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The subsidiary GntII system for gluconate metabolism in *Escherichia coli:* Alternative induction of the *gnt*V gene

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ABSTRACT

Two systems are involved in the transport and phosphorylation of gluconate in *Escherichia coli*. GntI, the main system, consists of high and low-affinity gluconate transporters and a thermoresistant gluconokinase for its phosphorylation. The corresponding genes, *gnt*T, *gnt*U and *gnt*K at 76.5 min, are induced by gluconate. GntII, the subsidiary system, includes IdnT and GntV, which duplicate activities of transport and phosphorylation of gluconate, respectively. Gene *gnt*V at 96.8 min is divergently transcribed from the *idn*DOTR operon involved in L-idonate metabolism. These genetic elements are induced by the substrate or 5-keto-D-gluconate. Because *gnt*V is also induced in cells grown in gluconate, it was of interest to investigate its expression in this condition. *E. coli gnt*K, *idn*O<>*kan* mutants were constructed to study this question. These *idn*O *kan*-cassete inserted mutants, unable to convert gluconate to 5-keto-D-gluconate, permitted examining *gnt*V expression in the absence of this inducer and demonstrating that it is not required when the cells grow in gluconate. The results suggest that *E. coli gnt*V gene is alternatively induced by 5-keto-D-gluconate or gluconate in cells cultivated either in idonate or gluconate. In this way, the control of *gnt*V expression would seem to be involved in the efficient utilization of these substrates.

Key terms: *E. coli*, gluconate, GntII, *gnt*V.

INTRODUCTION

The genetics and physiology of transport and phosphorylation of gluconate (Gnt) in *Escherichia coli* have turned out to be highly complex (Fig. 1). Previous work has described the genes involved, as well as their regulation. There are two systems encoded by operons distinctly regulated and located in different regions of the bacterial chromosome (Bächi and Kornberg, 1975, Istúriz et al., 1979). GntI, the main system, consists of high and low affinity gluconate transporters (GntT, GntU) and a thermoresistant gluconate kinase (GntK, Fig.1A). The *gnt*T and the *gnt*KU genes constitute two operons located in the *bio*H-*asd* region of the chromosome at 76.4 and 77.1 min, respectively, on the *E. coli* map (Fig.1B). These operons, as well as that of the Entner Doudoroff pathway (EDP, *edd-eda*), are induced by gluconate and negatively controlled by the *gnt*R gene product (77.1 min) in a regulatory network known as the gntR regulon (Zwaig et al., 1973, Tong, et al., 1996, Izu et al., 1997, Peekhaus and Conway, 1998).

GntII, the subsidiary system, contains another high affinity gluconate transporter (IdnT) and also a thermosensitive gluconate kinase (GntV, Fig.1A). It was revealed by the gluconate negative phenotype of BBI, an *E. coli* mutant carrying two lesions linked to *fdp* and *mal*A markers, affecting a subsidiary gluconate transporter and the regulatory *gnt*R gene respectively (Bächi and Kornberg, 1975). Later, it was confirmed by the selection in mineral medium with gluconate of spontaneous fermenting pseudorevertants of *E. coli* HfrG6ΔMD2, a *bio*H-*gnt*TKUR-*asd* deleted mutant that cannot grow in gluconate. One representative pseudorevertant, mutant C177 (Δ*gnt*R), expressed the dehydratase (*edd,* 41 min) in mineral medium with glucose and formed when grown in media with gluconate, both a high-affinity transporter for this substrate and the thermosensitive gluconokinase. The lesion responsible for this gluconate positive phenotype designated as *gnt*177 was located at 96 min on the map, 76% linked to *pyr*B (Istúriz et al., 1979).

