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Steven C. Quay
Western Michigan University

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The Role of D-Gluconic Acid in the Synthesis of the Enzymes of the Entner-Doudoroff Pathway in Pseudomonas fluorescens

Steven C. Quay

A Thesis presented to the Honors College in partial fulfillment of the Bachelor of Arts Degree
ACKNOWLEDGMENTS

I would like to thank a number of people, without whom this work could not have been performed. I would like to thank Dr. D. Fowler for initiating this project. To the latter I express my extreme gratitude.

Drs. S.B. Friedman and R.C. Eisenberg, for their continuous time, energy, and patience. At every point of this investigation they were instrumental in the designing and directing of experiments. This investigation was supported in part with funds from an Honors College assistantship. Finally I would like to acknowledge the loving concern of my family who supported me throughout this work and to whom this work is dedicated.
INTRODUCTION

Our understanding of microbial carbohydrate metabolism appeared complete following the elucidation of the important catabolic pathways, i.e., the Embden-Meyerhof, hexose monophosphate, and the Entner-Doudoroff pathways. However, recent advances in both the elucidation of new intermediates and the important question of regulation has kept this field open to investigation. A role for gluconate in the formation of the Entner-Doudoroff (ED) pathway enzymes, first discovered in *Pseudomonas saccharophila* (7), has been implied in numerous organisms, including, *Escherichia coli* (6), *Enterobacter aerogenes* (6), *Salmonella typhimurium* (8), *Pseudomonas natriegans* (5), and *Streptococcus faecalis* (17).

This study was therefore undertaken to determine the specific inducer of the ED pathway enzymes. The fluorescent pseudomonads seemed an ideal group to investigate since they generally lack fermentative mechanisms for glucose dissimilation. *Pseudomonas fluorescens* ATCC 13525 was selected for this work since it has been designated a neotype strain for *P. fluorescens* (16,18). A preliminary report of these investigations was presented (S.C. Quay, S.B. Friedman, and R.C. Eisenberg, Bacteriol. Proc., p. 152, 1971).
MATERIALS AND METHODS

Chemicals

The following were obtained from the Sigma Chemical Co., (St. Louis, Mo.): 6-phosphogluconic acid (6-PG), trisodium salt; glucose-6-phosphate (Glu-6-P); glutathione, reduced form; adenosine triphosphate (ATP); reduced nicotinamide adenine dinucleotide (NADH); oxidized nicotinamide adenine dinucleotide phosphate (NADP); tris-(hydroxymethyl)-aminomethane (Tris) buffer; D-glucono-δ-lactone, 2-keto-3-deoxy-6-phosphogluconate (KDPG), barium salt, was the generous gift of Dr. W. A. Wood, Department of Biochemistry, Michigan State University. Other chemicals were of reagent grade obtained from commercial sources.

Bacteria and Growth Media

Pseudomonas fluorescens ATCC 13525 was used throughout. All strains were maintained on Luria agar slants and transferred approximately every 2 months to the same media. The ability of cells to oxidize glucose was determined by growth of organisms on modified eosin y-methylene blue (EMB-glucose) agar which contained (grams per liter): eosin y, 0.2; methylene blue, 0.033; Difco (Detroit) yeast extract, 10.0; Difco Bacto-agar, 15.0; D-glucose, 10.0. Cells with defective glucose metabolism produced pink colonies, while normal cells gave rise to dark purple colonies with a typical metallic green sheen. For enzymatic studies cells were grown at 25°C
on rotary shakers in basal media (previously described) which contained (grams per liter): $K_2HPO_4$, 48.2; $KH_2PO_4$, 8.0; $MgSO_4\cdot7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0. Bacto casamino acids, vitamin-free (Difco) was added to the media (2.5 mg per ml) prior to sterilization. This media was altered for growth rate studies by reduction of the level of casamino acids to 1 mg per ml. Carbohydrates were sterilized separately and added aseptically as indicated in the text. Growth rates were determined from turbidity measurements using a Klett-Summerson Photoelectric Colorimeter, fitted with a 660 nm filter.

