The Characterization and Cloning of a Gluconate (gnt) Operon of Bacillus subtilis

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The enzymes involved in gluconate utilization in Bacillus subtilis seemed to be gluconate permease and gluconate kinase. Several mutants unable to grow on gluconate were isolated. The mutations they harboured (gnt) were clustered between iol-6 and fdp-74 on the B. subtilis chromosome (a tentative map order of gnt-f0, gnt-4, gnt-26, gnt-23 and gnt-9 was obtained). The gnt-10 mutation seemed to be located within the structural gene of the kinase, and the gnt-23 and gnt-26 mutations seemed to be within that of the permease. An EcoRI fragment (4.5 MDal) containing an intact gluconate (gnt) operon consisting of these two structural genes was cloned in phage φ105 by prophage transformation and was mapped physically. The physical location of the mutations coincided with their order on the genetic map. The HindIII-A fragment (2.4 MDal), which corrects all the gnt mutations, was subcloned in plasmid pC194. The fragment contained the structural genes for the gluconate permease and kinase, but not the regulatory region of the gluconate operon.

INTRODUCTION

After entering the Bacillus subtilis cell, gluconate is phosphorylated to gluconate 6-phosphate, which, because B. subtilis lacks the Entner–Doudoroff pathway, can only be metabolized through the pentose cycle. Therefore, only two enzymes may be needed for the utilization of gluconate as a carbon source: one is gluconate permease, which transports gluconate into the cell, and the other is gluconate kinase, which phosphorylates gluconate. Both enzymes are induced in response to gluconate and their induction is repressed in the presence of rapidly metabolizable carbohydrates such as glucose (Dowds et al., 1978; Nihashi & Fujita, 1984). We have tentatively reported the cloning of the genes for gluconate utilization in a temperate phage, ρ11 (Fujita et al., 1983). In this paper, we communicate the genetic mapping and characterization of several gnt mutations that render the cell unable to grow on gluconate and we also report the recloning of an EcoRI fragment cloned in ρ11 in another temperate phage, φ105. This fragment encodes an intact gluconate operon. To our knowledge, this work is the first example of the use of a B. subtilis host–vector system to clone an intact catabolic operon of this species.

METHODS

Bacteria, phage and plasmids. Strain 60015 (trpC2 metC7) is our standard strain. Among Gnt+ mutants, the isolation of strain 61656 (Aigj hisA1 leuA8 metB5 trpC2), YF127 (gnt-4 trpC2 metC7) and YF029 (gnt-9 purA16 leuA8 metB5 hisA3) has already been reported (Fujita & Freese, 1981; Fujita & Fujita, 1983). Strain YF158 (gnt-4 recE4 trpC2) was constructed from YF127 by congression using the DNA of strain 1A423 (leuA8 thr-5 argA15 recE4 r- m+), which was obtained from the Bacillus Genetic Stock Center, Ohio State University, Columbus, USA. Strain YF160 (gnt-10 trpC2 metC7) was isolated from strain 60015 that had been treated with

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ethylmethane sulphonate, according to the method previously described (Fujita & Fujita, 1983). Strains YF161 (gnt-23 trpC2 metC7) and YF162 (gnt-26 trpC2 metC7) were isolated as follows. After strain 60015 had been treated with ethylmethane sulphonate, cells were grown in a liquid minimal medium (S6) (Fujita & Fujita, 1983) containing glucose as sole carbon source and were plated on a solid minimal medium (N) (Fujita & Fujita, 1983) containing gluconate (0.1%), and glucose (0.01%) as carbon sources. Among approximately 20000 colonies screened, 1500 tiny colonies were picked and tested for growth on glucose and gluconate. Only two colonies, YF161 and YF162, showed a Gnt- phenotype. The temperate phage, φ105, was provided by F. Kawamura (Institute of Applied Microbiology, Tokyo University, Japan). Plasmids pUB110 and pC194 were obtained from Y. Sadaie (National Institute of Genetics, Mishima, Japan) and the Bacillus Genetic Stock Center, respectively.

**Enzyme assays.** Glucanase kinase (EC 2.7.1.12) was assayed as described previously (Niihashi & Fujita, 1984).

The enzyme was induced by exposure of cells to 10 mM-gluconate for 2 h.