Much has been learned about the GntII system over the present and past two decades. Gene *gnt*V, located at 96.8 min (Istúriz et al., 1986, Burland et al., 1995), is monocistronic and divergently transcribed from the *idn*DOTR operon (Fig.1B), which encodes enzymes that metabolizes L-idonic acid (Idn) to D-gluconate (Bausch et al., 1998). Enzyme IdnD, an L-idonate 5-dehydrogenase, converts incorporated idonate to 5-keto-D-gluconate (5KG), which in turn is transformed to gluconate by IdnO, a 5-keto-D-gluconate 5-reductase. Gluconate is then phosphorylated to 6-phosphogluconate by GntV (IdnK), whose gene is coordinately induced with the *idn*DOTR operon. IdnT was found to function as a permease for transport of both idonate and gluconate, indicating that the GntII system contains the enzymes of a pathway for idonate catabolism where gluconate is an intermediate (Fig. 1A). IdnR was identified as a positive regulator of the idnR regulon with 5KG as the inducer (Bausch et al., 1998). A negative regulatory effect of IdnR on the *gnt*T, *gnt*KU and *edd-eda* operons (not shown in Fig.1) is indicative of a cross-regulation between the gntR and idnR regulons (Tsunedomi et al., 2003a, Ramírez et al., 2007).

The fact that *gnt*V is also induced in wild type cells grown in gluconate led us to wonder whether this expression is also under the positive control of the 5KG-IdnR complex. Previous reports do not favor this possibility, so the present work was undertaken to resolve the question. Although cells grown in gluconate display a poor induction of the *idn*DOTR operon, there is strong induction of GntK and GntV expression (Istúriz et al., 1986, Bausch et al., 2004). Moreover, in gluconate-limited mineral medium continuous culture, the total gluconate

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kinase activity, consisting of GntV at very low dilution rates (D), is gradually repressed as the induction of GntK increases as a consequence of the progressive increase of D and corresponding increment in the concentration of limiting substrate. These findings indicated that *gnt*V induction, contrary to that of *gnt*K, occurs mainly at low gluconate concentrations (Coello and Istúriz, 1992).

The possibility that *gnt*V expression occurs independently of the IdnR-5KG complex is also suggested by the complexity of the intergenic regulatory region between *gnt*V and *idn*D. This region includes promoter-operator sequences for the *gnt*V gene and the *idn*DOTR operon, each with a binding element for GntR or IdnR. These regulators have 42% similarity between their entire primary sequences and 70% identity between their DNA-binding motifs. In addition, there are two binding sites for cAMP-CRP complex and another for GntR or IdnR (Izu et al., 1997, Tsunedomi et al., 2003b).

Here we demonstrate that the induction of *gnt*V in *E. coli* grown in gluconate occurs in the absence of 5KG and is not coordinated with that of the *idn*DOTR operon; furthermore, in this condition gluconate is suggested as the inducer. The study involved the construction and analysis of two *E. coli* *gnt*K, *idn*O<>*kan* sets of mutants, differentiated by the presence of the mutation *gnt*177 in one of them. In the presence of this mutation the *E. coli* idnR regulon is induced by 5KG formed from gluconate by IdnO activity (Ramírez 2004, Ramirez et al., 2007). The fact that *gnt*K, *idn*O<>*kan* mutants grow in gluconate despite being disabled from converting it to the inducer 5KG, revealed that in this case g*nt*V expression is independent of that of the *idn*DOTR operon and presumably is induced by gluconate. This alternative induction displayed by *gnt*V seems to be important for an efficient utilization of the involved substrates. The results permit a better understanding of the cross-regulation between GntI and GntII systems.

MATERIALS AND METHODS

Bacterial strains

The *Escherichia coli* strains used in this study are listed in Table I. The genetic markers were previously reported (Berlyn et al., 1996).

Media

E. coli strains were grown in Luria-Bertani broth (LB) or mineral medium [MM (Tanaka et al., 1967)] or on LB plates, MM plates or gluconate bromthymol blue indicator plates