Dry weights of bacterial suspensions were estimated from turbidity measurements at 660 nm in side-arm flasks with a Klett-Summerson Colorimeter; 100 Klett units were taken to be equivalent to 0.262 mg of bacteria, dry weight, per ml of culture.

**Mutagenesis**

This procedure is essentially as described by Adelberg, Mandel, and Chen (1). *P. fluorescens*, grown on 25 ml of Difco nutrient broth and 1% D-glucose on a rotary shaker at 25°C, was harvested in mid-log phase of growth (0.5 mg per ml) and washed twice following centrifugation with potassium phosphate buffer, 0.03 M (pH 7.0). N-methyl-N'-nitro-N-nitrosoguanidine (NTG), 60 μg per ml, final concentration was added to 4.0 ml of a potassium phosphate buffer suspension of cells (0.2 mg per ml). The resulting suspension
was incubated for 40 min at 30°C with shaking. The cell suspension was then centrifuged, washed twice with potassium phosphate buffer, and resuspended in 5.0 ml of buffer. One half ml of this suspension was used to inoculate 25 ml of Difco nutrient broth containing 1% D-glucose. Cells from the above were incubated at 25°C with shaking. The culture was serially diluted in sterile saline when in mid-log phase of growth and plated on EMB-glucose agar. Cells with defective glucose metabolism were isolated in pure culture and retained for further characterization. The NTG concentration indicated above produced approximately a 50% kill by the same procedure. Bacteriological characterization tests were performed as outlined for P. fluorescens in Bergey's Manual of Determinative Bacteriology (3).

Preparation of cells and cell-free extracts.

Cultures were harvested by centrifugation at 10,000 xg for 10 min and washed twice with either 0.03 M potassium phosphate buffer, (pH 7.0) or 0.01 M Tris-hydrogen chloride (Tris-Cl) buffer (pH 7.3). For the preparation of extracts, the cells were resuspended in 5 mM 2-mercaptoethanol and disrupted by sonic oscillation at 4°C in three 10-sec intervals using a Branson model S-125 Sonifier (Branson Instruments, Stamford, Conn.), followed by extraction of the supernatant fraction after centrifugation at 20,000 xg for 30 minutes. This supernatant was called the "crude extract." In the preparation of cell membranes and cell-free extracts, the
The crude extract was subjected to further centrifugation at 200,000 xg for 2.5 hrs. The high-speed supernatant, called the high-speed supernatant, was called the high-speed supernatant, and the cell-free extract was retained for soluble enzyme assays. The pellet, "the particulate fraction," was washed twice in 0.03 M phosphate buffer (pH 7.0) and recentrifuged following each washing at 200,000 xg for 2.5 hrs. All manipulations were carried out at 0-4°C.

Enzymatic assays.

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) and 6-gluconate dehydrogenase (6-phospho-D-gluconate: NADP oxidoreductase, EC 1.1.1.43) were determined spectrophotometrically, using a Gilford recording spectrophotometer (Gilford Instruments, Oberlin, Ohio). The assay involved the reduction of nicotinamide adenine dinucleotide phosphate (NADP) at 340 nm as a function of time. The values obtained were converted to nanomoles of NADP reduced per minute per milligram of protein, assuming an extinction coefficient of 6.21 mM⁻¹ cm⁻¹ for NADPH. The reaction mixtures included: Tris-Cl (pH 7.68), 600 μmoles; glucose-6-phosphate or 6-phosphogluconate, 10 μmoles; NADP, 1 μmole; cell-free extract and water to 3.0 ml. The reaction was conducted at room temperature.