Glucanase permease was assayed essentially by the method described by Dowds et al. (1978). When cells had grown at 37 °C to an OD_600 of 1.0 in S6 medium containing 0.5% Casamino acids (Difco), 3 ml of the culture were harvested and washed in S6 medium plus chloramphenicol (100 μg ml⁻¹). Washed cells were suspended in 2 ml of the same medium plus chloramphenicol (100 μg ml⁻¹) and used for the permease assay as an uninduced sample. In order to induce the permease, the above culture was diluted four times with the same growth medium and the cells allowed to grow to an OD_600 of 1.0 for approximately 2 h in the presence of 10 mM-gluconate. Samples (3 ml) of the culture were harvested, washed and resuspended as described above. The suspension (2 ml) was used as an induced sample. The assay tube held, at 37 °C, 0.7 ml S6 medium plus chloramphenicol (100 μg ml⁻¹) containing D-[U-¹⁴C]gluconate (0.05 μCi, 19.9 kBq; 5.6 μCi μmol⁻¹, 210 MBq μmol⁻¹) (Amersham) and 93 μM-gluconate. Incorporation was initiated by the addition of 0.6 ml of the cell suspension to the assay tube. After incubation at 37 °C for 30 s, the suspension was filtered on a premoistened membrane (25 mm diameter, 0.45 μm pore size) (Toyo Roshi Co.). The filter pad was immediately washed three times with 5 ml S6 medium plus chloramphenicol (100 μg ml⁻¹). The pad was dried and counted in 10 ml scintillant (ACSII; Amersham).

**Transduction and transformation.** PBS1-mediated transduction and DNA-mediated transformation were done as described by lijima et al. (1977) and Shibata & Saito (1973), respectively. Specialized transduction by phages derived from φ105 was done as described by lijima et al. (1980). For selection of Gnt' recombinants, N medium containing 25 mM-gluconate and enriched with 0.002% yeast extract (Difco) was used.

Transformation of Gnt' strains by restriction enzyme fragments was done as follows. The gnt' fragment (1 μg) was digested with BglII, MscI, PstI, Smal, Stul and HindIII. The digested samples were electrophoresed through a 1% gel of low melting point agarose (BRL) and then the bands stained with ethidium bromide (0.5 μg ml⁻¹ in H₂O) were excised. After melting the gels (65 °C; 30 min), a portion (10 μl) of the gel containing each fragment was used for transformation.

**Preparation of DNA.** *B. subtilis* chromosomal DNA was purified as described by Saito & Miura (1963). Phage particles (φ1 and φ105) and their DNA were prepared by methods essentially similar to those described by lijima et al. (1980). After phages had been induced with mitomycin C, phage particles were sedimented in the presence of polyethylene glycol (Yamamoto et al., 1970) and further purified by centrifugation through discontinuous CsCl gradients. Viral DNA was prepared from purified phage particles by phenol treatment. Plasmid pC194 was prepared in strain YF029 (φ105) purA16) was transduced with PBS1 propagated in strain YF127 (gnt-4) and then Pur' recombinants were selected, two Gnt' colonies were found among 161 Pur' recombinants. These results suggested that gnt-9 is very close to gnt-4, perhaps located between gnt-4 and fdp-74. All of the gnt mutations in our collection, including those that had been newly isolated, gnt-10, gnt-23 and gnt-26, were mapped by two-factor transformation crosses between Gnt' strains measuring recombination indexes (Fig. 1). The crosses suggested a map order of gnt-10, gnt-4, gnt-26, gnt-23 and gnt-9.
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Fig. 1. Map of the gnt locus deduced from transformation crosses. In the crosses, YF141 (gnt-4 trpC2 metB5 hisA1) and YF029 (gnt-9 purA16 leuA8 metB5 hisA3) were used as recipients and strains YF160, YF161, YF162, YF127 and YF164 (gnt-9 purA16 leuA8 metB5) as donors. Distances are expressed as recombination indexes (Chasin & Magasanik, 1968). The ratio of gnt+ to his+ (YF141 as recipient) or leu+ (YF029 as recipient) transformants was a measure of the relative frequency of transformation to gluconate utilization.