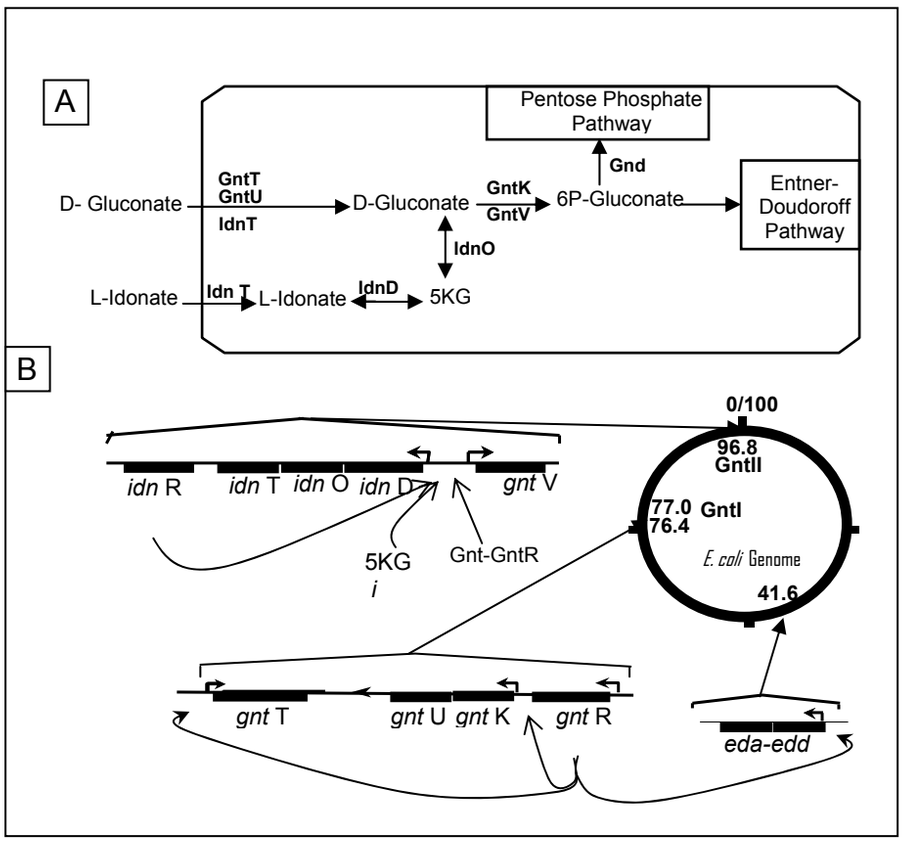

Fig. 1. Enzymes and respective genes of L-Idonate (Idn) and Gluconate (Gnt) catabolisms. Abreviations: 5KG, 5-keto-D-gluconate; GntT and GntU, GntI permeases; GntK and GntV, GntI and GntII gluconate kinases respectively; IdnT idonate and gluconate permeases; IdnD, idonate dehydrogenase; IdnO, 5KG reductase; IdnR, idnR regulon regulator; GntR, gntR regulon regulator; Edd, Entner-Doudoroff dehydratase; Eda, Entner-Doudoroff aldolase.

[GBTB (Istúriz, et al., 1986)]. MM was supplemented with carbon source as indicated at 2 g l^{-1}, 5 μg ml^{-1} of thiamine hydrochloride, and 20 μg ml^{-1} of L-amino acids as required. If necessary, MM and rich media were supplemented with 500 and 40 μg ml^{-1} DL-a-e-diaminopimelic acid (DAPA) respectively. When required tetracycline (Tet) was used at 15 μg ml^{-1}, kanamycin (Kan) at 30 mg ml^{-1}, and 5KG at 0.4%.

Growth conditions

Cells were routinely grown aerobically at 37 °C, in volumes of 10 ml for growth curves and 20 ml for enzyme assays in 125 ml flasks fitted with side arms, on a gyratory water bath (model G76, New Brunswick) at about 200 cycles min^{-1}. In each case, the growth was monitored by reading the optical density in a Klett colorimeter with a N° 42 filter.

Preparation of crude extracts

Cells were harvested by centrifugation, resuspended in 50 mM Tris-HCl 10 mM $MgCl_2$ (pH 7.6) and disrupted with a Braun Sonic 2000 (12T probe, 45 wattage level) by three 20s sonication pulses (3 pulses) separated by 30s cooling periods. Cell debris was, in each case, removed by centrifugation at 27000xg for 15 min.

Enzyme assays

Gluconokinase activity and its heat inactivation were assayed as previously described (Fraenkel and Horecker, 1964, Istúriz et al., 1986). Activities are reported as nmol min^{-1} (mg protein)$^{-1}$.

Phages and generalized transductions

The preparation of P1 lysates and generalized transductions were performed as reported (Miller, 1992) using a P1 phage stock kept in the laboratory.

DNA manipulations and transformations

Conventional and standard recombinant DNA techniques were employed (Sambrook et al., 1989).