Glucokinase (ATP: D-glucose-6-phosphotransferase, EC 2.7.1.2) and gluconokinase (ATP: D-gluconate 6-phosphotransferase, EC 2.7.1.12) activities were measured by coupling formation
of glucose-6-phosphate or 6-phosphogluconate to NADP reduction in the presence of extracts of glucose-grown *Escherichia coli* as a source of glucose-6-phosphate:NADP oxidoreductase or 6-phosphogluconate: NADP oxidoreductase. Glucokinase and gluconokinase were rate-limiting in these assays. The reaction mixtures (3.0 ml) were the same as in the assay for glucose-6-phosphate dehydrogenase except that glucose-6-phosphate was omitted and the mixtures were supplemented with 10 μmoles MgCl₂; 2.5 μmoles ATP and 10 μmoles of D-glucose or D-gluconate. 6-phosphogluconate dehydratase (6-phospho-D-gluconate hydrolyase, EC 4.2.1.12) and KDPG aldolase (6-phospho-2-keto-3-deoxy-D-gluconate D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.14) were assayed essentially as described by Kovachevich and Wood (10). Particulate glucose dehydrogenase (D-glucose:2,6-dichloroindophenol oxidoreductase, EC 1.1.99.a) was determined spectrophotometrically by following the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm as a function of time. The extinction coefficient employed for DCIP was 20 mM⁻¹ cm⁻¹ at 600 nm and pH 7.0. The reaction mixture included: DCIP, sodium salt, 0.18 μmoles; phenazine methosulphate, 0.3 μmoles; sodium cyanide, 3 μmoles; potassium phosphate buffer, pH 7.0, 130 μmoles; D-glucose, 10 μmoles; particulate fraction and water to 3.0 ml. The reaction was conducted at room temperature. Particulate glucose oxidase (D-glucose: oxygen oxidoreductase, EC 1.1.3.4) and gluconate oxidase
(D-gluconate: oxygen oxidoreductase, EC 1.1.99.3) were determined by following the consumption of dissolved oxygen at 25 C as measured in a Gilson Oxygraph fitted with a Clark-type electrode. Oxygen saturation in distilled water at 25 C was assumed to be 250 nmoles per ml. The reaction mixture contained: D-glucose or D-gluconate, 4 μmoles; potassium phosphate buffer, pH 7.0, 45 μmoles; particulate fraction and water to 1 ml. Non-growing cells were used to measure oxygen consumption in the presence of glucose or gluconate in a reaction mixture which contained: D-glucose or D-gluconate, 4 μmoles; potassium phosphate buffer, pH 7.0, 27 μmoles; cells and water to 1 ml. The reaction mixture was allowed to equilibrate for 4-5 min at 25 C with stirring before the reaction was started by addition of substrate. Activity was corrected for endogenous O_2 consumption, which was always less than 5% exogenous activity. Cells were suspended to the same absorbance by using a klett-Summerson Colorimeter fitted with a 660 nm filter. Specific activity was determined from a standard curve relating absorbancy and whole cell dry weight.

Miscellaneous.

Protein concentrations were estimated by a modification of the procedure described by Lowry et al (12). Since Tris-HCl gives a positive test with this method, appropriate blanks were used when Tris-HCl buffer was employed. Membrane protein was estimated by heating membranes in 0.5 N NaOH for 15 min.
at 100°C and assaying the supernatant solution by the Lowry method (12). Crystalline bovine serum albumin (Sigma), treated in the same manner as the membranes, was used as a protein standard.

Pyruvate was determined by the method of Friedmann and Haugan (9) and identified by the absorption spectrum of its 2,4-dinitrophenylhydrazone chromogen.
RESULTS

Characterization of glucose oxidase deficient mutants.

Glucose oxidase deficient mutants of *Pseudomonas fluorescens* ATCC 13525 were obtained as described in Materials and Methods. Tests were performed (Table 1) to verify that the isolated mutants were *P. fluorescens*. It was concluded on the basis of these tests that our isolates were homogenous and were *P. fluorescens*. The nature of the lesion in these mutants was investigated by measuring whole cell oxygen consumption and particulate glucose: oxygen oxidoreductase and glucose dehydrogenase activities. All tests were performed at pH 7.0 and 25 C. Table 2 depicts results from such experiments. Mutant 7 showed no ability to reduce either oxygen or DCIP at the expense of glucose. Mutant 17 showed no glucose oxidase or glucose dehydrogenase in particulate fractions though it showed about 17% of the parent strain consumption in the whole cell. The effect of varying hydrogen ion concentrations on whole cell oxygen consumption was determined (Fig 1). Alkaline conditions reduced activity in all three strains, most dramatically in the mutants. When particulate fractions of the parent and mutant 7 strains were examined for glucose: oxygen oxidoreductase activities as a function of pH the mutant showed no activity over a pH range of 6.0 to 8.0 though the parent strain showed
activity comparable to whole cell activity over the same hydrogen ion concentration range.

