and 65–70% at 10 mM; Table 1, Fig. 3d, e). The initial rates of inhibition of GDH$_I$ and GDH$_A$ were low compared with GDH$_{II}$, GDH$_B$ and GDH$_C$ (Fig. 3d).

In strain ISP4, isophthalate-grown cells showed a threefold higher activity of GDH$_P$ compared with 2YT-grown cells; this observation was supported by the activity staining data.

**Table 1. Specific activities of GDHs from strains PP4, PPD and ISP4 grown on different carbon sources**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>Form of GDH</th>
<th>GDH activities with:*</th>
<th>NADP-GDH inhibition (%)‡</th>
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<tr>
<td></td>
<td></td>
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<td>NADH + Iso†</td>
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<td>Iso</td>
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*Amination activity of GDH was monitored. See Methods for details.

†NAD- and NADP-GDH activities monitored in the presence of isophthalate (1 mM).

‡To calculate the percentage inhibition by isophthalate at 1 mM, the respective NADP-GDH activities in the absence of isophthalate were taken as 100%.

§Abbreviations: Iso, isophthalate; Pht, phthalate; Tere, terephthalate; Glc, glucose; Glu, glutamate.

||Enzymes are named as denoted in Fig. 2.

§ND, Activity could not be detected.
(Fig. 2c). The activity of GDH_p from isophthalate-, isophthalate plus glutamate- (1 mM), benzoate- and 2YT-grown cells showed 52–58 % inhibition at 1 mM and ~80 % at 10 mM isophthalate (Fig. 3f).

**DISCUSSION**

*P. aeruginosa* strain PP4 degrades isophthalate slowly compared with *Pseudomonas* strain PPD, while *A. lwoffii* strain ISP4 utilizes it rapidly as the carbon source (Vamsee-Krishna et al., 2006). The results demonstrate that the exogenous addition of α-KG or glutamate-family amino acids to PP4 and PPD cultures led to faster growth on isophthalate; however, such an effect was not observed in strain ISP4 (Fig. 1). This could be mediated through rapid uptake of α-KG or glutamate-family amino acids, leading to an increase in the intracellular concentration of these compounds, thus lowering the effective inhibition of GDH_I and GDH_A by isophthalate. To the best of our knowledge, these growth-promoting effects are unique and have not been reported so far. Interestingly, in strain PP4, exogenous addition of glutamate promoted faster growth on mHB and showed a twofold higher activity of GDH_I compared with isophthalate-grown cells, while pHB-grown cells showed a...
higher activity of GDHII (Fig. 1, Table 1). These results are intriguing; one possible explanation is that although mHB and pHB have the same functional groups (-OH and -COOH), their positions are different on the aromatic ring. In that respect, mHB is structurally closer to isophthalate than pHB.

Depending on the carbon and nitrogen sources, different forms of GDH [NADP-, NAD- or NAD(P)-] have been reported from Clostridium symbiosum (Syed et al., 1991), Laccaria bicolor (Garnier et al., 1997), Escherichia coli (Maurizi & Rasulova, 2002), P. aeruginosa (Smits et al., 1984), Sulfolobus solfataricus (Schinkinger et al., 1991) and Salinibacter ruber (Bonete et al., 2003). The induction of NAD-, NADP- or NAD(P)-GDH has also been reported under varying environmental conditions such as salt (Bonete et al., 1990, 2003), temperature (Camardella et al., 2002) and nitrogen source (Abrahams & Abratt, 1998; Brown et al., 1973; LeJohn & McCrea, 1968). In Neisseria meningitidis, transcriptional analysis has shown carbon source-dependent expression of gdhA, leading to a difference in the growth of the strain (Pagliarulo et al., 2004).