Construction of mutants

To construct the mutants required for this work, we first constructed *E. coli* TK412 (*idn*O<>*kan*) using recombineering [Yu, et al., 2000 (Fig. 2A)]. First, an 1150 bp kanamycine recombinant cassette with *idn*O internal sequences on its flanks was generated by PCR from *E. coli* Y1088 *pro*A::Tn5, *kan* (Young and Davis, 1983) chromosomal DNA, using PCR primers

TABLE I
Strains of E. coli

Strain Source Phenotypes on Plat	Sex	Relevant Characteristics	Phenotypes on plates (24 h) BTB Mineral		Source
M1	HfrC	Prototrophic	Y	Gnt^+	[6]
C177	HfrG	*gnt*177, Δ (*bio*H –*asd)*	Y	Gnt^+	This Lab.
Y1088	F^-	*pro*A::Tn*5*, Kan^R	Y	Gnt^+	[29]
TAF394	HfrC	λcI857 Δ(*cro-bioA*)	Y	Gnt^+	This Lab.
DY329	F^-	Δ *lac* U169 *nad*A::Tn*10*, *gal*490 λcI857 Δ (*cro-bioA*)	Y	Gnt^+	[28]
TK411	HfrC	λcI857 Δ(*cro-bioA*), *idn*O<>*kan*	Y	Gnt^+	This study
TK412	HfrC	*idn*O<>*kan*	Y	Gnt^+	This study
TK416	HfrG	Δ(*bio*H-*gnt*T-*mal*A-*glp*D, *gntKU*, *gnt*R *asd*) *his*, *gnt*177, *idn*O<>*kan*	W	Gnt^-	This study
TGN282	F^-	*gntK*, *gnt*V, *his*, *trp*, *xyl*, *gal*	W	Gnt^-	This Lab.
TK424	HfrG	*gnt*K, *gnt*177, *his*, *idn*O<>*kan*	W	Gnt^-	This study
TK425	HfrG	*gnt*K, *gnt*177, *his*	Y	Gnt^+	This study
TUR285	F^-	*mal*A-*glp*D-*asd*, *gnt*V zhg21::Tn*10*, *his*, *trp*, *xyl*, *gal*	Y	Gnt^+	This Lab.
TK414	HfrC	*idn*O<>*kan*, Tet^R, *mal*A	Y	Gnt^+	This study
TK428	HfrC	*idn*O<>*kan*, Tet^S, Mal^+, *gnt*K	W[a]	Gnt^{-b}	This study
TK430	HfrC	*idn*O<>*kan*, Tet^S, Mal^+	Y	Gnt^+	This study

All the strains are ***E. coli*** K12 derivatives. The genetics markers were as previously described (Berlyn et al., 1996). Y (yellow) and W (white) colonies on BTB gluconate plates indicate fermenting and non-fermenting phenotypes respectively. The colonies were tested by streaking fresh colonies and scoring after 24 h incubation. Gnt^+ and Gnt^- indicate growth and no growth, respectively, on mineral agar plates with gluconate. [a]Fermenting phenotypes at 48 h. [b]Growth at about 48 h. <> to indicate a replacement generated by homologous recombination techniques.

PAHNO1 (5′CAGGTGGCCGTTTACGAAATCAGAGGCTTTTGAAGAAAGGAACACCGCATCAGAAGAACTCGTCAAGAAG3′) and PAHNO2 (5′GCAGCAAAAGTCCAGCTTGTTTTCTAAGAGATAAATAAAGAAATAATACACATGGACAGCAAGCGAACCG3′). Second, the recombinant cassette was used to transform *E. coli* TAF394 [(λcI857Δ(*cro-bio*A)], a suitable lambda lysogen for promoting linear recombination. Third, *E. coli* TK411 [(λcI857Δ(*cro-bio*A) *idn*O<>*kan*] a lysogen Kan^R transformant, was cured from the defective prophage by transducing it to Bio$^+$ at 42 ^{0}C with P1 phage grown in *E. coli* M1. Finally, we selected *idn*O<>*kan* transductant *E. coli* TK412 (*idn*O<>*kan*) for later work.

The *idn*O gene (850 bp) only or *kan* inserted (1250 bp) were amplified with primers PAH5: 5′CGGAATTCCGGGGGGCTGTTAAACAGCCAC3′ and PAH6: 5′CGGGATCCCGAGATAAATAAAGGAATAATA3′(Fig. 3).