Table 1. *Induction of gluconate permease and gluconate kinase in gnt mutants*

Gluconate kinase and permease were induced and assayed as described in Methods. Values are means of duplicate assays. Data are from a representative experiment using a complete set of the gnt mutants. Similar independent experiments were repeated at least twice.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gluconate permease*</th>
<th>Gluconate kinase†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not induced</td>
<td>Induced</td>
</tr>
<tr>
<td>60015 (gnt+)</td>
<td>1.14</td>
<td>12.39</td>
</tr>
<tr>
<td>61656 (Δigf')</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>YF029 (gnt-9)</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>YF127 (gnt-4)</td>
<td>34.45</td>
<td>26.14</td>
</tr>
<tr>
<td>YF160 (gnt-10)</td>
<td>2.56</td>
<td>12.82</td>
</tr>
<tr>
<td>YF161 (gnt-23)</td>
<td>0.06</td>
<td>0.48</td>
</tr>
<tr>
<td>YF162 (gnt-26)</td>
<td>0.37</td>
<td>5.25</td>
</tr>
</tbody>
</table>

*nmol min⁻¹ per unit OD₆₀₀.
†nmol min⁻¹ mg⁻¹.

Since the enzymes involved in gluconate metabolism are supposed to be gluconate kinase and permease, the inducibility of these two enzymes in the Gnt⁻ mutants was investigated (Table 1). As expected, strain 61656 (Δigf') could induce neither the kinase nor the permease; the Δigf mutation is a large deletion covering the gnt locus. Strain YF029 (gnt-9) could not induce the two enzymes as normal. Since the gnt-9 mutation was considered to be a point mutation, this strain seemed to be impaired in a regulatory function required for the induction of both enzymes. Strain YF160 (gnt-10) was impaired only in the induction of the kinase, whereas strains YF161 (gnt-23) and YF162 (gnt-26) were impaired only in the induction of the permease. The former mutation seemed to be located within the structural gene of the kinase and the latter mutations were likely to be within that of the permease. Strain YF127 (gnt-4) could not induce the kinase at all, but unexpectedly this strain synthesized the permease constitutively.

**Cloning of the gnt⁺ fragment in the temperate phage φ105**

An EcoRI gnt⁺ fragment from *B. subtilis* Marburg 168 (trpC2) DNA containing the genes for gluconate utilization has been cloned in a temperate phage, ρ11, by prophage transformation.
Table 2. Enzyme induction and repression by glucose in the Φ105gnt
lysogenic strain

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gluconate permease*</th>
<th>Gluconate kinase†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not induced</td>
<td>Induced (+glucose)†</td>
</tr>
<tr>
<td>60015 (gnt+)</td>
<td>0.71</td>
<td>10.00</td>
</tr>
<tr>
<td>61656 (Aigf) (Φ105gnt+)</td>
<td>0.61</td>
<td>8.66</td>
</tr>
</tbody>
</table>

*nmol min⁻¹ per unit OD₆₀₀.
†nmol min⁻¹ mg⁻¹.
‡Glucose (10 mM) was added to the medium to repress enzyme induction.

using strain 61656 as recipient (Fujita et al., 1983). Moreover, Fujita et al. (1983) tentatively suggested that the gnt⁺ fragment contains the structural gene of the gluconate kinase and its regulatory region. However, it was very difficult to isolate the gnt⁺ fragment of the recombinant phage by agarose gel electrophoresis because EcoRI digestion of Φ11 DNA produces more than 25 fragments (Mizukami et al., 1980). To isolate and analyse the gnt⁺ fragment, it was recloned in another temperate phage, Φ105, that is relatively small, and convenient for isolation of this fragment (Iijima et al., 1980). DNA (5 μg) of phage Φ11hisA⁺ gnt⁺E (a recombinant Φ11 possessing the gnt⁺ fragment) and DNA (10 μg) of phage Φ105 were each completely digested with EcoRI. The digestion products were mixed and ligated using T4 DNA ligase and then used to transform strain 61656 (Aigf), lysogenic for Φ105, to Gnt⁺. All of the 250 Gnt⁺ transformants obtained were considered to result from the integration of the gnt⁺ fragment at the site of the Φ105 prophage genome, because of the absence of homology between this fragment and the chromosome of the Φ105 strain. Five transformants were treated with mitomycin C and at the same time infected with wild-type Φ105. All the lysates obtained exhibited the ability to transduce strain 61656 to Gnt⁺. One of the Gnt⁺ transductants was used for further experiments after single colony isolation. This transductant produced, upon induction, a gnt⁺ specialized transducing phage (Φ105gnt⁺) in the absence of helper phage Φ105, but the resulting phage required helper for transduction of a Gnt⁻ recipient to Gnt⁺. Φ105gnt⁺ could transduce not only the Aigf mutant but also strains carrying all the gnt mutations (gnt-4, gnt-9, gnt-10, gnt-23 and gnt-26) to Gnt⁺.