The mutant and parent strains showed similar growth rates in minimal media containing glycerol or gluconate as sole carbohydrate, while on glucose the mutants grew far more slowly than the parent strain (Table 3). This is consistent with the inability of the mutants to oxidize glucose. The mutants were found to also be defective in some aspect of amino acid biosynthesis. It was observed that ammonium sulfate as sole nitrogen source in a basal salts and gluconate media could not support growth of either mutant strain, though good growth was observed in the parent strain. For this reason growth rates with carbohydrates as sole carbon source could not be determined since all growth was conducted with casamino acids present.

**Induction of catabolic enzymes for glucose dissimilation.**

The effect of glucose and gluconate pulsing during growth on the synthesis of the Entner-Doudoroff (ED) enzymes, 6-PG dehydratase and KDPG aldolase, is shown in Table 4. These data represent experiments in which logarithmically growing cells were pulsed with 0.02 M glucose or gluconate and after 3 hrs of additional growth the cells were harvested and crude extracts prepared as described in Materials and Methods. Enzyme analysis was based on the conversion of 6-phosphogluconate to pyruvate by the crude extracts. It is apparent that gluconate induces the ED enzymes in the
strains tested. Glucose, however, fails to induce these enzymes in mutant 7 and to a lesser extent in mutant 17. This is apparently due to the lack of particulate glucose: oxygen oxidoreductase activities in mutant 7 and 17. Mutant 17, which has a small amount of whole cell oxygen consumption with glucose, has a corresponding low level of ED enzymes. It should be noted that gluconate produces a higher specific activity of ED enzymes than glucose in the parent strain. This is presumably related to the need to form gluconate from glucose prior to induction of the ED enzymes. Eisenberg and Dobrogosz (6) found approximately equivalent levels of these enzymes in this P. fluorescens parent strain when glucose or gluconate was present throughout the entire growth period.

The effect of substrate concentration on ED enzyme induction is indicated in Fig 2. The experiment was conducted as described above but with the induction period reduced to 1 hr. It is clearly seen that enzyme induction increases with substrate concentration over the range shown. Again, there is little or no induction of the enzymes in question when mutant 7 is exposed to glucose.

To determine the effect of gluconate on the synthesis of other enzymes of glucose and gluconate dissimilation an experiment was performed in which cell-free extracts of glucose and gluconate pulsed cells were tested for a number of enzymatic activities. The results are shown in Table 5.
The particulate gluconate oxidase, gluconokinase, 6-PG dehydratase, and the KDPG aldolase were all induced in the presence of gluconate. Since all strains had a fairly high level of KDPG aldolase activity when grown on glucose a subsequent experiment was performed (not shown) which indicates that this enzyme is present when cells are grown on casamino acids as sole carbon and energy source. Constitutive KDPG aldolase activity has also been observed in Salmonella typhimurium grown on fructose or glucose (8) and in E. coli, which contains negligible amounts of 6-PG dehydratase.

The presence of glucokinase could not be detected in either parent or mutant strains, in support of the previously shown observation that P. fluorescens does not possess a functional glucokinase (19,20).

The data indicates that the soluble dehydrogenases, glucose-6-phosphate: NADP oxidoreductase and 6-phosphogluconate: NADP oxidoreductase, are not induced by gluconate under the experimental conditions. This is similar to observations made in Salmonella typhimurium (8). However, these enzymes have been shown to be induced by growth on glucose, gluconate, or glycerol but not succinate in Pseudomonas aeruginosa (13). Our data does indicate that growth on gluconate results in a two-fold increase in glucose-6-phosphate and 6-phosphogluconate dehydrogenases in both the parent and the mutant
strains. One explanation for the increased activity with gluconate, discussed in connection with *Salmonella typhimurium* (8), is that the rate of carbohydrate oxidation through the hexose monophosphate shunt is dependent on the availability of NADP. Since gluconate forms only one equivalent of NADPH while 2 equivalents are formed and must be reoxidized during glucose metabolism, the levels of NADP-linked enzymes should be lower under conditions of glucose growth. A second notion is that an increase in demand for reduced pyridines, i.e., NADPH, for the catabolism of 2-ketogluconate (which is known to accumulate during growth on gluconate) via 2-keto-6-phosphogluconate reductase is met by an increase in NADP-linked dehydrogenases. Our data does not indicate which is the correct interpretation nor if other alternatives exist.