To obtain isogenic *gnt*K, *idn*O<>*kan E. coli* strains with or without the *gnt*177 mutation, phage P1 grown in *E. coli* TK412 was used to transduce *E. coli* C177 to Kan^R (Fig. 2B). *E. coli* TK416, a Kan^R transductant and *E. coli* C177, were in turn made *gnt*T$^+$, *gnt*K, *gnt*U$^+$ by transducing them to Mal$^+$, Asd$^+$ and restoring their *bio*H-*asd* regions with phage P1 grown in *E. coli* TGN282 (*gnt*K). Two transductants were selected, *E. coli* TK424 (*gnt*K, *idn*O<>*kan*, *gnt*177) and *E. coli* TK425 (*gnt*K, *gnt*177) and saved for use in this work.

In order to obtain a second and similar pair of *E. coli* mutants that lacked the *gnt*177 mutation, strain TK412 was made GntV dependent to growth in gluconate by incorporating it a *gnt*K allele in two steps (Fig. 2A). First, it was transduced to Tet^R, Mal$^-$ with P1 phage grown in *E. coli* TUR285. Second, a selected transductant *E. coli* TK414, was in turn transduced to Mal$^+$ with phage P1 grown in *E. coli* TGN282 (*gnt*K). Among the Tet^S transductants, two phenotypes arose on BTB gluconate plates: one formed white non-fermenting colonies after 24 h of incubation that became yellow after 48 h incubation. The other formed unchanging, yellow fermenting colonies. It is known (Istúriz et al., 1986) and demonstrated below, that such phenotypes indicate the functioning of the thermosensitive (GntV) and thermoresistant (GntK alone or mixed with GntV) gluconate kinases respectively. A transductant of each type, *E. coli* TK428 and *E. coli* TK430 respectively, was saved for subsequent studies. PCR analysis confirmed the genotypes of mutants (not shown).

Chemicals

D-gluconic acid (potassium salt), pyrimidine nucleotides, sugars, amino acids and most other chemicals were purchased from Sigma. Media were from L-Himedia Lab. Primers were from Promega and GIBCOBRL.

RESULTS

Characteristics of E. coli mutants TK424 (idnO<>kan, gntK, gnt177) and TK425 (gntK, gnt177)

Because *E. coli* mutant TK425 carries the *gnt*177 and *gnt*K mutations, its growth in gluconate must depend on the inducible expression of the idnR regulon (*idn*DOTR and *gnt*V

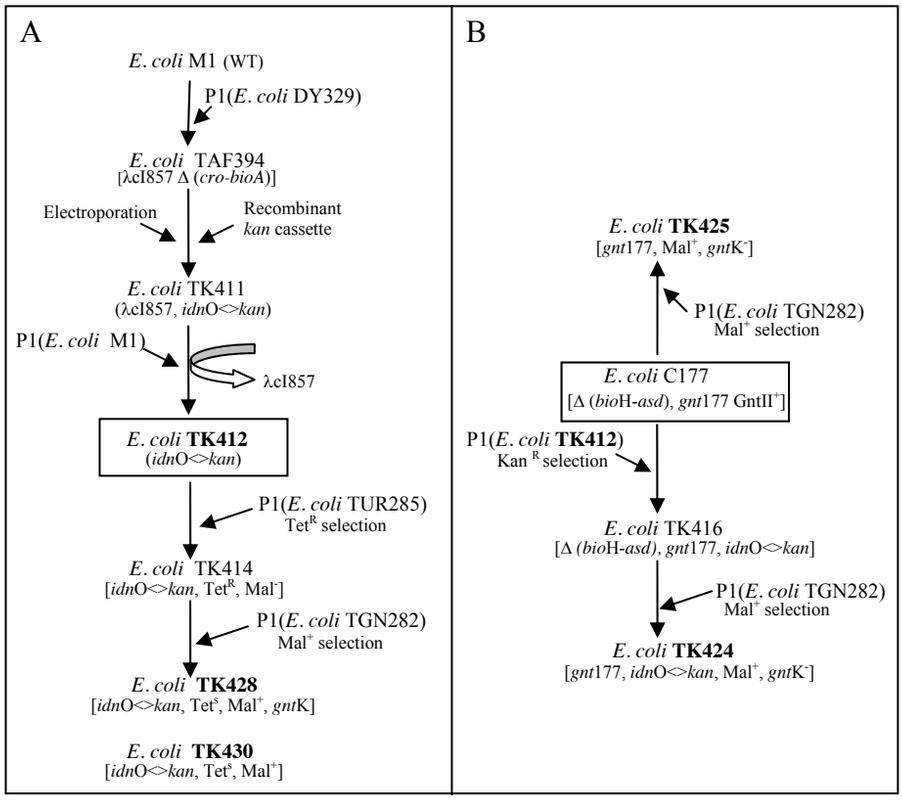

Fig. 2. Construction of *E. coli* mutants: For markers not indicated, see Table I.