The inducibility of the gluconate kinase and permease in a Φ105gnt⁺ lysogen of strain 61656 (Aigf) was compared with that in our standard strain 60015 (Table 2). Strain 61656 (Φ105gnt⁺) induced the two enzymes at normal levels upon addition of gluconate to medium, and induction was repressed by the addition of glucose. The results clearly indicated that the structural genes of the gluconate kinase and permease and regulatory regions for their expression had been cloned in Φ105.

DNA from Φ105 and Φ105gnt⁺ was digested with EcoRI and analysed by agarose gel electrophoresis (Fig. 2a). Φ105 DNA produced nine fragments (from A to I) whereas Φ105gnt⁺ DNA produced a new fragment of 4.5 MDal (the gnt⁺ fragment) and three of the EcoRI fragments of Φ105 DNA (E, G and I) were missing. [The EcoRI cleavage map of Φ105gnt⁺ DNA is shown with that of Φ105 in Fig. 2(b).] It was ascertained that the gnt⁺ fragment was sited between fragments B and D as follows. Φ105 DNA does not contain any BglII cleavage sites whereas Φ105gnt⁺ DNA had one BglII cleavage site in the gnt⁺ fragment. After digestion of Φ105gnt⁺ DNA with BglII, the smaller fragment was isolated by agarose gel electrophoresis. EcoRI digestion of this fragment produced the EcoRI-D fragment, and the BglII-B fragment indicated in Fig. 3(a).

Construction of the restriction enzyme map of the gnt⁺ fragment and physical localization of the gnt mutations

The restriction enzyme map of the gnt⁺ fragment was constructed by digestion with BglII, HindIII, MluI, PstI, PvuII, SmaI and StuI (Fig. 3a). Since the gnt⁺ fragment had the ability to
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Fig. 2. (a) Agarose gel electrophoresis of EcoRI-digested samples of φ105 and φ105gnf+ DNA. The DNA (2 µg) was digested with EcoRI and electrophoresed in a 0.8% agarose gel. (b) EcoRI cleavage maps of φ105 and φ105gnf+ DNA, with sizes of fragments expressed as MDal. The map of φ105 DNA has recently been corrected (Anaguchi et al., 1984; Lampel et al., 1984; Bugaichuk et al., 1984). The cloned gnf+ fragment (4.5 MDal) is indicated as a bold line.

transform all the Gnt− mutants except the Δigf deletion mutant to Gnt+, we attempted to localize physically each gnt mutation on a particular restriction enzyme fragment by assaying fragments for their ability to transform the respective gnt mutant to Gnt+ (Table 3). The BglII-A, MluI-A, PstI-A, Smal-A, SphI-A and HindIII-A fragments transformed the gnt-4 mutant to the wild-type, while the BglII-A, MluI-A, PstI-A, Smal-B, SphI-B and HindIII-A fragments transformed the gnt-9 mutant to Gnt+. The gnt-10 mutant was transformed with the BglII-B, MluI-B, PstI-B, Smal-A, SphI-A and HindIII-A fragments, whereas the gnt-26 mutant was transformed with the BglII-A, MluI-A, PstI-A, Smal-A, SphI-B and HindIII-A fragments. The gnt-23 mutant was transformed with BglII-A, MluI-A, PstI-A and HindIII-A fragments. The reversion frequency of the gnt-23 mutation was extraordinarily high so that we could not conclude with confidence which of the SphI and Smal fragments exhibited the transforming activity. From these results, each gnt mutation was localized on the respective enzyme fragment (Fig. 3b). (The gnt-23 mutation could be localized approximately between the MluI site and the right-hand HindIII site.)
Fig. 3. (a) Restriction enzyme map of the gnt+ fragment. Fragments generated by BglII, HindIII, MluI, PstI, PvuII, SmaI and StuI digestion were alphabetically named; their sizes are expressed as MDal. (b) Localization of gnt mutations. This was deduced from the results of transformation experiments using fragments shown in (a) (Table 3). Transformation experiments were independently repeated at least twice using each recipient and various sets of restriction enzyme fragments; all the results obtained supported the locations shown.

Table 3. Gnt+ transforming activities of restriction enzyme fragments of the gnt+ fragment

The transformation was done as described in Methods. Independent transformation experiments were done for each recipient. Values are means of duplicate assays.