The observation that the particulate gluconate oxidase is induced by gluconate in the parent and mutant strains but not in cells of mutant 17 is important in designating the specific inducer of this catabolic series. Since gluconate can also be oxidized by the particulate gluconate oxidase to 2-keto-gluconate and subsequently phosphorylated to 2-keto-6-phosphogluconate before being reduced to 6-PG the possibility existed that one of these intermediates, 2-ketogluconate or 2-keto-6-phosphogluconate might be the specific inducer. This possibility is untenable for the following reasons: First, the gluconate oxidase is itself induced by gluconate.
Secondly, mutant 17, which lacks the particulate gluconate oxidase and the ability to oxidize gluconate as nonproliferating cells, retains the capacity to induce the ED enzymes in the presence of gluconate.
DISCUSSION

Catabolism of glucose in the aerobic heterotrophic pseudomonads has been viewed as occurring via the phosphorylation of glucose and its subsequent oxidation to 6-PG as indicated in Scheme I. Through the use of glucose oxidase deficient mutants an alternate sequence, in which 6-PG is formed by phosphorylation of gluconate derived from a membrane associated glucose oxidase, has been confirmed in *Pseudomonas fluorescens*. This conclusion, summarized in Scheme 2, is based on the lack of glucokinase in this organism and the requirement for gluconate as specific inducer of the Entner-Doudoroff pathway enzymes.

The effect of mutations on the utilization of specific metabolic sequences when branched pathways exist, i.e., glucose dissimilation via Embden-Meyerhof and Entner-Doudoroff pathways, can be illustrated by viewing glucose metabolism in a number of organisms. At one extreme is *E. coli* which possesses primarily the enzymes of anaerobic glycolysis. The presence of the ED pathway enzymes is induced by growth on gluconate but not glucose, since the latter participates chiefly in kinase-mediated phosphorylations. *P. fluorescens* on the other hand, lacking a glucokinase, could form only gluconate from glucose. The aerobic nature of these processes implies their development after the establishment of an amply supply of molecular oxygen. *P. aeruginosa*, while able
to oxidize glucose via molecular oxygen **in vitro**, appears to do so at a three-fold lower level than **in vivo** glucose utilization (13). Further, this organism contains a functional glucokinase. These observations would imply that *P. aeruginosa* is facultative in relationship to oxygen. However, due to the lack of a functional fructose-1,6-diphosphate aldolase, this organism is obliged to utilize only the ED pathway for glucose catabolism. Thus the diversity in the mechanisms of glucose dissimilation in these organisms can be traced to the nature and extent of enzymatic lesions.

The role of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the regulation of the branch point between the hexose monophosphate shunt and other routes of glucose catabolism has been implied from a number of observations. Among these was the observation that ATP inhibited glucose-6-phosphate dehydrogenase in *P. aeruginosa* (13) and more recently, the fructose-1,6-diphosphate-mediated inhibition of 6-phosphogluconate dehydrogenase in a number of procaryotic and eucaryotic organisms (4). The latter observation is difficult to interpret for *P. fluorescens*, since this organism does not have a phosphohexokinase. It is presumed that fructose-1,6-diphosphate can be formed by other mechanisms in this organism. Another mechanism for the regulation of branched metabolic pathways involves enzyme synthesis, mediated at the transcriptional level. This approach can be used to
interprete the functional implications of the fact that the glucose-6-phosphate dehydrogenase and the 6-PG dehydratase are coded for in adjacent, i.e., cotransduceable, regions of the chromosome of E. coli (14).