operons) by the 5KG-IdnR complex (Ramírez, 2004). In this case, the inducer 5KG is formed from gluconate by the IdnO activity. Consequently, the set formed by this mutant and its isogenic *E. coli* TK424 was suitable to confirm the efficiency of the inserted *kan* cassette to abort the IdnO activity.

The above mutants grew on LB plates and mineral plates supplemented with maltose or fructose but in contrast to the *gnt*K, *gnt*177 control strain TK425, *E. coli* TK424 (*idn*O<>*kan*, *gnt*K, *gnt*177) was Kan^R and required 5KG to grow on mineral plates with gluconate. Moreover, the colonies of *E. coli* TK424 were white nonfermenting on BTB-gluconate plates, but yellow fermenting after 48 h incubation if supplemented with 5KG (Table II). In agreement with these results, although both mutants displayed normal generation times in MM with fructose (57 and 59 min) and fructose plus gluconate (58 and 65 min) , *E. coli* TK424 required 5KG to grow in gluconate and this growth had a lag period and a doubling time (300 and 242 min, respectively) that were longer than those of *E. coli* C177 (240 and 180 min) and the isogenic *E. coli* TK425 (30 and 80 min) grown in MM supplemented with gluconate (Table III).

The gluconate kinase activity was also measured in *E. coli* TK424 and TK425 grown in MM with fructose, fructose plus gluconate, and this substrate, with and without 5KG. *E. coli* C177 grown in MM with gluconate, was used as an additional control (Table IV). Where this activity was detected, it was thermosensitive and expressed in inducible form. The level displayed by *E. coli* TK424 grown in gluconate supplemented with 5KG [44 nmol min^{-1} (mg protein)$^{-1}$] was lower than those expressed in *E. coli* C177 and TK425 [62 and 106 nmol min^{-1} (mg protein)$^{-1}$] grown in the same medium without 5KG respectively (Table IV).

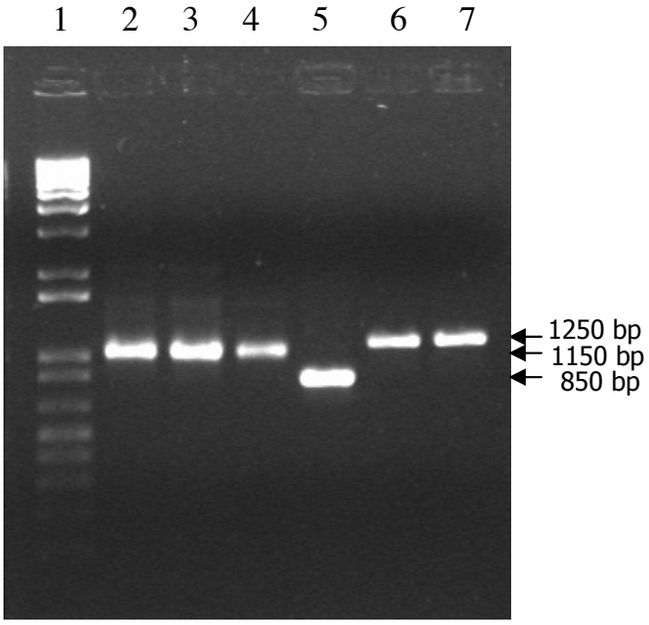

Fig. 3. Electrophoretic analysis in 0.8 % agarose gel of PCR products. Lane 1, 1 Kb DNA ladder; lanes 2, 3 and 4, *kan* cassettes from *E. coli* Y1088, TK411 and TK412; lane 5, *idn*O from *E coli* TAF394; lanes 6 and 7, *idn*O<>*kan* from *E. coli* TK411 and TK412.