<table>
<thead>
<tr>
<th>Donor fragment</th>
<th>YF127 gnt-4</th>
<th>YF029 gnt-9</th>
<th>YF160 gnt-10</th>
<th>YF161 gnt-23</th>
<th>YF162 gnt-26</th>
</tr>
</thead>
<tbody>
<tr>
<td>BglII-A</td>
<td>600</td>
<td>1300</td>
<td>25</td>
<td>810</td>
<td>1100</td>
</tr>
<tr>
<td>BglII-B</td>
<td>1</td>
<td>95</td>
<td>380</td>
<td>186</td>
<td>76</td>
</tr>
<tr>
<td>MluI-A</td>
<td>65</td>
<td>550</td>
<td>37</td>
<td>450</td>
<td>660</td>
</tr>
<tr>
<td>MluI-B</td>
<td>8</td>
<td>128</td>
<td>900</td>
<td>210</td>
<td>110</td>
</tr>
<tr>
<td>PstI-A</td>
<td>442</td>
<td>1170</td>
<td>44</td>
<td>280</td>
<td>300</td>
</tr>
<tr>
<td>PstI-B</td>
<td>2</td>
<td>83</td>
<td>440</td>
<td>175</td>
<td>57</td>
</tr>
<tr>
<td>SmaI-A</td>
<td>1000</td>
<td>83</td>
<td>4000</td>
<td>230</td>
<td>180</td>
</tr>
<tr>
<td>SmaI-B</td>
<td>7</td>
<td>216</td>
<td>18</td>
<td>190</td>
<td>91</td>
</tr>
<tr>
<td>StuI-A</td>
<td>192</td>
<td>95</td>
<td>2800</td>
<td>220</td>
<td>56</td>
</tr>
<tr>
<td>StuI-B</td>
<td>1</td>
<td>212</td>
<td>38</td>
<td>250</td>
<td>174</td>
</tr>
<tr>
<td>HindIII-A</td>
<td>630</td>
<td>340</td>
<td>1500</td>
<td>770</td>
<td>910</td>
</tr>
<tr>
<td>No addition</td>
<td>0</td>
<td>80</td>
<td>8</td>
<td>193</td>
<td>95</td>
</tr>
</tbody>
</table>
Table 4. Induction of gluconate permease and gluconate kinase in ΔIgf strains bearing plasmids or φ105 derivatives containing the HindIII-A fragment

<table>
<thead>
<tr>
<th>Plasmid or phage</th>
<th>Gluconate permease*</th>
<th>Gluconate kinase†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not induced</td>
<td>Induced</td>
</tr>
<tr>
<td>pCG1</td>
<td>5.36</td>
<td>4.02</td>
</tr>
<tr>
<td>pCG8</td>
<td>16.08</td>
<td>9.15</td>
</tr>
<tr>
<td>φ105gnt+H2</td>
<td>2.76</td>
<td>2.85</td>
</tr>
<tr>
<td>φ105gnt+H4</td>
<td>3.71</td>
<td>3.05</td>
</tr>
</tbody>
</table>

* nmol min⁻¹ per unit OD₆₀₀.  † nmol min⁻¹ mg⁻¹.

Cloning of the HindIII-A fragment with pC194 and φ105 and its expression

To investigate the effect of high copy number on the regulation of the expression of the cloned genes, we attempted to clone the gnt⁺ fragment in plasmid pUB110; these experiments failed, perhaps because the fragment was too large to be cloned in this plasmid. As an alternative approach we decided to clone the HindIII-A fragment (2.4 MDal) in another plasmid, pC194, because this fragment was smaller, but nevertheless was able to correct all the gnt mutations in transformation experiments. The HindIII-A fragment (2 μg) was isolated electrophoretically from a HindIII-digested sample of the gnt⁺ fragment, and was ligated with pC194 (2 μg) that had been digested with HindIII. The ligated DNA was used to obtain Gnt⁺ transformants of strain YF158 (gnt-4 recE4 trpC2). Out of 187 Gnt⁺ colonies obtained, ten colonies were used for plasmid extraction and analysis. Two kinds of plasmid (represented by pCG1 and pCG8) were found in which the HindIII-A fragment was cloned in opposite orientations. Strain 61656 (Aid) transformed with each plasmid was used for further experiments. The inducibility of the gluconate permease and kinase in strain 61656 (ΔIgf) harbouring pCG1 or pCG8 ([61656(pCG1 or pCG8)] was investigated. The two enzymes were synthesized constitutively in both 61656(pCG1) and 61656(pCG8) (Table 4). Specific activities of the enzymes synthesized in 61656(pCG8) were 2–3 times higher than those in 61656(pCG1).