The transport of carbohydrates in procaryotic organisms has been extensively studied in recent years. One consequence of such studies was the identification and characterization of a phosphoenolpyruvate (PEP): hexose phosphotransferase system by Kundig and coworkers (11). Of particular interest was the recent work by Romano et al (15) demonstrating that the PEP: hexose phosphotransferase system does not generally occur in aerobic bacteria but that a fundamentally different system exists for those organisms. As depicted in Scheme 2, the concept that transport of glucose in P. fluorescens is by means of an oxidative mechanism is consistent with the inability of glucose oxidase deficient mutants to produce an inducer of the ED pathway enzymes. Work by Eagon and Phibbs (in press) however, indicates that glucose-grown cells of P. aeruginosa fail to accumulate \(^{14}\)C-gluconate when incubated with \(^{14}\)C-glucose, although sugar phosphates were found. This they imply would indicate that glucose transport was by other than an oxidative mechanism. However, our data indicates (Table 5) that the glucose oxidase is rate limiting with respect to the gluconokinase. Under these conditions gluconate would not accumulate. The fact that \(\gamma\)-methyl glucoside is not
transported by the glucose transport system in *P. aeruginosa* is further evidence that the oxidation of glucose is obligatory to glucose transport. These observations do not however rule out the possibility that glucose is transported as free sugar and is trapped intracellularly by oxidation and subsequent phosphorylation, as forwarded by Eagon and Phibbs (*in press*).


Table 1. Bacteriological tests of isolated mutants

<table>
<thead>
<tr>
<th>Character Tested</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parent Strain</td>
</tr>
<tr>
<td>Relation to oxygen</td>
<td>obligate aerobe</td>
</tr>
<tr>
<td>Production of fluorescent pigment</td>
<td>positive</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>negative</td>
</tr>
<tr>
<td>Indole production</td>
<td>negative</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>positive</td>
</tr>
<tr>
<td>Reaction in Litmus milk</td>
<td>alkaline</td>
</tr>
</tbody>
</table>

*Procedures as indicated in Materials and Methods*
Table 2. Glucose dehydrogenase and oxidase activities in particulate fractions from parent and mutant strains of P. fluorescens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Whole cell $O_2$ consumption</th>
<th>Particulate glucose oxidase</th>
<th>Particulate glucose dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent strain</td>
<td>26.0</td>
<td>7.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Mutant 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mutant 17</td>
<td>3.6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Cells were grown to midlog phase on basal salts plus casamino acids media and pulsed for 3 hrs with 0.02 M D-glucose before harvesting. Whole cells and particulate fractions prepared as described in Materials and Methods.

*b* Non-proliferating cell suspensions were in potassium phosphate buffer at pH 7.0. Activities represent nmoles $O_2$ consumed per min per mg dry weight.

*c* D-glucose:$O_2$ oxidoreductase activities represent nmoles consumed per min per mg protein.

*d* D-glucose:DCIP oxidoreductase activities represent nmoles DCIP reduced per min per mg protein, assuming an extinction coefficient for DCIP at 600 nm as 20 mM$^{-1}$ cm$^{-1}$. 
Table 3. Growth rates of parent and mutant strains on various carbon and energy sources

<table>
<thead>
<tr>
<th>Growth Conditionsa</th>
<th>Exponential Doubling Times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parent</td>
</tr>
<tr>
<td>Basal Media (BM)</td>
<td>193</td>
</tr>
<tr>
<td>BM + glucose</td>
<td>96</td>
</tr>
<tr>
<td>BM + gluconate</td>
<td>91</td>
</tr>
<tr>
<td>BM + glycerol</td>
<td>100</td>
</tr>
</tbody>
</table>

aBasal media contained basal salts, 1 mg casamino acids per ml, and (where indicated) 0.03 M carbohydrates.
Table 4. Comparison of coupled 6-PG dehydratase and KDPG aldolase activities from parent and mutant cells grown on glucose and gluconate