Characteristics of E. coli mutants TK428 (idnO<>kan, gntK, Tet^S) and TK430 (idnO<>kan)

Since these mutants lack the mutation *gnt*177, the control on the *gnt*V expression should be as in *E. coli* wild type, therefore, they were suitable to investigate whether this expression in cells grown in gluconate depends on 5KG as inducer and is coordinated with that of the operon *idn*DOTR. This is just what occurs when *E. coli* is grown in idonate and the inducer 5KG is formed from idonate by the IdnD activity (Bausch et al., 1998). Both mutants grew on MM plates supplemented with maltose, fructose, LB plus kanamycine but, as expected, did not grow on LB plus tetracycline (Table II). Interestingly, in agreement with their fermenting phenotypes displayed on BTB-gluconate plates (Table II), these mutants grew in MM supplemented with gluconate without requiring 5KG (Table III); however, the lag period (245 min) and doubling time (155 min) showed by *E. coli* TK428 were notably higher than those in *E. coli* TK430 (50 and 60 min respectively).

The level of specific thermosensitive (70% heat inactivated) gluconate kinase expressed in *E. coli* TK428 [40 nmol min^{-1} (mg protein)$^{-1}$] did not increase with the addition of 5KG to the medium and was significantly lower than that displayed by *E. coli* TK430 [141 nmol min^{-1} (mg protein)$^{-1}$] expressing mainly the thermoresistant GntK (7% heat inactivated; Table IV).

DISCUSSION

The idnR regulon, induced by 5KG in *E. coli* grown in idonate, includes the *gnt*V gene encoding a thermosensitive gluconate kinase which is also induced in gluconate grown cells. As it was not known whether in this case 5KG is the inducer, the research presented here was addressed to elucidating this question. Our results indicate that *gnt*V is expressed in the absence of 5KG when gluconate is the substrate. They were obtained through the construction and comparative analysis of two sets of isogenic *E. coli gnt*K, *idn*O<>*kan* mutants, differing by the presence of the mutation *gnt*177 in one of them.

Because idonate is not commercially available, the gluconate phenotype displayed by the set of *E. coli idn*O *kan*–cassette inserted mutants carrying the *gnt*177 mutation, was of central importance in the present work. Since in these mutants the idn regulon (*idn*DOTR operon plus *gnt*V) is induced by 5KG in cells growing in gluconate, they were not only suitable to demonstrate the efficiency of the *idn*O *kan*–cassete insertion to abort the IdnO activity, but also permitted determining that in similar mutants lacking the *gnt*177 mutation, *gnt*V expression does not involve 5KG as the inducer in cells cultivated in gluconate.

In the above context, it was demonstrated that while 5KG is essential for *E. coli* TK424 (*idn*O<>*kan*, *gnt*K, *gnt*177) to grow in gluconate, it is not required by the isogenic *E. coli* TK425 (*idn*O^+) control (Table III). Contrary to *E. coli* TK424, *E. coli* TK428 (*idn*O<>*kan*, *gnt*K) grows in gluconate without requiring 5KG despite being blocked in the synthesis of this inducer. This indicates that under this condition *gnt*V is expressed in absence of *idn*DOTR operon induction (Table III).The 5KG requirement of *E. coli* TK424 for growth on MM with gluconate indicated that the inserted *kan* cassette eliminated IdnO activity so it could be assumed that it is also absent in both *E. coli* TK428 and *E. coli* TK430.

TABLE II
Phenotypes on plates of strains of *E. coli*

Media	TK424	TK425	TK428	TK430	C177
LB Tetracycline	-	-	-	-	-
Maltose MM	+	+	+	+	-
Fructose MM	+	+	+	+	+
Gluconate MM	Gnt^-	Gnt^+	Gnt^{+b}	Gnt^+	Gnt^+
Gluconate MM + 5 KG	Gnt^{+b}	Gnt^+	n.d	Gnt^+	Gnt^+
BTB, Gluconate	W	Y	W^a	Y	Y
BTB, Gluconate + 5 KG	W^a	Y	W^a	Y	Y
LB Kanamycine.	+	–	+	+	–

Y (yellow), W (white), Gnt^+, $Gnt^–$, [a] and [b] respectively, indicate as described in Table I. + and – signs indicate growth and no growth on the respective plates. n.d, not determined.