In strains PP4, PPD and ISP4, both NAD- and NADP-GDHs were detected (Table 1, Fig. 2). With fixed nitrogen and varying carbon sources, two forms of NADP-GDH, GDH1 and GDHII, in strain PP4, and three forms of NADP-GDH, GDHA, GDHB and GDHC, in strain PPD, were detected, and showed differences in thermal stability, activity staining pattern and susceptibility to inhibition by isophthalate (Table 1, Figs 2–4). To the best of our knowledge, this is the first biochemical report of the carbon source-dependent presence of different forms of NADP-GDH in a bacterial system. Carbon source-dependent induction of two isozymes (NADP-GDH) has been reported in Saccharomyces cerevisiae; GDH1 is induced when cells are grown either on glucose or ethanol, while GDH2 is induced on ethanol only (Deluna et al., 2001). The co-ordinated expression of these enzymes with different carbon sources, and the varying allosteric properties and rates of ox-KG utilization account for the role of isozymes of NADP-GDH. Similarly, in the green alga Chlamydomonas reinhardtii, the presence of three different isozymes of NAD(P)-GDHs, GDH1, GDH2 and GDH3, is induced under dark, CO2 plus light and light phases, respectively (Moyano et al., 1992; Muñoz-Blanco et al., 1989).

NADP-GDH from strains PP4, PPD and ISP4 was inhibited by isophthalate (75–80% at 10 mM). Similar results have been observed for NADP-GDH from A. niger (Noor & Punekar, 2005). Interestingly, no inhibitory effect of isophthalate was observed with NAD-GDH from the three strains (Table 1); this is in contrast to bovine NAD-GDH, which is susceptible to inhibition by isophthalate, terephthalate and phthalate (Caughey et al., 1957). The activity of GDH has been reported to be modulated (activation or deactivation) by nucleotides such as ATP, ADP, AMP and GTP (Bonete et al., 2003; LeJohn & Jackson, 1968; Smith et al., 2001, 2002). However, no such modulatory effect was observed with NADP-GDH from the three strains in this study (data not shown). Mixing and incubation of cell-free extracts of isophthalate- and glucose-grown cells did not vary the electrophoretic mobility of GDH, suggesting that post-translational protein modification(s) is/are absent, and that the forms of the enzymes are indeed different, as seen in Fig. 2.

Fig. 4. Time-dependent changes in the activity of NADP-GDH at 60 °C from P. aeruginosa strain PP4 (a), Pseudomonas strain PPD (b) and A. lwoffi strain ISP4 (c). Cells were grown on different carbon sources: isophthalate (0.1 %, ●), glucose (0.25 %, ▽) or 2YT (▲), mHB (0.1 %, ×), pHB (0.1 %, ◊) and benzoate (0.1 %, ★). The cell-free extracts prepared from cells grown on isophthalate (0.1 %, ○, dotted line) and glucose (0.25 %, ▼, dotted line) or 2YT (▼, dotted line) without heat treatment were used as controls.
The difficulties in the successful isolation of isophthalate-degrading strains from the environment could be due to the fact that isophthalate acts as a potent competitive inhibitor of GDH, which interlinks carbon and nitrogen metabolism (Boots et al., 1976; Caughey et al., 1957; Cunliffe et al., 1983; Rogers et al., 1972; Stevens et al., 1989). The present study demonstrates that although GDH is inhibited by isophthalate, strains PP4, PPD and ISP4 have adopted different strategies, such as carbon source-dependent modulation of forms of NADP-GDH, for the effective utilization of isophthalate, and that they thrive in the environment. Strains PP4 and PPD when grown on isophthalate produce a form of NADP-GDH that is less sensitive to inhibition by isophthalate, while cells grown on glucose produce the other form, which is more sensitive to inhibition by isophthalate, whereas cells grown on isophthalate produce a form of NADP-GDH that is less sensitive to inhibition by isophthalate, while cells grown on glucose produce the other form, which is more sensitive to isophthalate inhibition. In strain ISP4, a single form of NADP-GDH was present, whose activity was increased threefold when grown on isophthalate; such modulation of enzymes helps to counteract the inhibitory effect of the carbon source, isophthalate. Characterization of these enzymes for their molecular and kinetic properties may help in the construction of novel strains suitable for effective bioremediation of recalcitrant aromatic compounds that interfere with the normal metabolic pathways of the cell.

ACKNOWLEDGEMENTS

We thank Dr Punekar N.S., Bioschool, IIT-Bombay, India, for fruitful discussions. A research grant from the Department of Biotechnology, Government of India to P. S. P. and a CSIR-Senior research fellowship to C. V.-K. are gratefully acknowledged.

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Edited by: H. L. Drake