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth Conditions</th>
<th>nmoles pyruvate formed from 6-phosphogluconate x min⁻¹ x mg protein⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent strain</td>
<td>Basal media (BM)</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>BM + glucose</td>
<td>40.9</td>
</tr>
<tr>
<td></td>
<td>BM + gluconate</td>
<td>71.7</td>
</tr>
<tr>
<td>Mutant 7</td>
<td>Basal media (BM)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>BM + glucose</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>BM + gluconate</td>
<td>65.7</td>
</tr>
<tr>
<td>Mutant 17</td>
<td>Basal media (BM)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>BM + glucose</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>BM + gluconate</td>
<td>54.3</td>
</tr>
</tbody>
</table>

*aBasal media (BM) consisted of basal salts and 2.5 mg per ml casamino acids. Glucose or gluconate were added to 0.02 M. Pyruvate was assayed as described in Materials and Methods.*
Table 5. Requirement of gluconate for induction of the Entner-Doudoroff pathway in P. fluorescens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth Conditions</th>
<th>Enzyme Activity $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
<tr>
<td>Parent</td>
<td>Glucose</td>
<td>7.1 5.2 4.5 19.0 40.7 33.0 2.30 55.1</td>
</tr>
<tr>
<td></td>
<td>Gluconate</td>
<td>9.8 6.1 23.8 64.0 89.3 4.64 106.0</td>
</tr>
<tr>
<td>Mutant 7</td>
<td>Glucose</td>
<td>0 0 0 0 2.2 26.2 1.99 60.6</td>
</tr>
<tr>
<td></td>
<td>Gluconate</td>
<td>7.1 &lt;0.1 19.0 61.3 71.7 5.14 94.2</td>
</tr>
<tr>
<td>Mutant 17</td>
<td>Glucose</td>
<td>0 0 0 1.3 9.3 49.0 2.23 61.5</td>
</tr>
<tr>
<td></td>
<td>Gluconate</td>
<td>0 0 &lt;0.1 22.2 59.2 139.7 3.77 101.0</td>
</tr>
</tbody>
</table>

$^a$Growth media consisted of basal salts, 2.5 mg per ml casamino acids, and glucose or gluconate at 0.03 M.

$^b$Enzymes are as follows: 1, glucose oxidase; 2, gluconate oxidase; 3, glucose:DCIP oxidoreductase; 4, gluconokinase; 5, 6-PG dehydrase; 6, KDPG aldolase; 7, 6-PG dehydrogenase; 8, glucose-6-P dehydrogenase.

$^c$Enzyme activity represents units per mg protein. Under the conditions of the assays, one unit represents: enzyme 1, nmoles $O_2$ consumed per min; 2, nmoles $O_2$ consumed per min; 3, nmoles DCIP reduced per min; 4, 7, 8, nmoles NADP reduced per min; 5, nmoles NADH oxidized per min; 6, nmoles pyruvate formed per min.

$^d$Several experiments indicate this enzyme to be present in variable amounts, though generally at a negligible level.
Fig. 1. Effect of pH on glucose oxidation by parent and mutant strains of *P. fluorescens*.

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Graph showing the effect of pH on glucose oxidation by different strains (X, parent strain; O, mutant). The x-axis represents pH values (10 to 6.8), and the y-axis represents glucose consumption (μmol/min/mg dry wt).
Fig. 2. Effect of substrate concentration on induction of coupled 6-phosphogluconate dehydrase and KDPG aldolase activities. (A) Parent strain grown on casamino acids and glucanate (○) or glucose (△). (B) Mutant 7. Casamino acids and glucanate (○) or glucose (△).
Scheme I. Enzymes involved in the dissimilation of glucose
Scheme 2. Summary of glucose and gluconate metabolism in Pseudomonas Fluorescens.

\[ \frac{1}{2} \text{O}_2 + \text{glucose} \rightarrow \text{gluconate} \rightarrow \text{6-phosphogluconate} \rightarrow 2\text{-keto-3-deoxy-6-phosphogluconate} \rightarrow \text{pyruvate + glyceraldehyde-3-P.} \]

Indicated enzymes are as follows: 0, glucose oxidase; 0, gluconate oxidase; 0, gluconokinase; 0, 2-keto gluconokinase; 0, 2-keto-6-phosphogluconate reductase; 0, 6-phosphogluconate dehydrase; 0, KDPG aldolase.

(---) indicates induction has not confirmed.