TABLE III
Doubling times (min) of strains of *E. coli*

Carbon Source	TK424		TK425		TK428		TK430		C177	
	LP	DT	LP	DT	LP	DT	LP	DT	LP	DT
Fructose	~20	57	~30	59	~20	55	~25	53	n.d	n.d
Fructose + Gluconate	~20	58	~20	65	n.d	n.d	n.d	n.d	n.d	n.d
Gluconate	no growth		~40	82	~245	155	~50	60	~240	180
Gluconate + 5KG (0.4 %)	~300	242	~30	80	~240	222	n.d	n.d	n.d	n.d

Cells were grown aerobically on MM with fructose, collected during the exponential phase, centrifuged (3000 rpm., Sorval SS34), resuspended in the same medium up to 300 Klett units (KU, about 10^9 cells ml^{-1}) and starved during 30 min at 37 °C. New cultures were initiated at about 10 UK (approximately 10^7 cells ml^{-1}) with the indicated carbon sources at 0.2%. LP, lag phase; DT, doubling time; ~ approximately; n.d, not determined.

TABLE IV
Gluconate kinase activities in *E. coli* strains

Carbon source	TK424	TK425	TK428	TK430	C177
Fructose	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fructose + Gluconate	< 0.01	47±5 (70)	n.d	n.d	n.d
Fructose + Gluconate + 5KG	< 0.01	61±7	n.d	n.d	n.d
Gluconate	no growth	106±8 (85)	40±4 (70)	141±7 (7)	62
Gluconate + 5KG	44±5 (90)	n.d	36±6 (97)	n.d	n.d

Cells were grown as indicated in Table III. New cultures without previous starvation were grown up to120 KU in MM with the indicated carbon sources at 0.2%.
n.d, not determined; Numbers in parenthesis indicate gluconokinase lability (percentage of activity lost after three hours preincubation at 30 °C). The values for the activities represent means ± standard deviations from two independent experiments. For units, see Materials and methods.

As demonstrated, *E. coli* TK428 *gnt*V expression is activated by gluconate in the absence of 5KG. Since GntR interacts with gluconate to control the gntR regulon, and GntR binding sites are included in the *gnt*V-*idn*D intergenic regulatory region of the idnR regulon, this same complex might be also involved in *gnt*V induction. Interestingly, GntR was found to have a negative effect on the expression of GntII genes in *gnt*R-disrupted strains carrying single copies of *gnt*V-*lac*Z or *idn*D-*lac*Z fusions. This effect was not observed by the addition of gluconate, presumably due to the formation of a Gnt-GntR complex (Tsunedomi et al., 2003b).

The lack of idnDOTR expression in E. coli does not alter the gluconate phenotype

E. coli TK430 (GntK$^+$, *idn*O<>kan) grows in MM gluconate with a shorter generation time (60 vs. 155 min; Table III) and has higher levels of gluconokinase [141 vs. 40 nmol min^{-1} (mg protein)$^{-1}$; Table IV] than *E. coli* TK428 (GntK$^-$, *idn*O<>*kan*). Notably, while the gluconate kinase expressed in this mutant is thermosensitive (70% inactivated), that expressed by *E. coli* TK430 is mainly thermoresistant GntK (7% inactivated; Table IV). Despite lacking the ability to form 5KG from the substrate, this characteristic of *E. coli* TK430 and its short lag period (25 min; Table III) in MM with gluconate are attributes of a wild type gluconate phenotype.

The idonate-gluconate crosstalk in E. coli wild type

The characteristics of the two sets of *E. coli* mutants used here would seem to reveal an important and novel physiologic aspect by which the gene *gnt*V is induced by 5KG or gluconate depending upon whether idonate or gluconate is metabolized. This alternative induction of *gnt*V might be of importance for the development of the bacteria in its natural environment where, as opposed to lab conditions, substrate concentrations are very low. In such a situation, it is not so obvious for energy-saving reasons that the utilization of either substrate requires the induction of both regulons; *i.e.*, idnR and gntR. The alternative induction of the *E. coli gnt*V gene reported here would seem to impede this situation by being coordinated with that of the *idn*DOTR operon or the *gnt*R regulon depending on which substrate, idonate or gluconate, is metabolized. It is of interest to advance in the molecular mechanisms associated with this alternative expression of *gnt*V.

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