The constitutive synthesis of the gluconate permease and kinase might possibly be attributed to the presence of multiple copies of the HindIII-A fragment containing pCG1 or pCG8. To test this possibility, the HindIII-A fragment was cloned in the genome of φ105, which can be integrated as only one copy in the host chromosome, and the expression of the genes in this fragment was investigated. The HindIII-A fragment was cloned by prophage transformation using 61656 (ΔIgf) as recipient and using similar procedures to those described above. From two Gnt⁺ transducing phages obtained (φ105gnt⁺H2 and φ105gnt⁺H4), DNA was prepared, digested with restriction enzymes and analysed by agarose gel electrophoresis. The electrophoretic patterns of the EcoRI- and the EcoRI + HindIII-digested samples of φ105gnt⁺H2 were different from those of φ105gnt⁺H4, but the doubly digested DNA samples gave a band of 2.4 MDal that corresponded to the HindIII-A fragment (data not shown). The results indicated that φ105gnt⁺H2 and H4 possess the HindIII-A fragment cloned in different HindIII sites of φ105. Table 4 shows that strain 61656 lysogenic for either φ105gnt⁺H2 or H4 synthesized the gluconate kinase and permease constitutively. The results imply that the constitutive synthesis of the two enzymes in strains carrying pCG1 or pCG8 was not due to the high copy number of the fragment when cloned in pC194, but rather that a regulatory function required for the induction of the enzymes was lacking in the fragment.

DISCUSSION

All of the mutations affecting the gluconate permease and kinase and the regulation of their biosynthesis were found to be clustered. On the basis of two factor crosses between Gnt- mutants (Fig. 1), we distinguished on the map the gnt-10 mutation and the gnt-23 and gnt-26 mutations in the structural genes of the kinase and the permease, respectively. The existence of
the gnt-9 mutation affecting both the permease and the kinase, and the fact that the Δigf strain lysogenic for φ105gnt+H2 or H4 constitutively synthesized the two enzymes, suggested that the two structural genes belong to a single transcription unit, a gluconate (gnt) operon. However, we have not excluded the possibility that there are two separate transcription units controlled by the same regulators. The presence of the gnt-4 mutation affecting the kinase and causing the constitutive synthesis of the permease is not completely understood. However, at present, we prefer one of the possible explanations, i.e. that the gluconate kinase protein itself may be involved in the regulation of the gluconone operon, so that the impaired gluconate kinase produced in the gnt-4 mutant might cause the constitutive synthesis of the permease.

We previously reported the cloning of the structural gene of the gluconate kinase and regulatory regions for its expression in a phage, φ11 (Fujita et al., 1983). In this investigation, an EcoRI gnt+ fragment generated from DNA of the specialized gnt+ transducing phage φ11 was recloned in another temperate phage, φ105. The fact that the induction of the gluconate permease and kinase in the Δigf strain lysogenic for φ105gnt+ was indistinguishable from that in the wild-type strain (Table 2), and that φ105gnt+ was able to transduce the Δigf deletion as well as all the gnt mutants to Gnt+, strongly indicated that an intact gluconate operon had been cloned in φ105. The HindIII-A fragment as well as the gnt+ fragment (Table 3) was able to transform all the gnt mutants to the wild-type. However, the Δigf strain lysogenic for φ105 bearing this fragment synthesized the gluconate permease and kinase constitutively. This suggests that the HindIII-A fragment probably contains the structural genes of the two enzymes, but does not contain any of the regulatory region of the gluconone operon.

The map order of gnt-10, gnt-4, gnt-26, gnt-23 and gnt-9 (Fig. 1) is considered to be tentative because it is based on data from two-factor crosses. However, it was not possible to carry out three-factor transformation crosses because at present there is no known easily selectable marker that is linked to the gnt mutations. Moreover, these mutations were too close to be ordered by PBS1 transduction crosses. All of the gnt mutations were successfully assigned to restriction enzyme fragments generated from the gnt+ fragment (Fig. 3b). Although the map order of the gnt mutations is tentative, it is likely to be reliable because it is reasonably consistent with the physical map. The gnt+ fragment does not complement the iol-6 and fdp-74 mutations (Fujita et al., 1983), but it did complement all the gnt mutations. From these results and those obtained from the genetic crosses, the following order from iol-6 to fdp-74 was deduced: iol-6, gnt-10, gnt-4, gnt-26, gnt-23, gnt-9 and fdp-74.

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REFERENCES


