The Metabolism of Sodium 2-Keto-D-gluconate by Micro-organisms

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SUMMARY: The ability of various bacteria, actinomycetes, yeasts and moulds to grow on a medium containing sodium 2-keto-D-gluconate as the major carbon source was investigated. The disappearance of 2-ketogluconate during growth was followed and the ability of washed unadapted cell suspensions to oxidize this substrate (as evidenced by O_2 uptake) was studied. Adapted strains were examined for the presence of a 2-ketogluconokinase; this enzyme was detected in organisms of the genera *Pseudomonas*, *Xanthomonas*, *Escherichia*, *Aerobacter*, *Paracolobactrum*, *Serratia*, *Erwinia*, *Bacillus*. Although some bacteria and yeasts consumed 2-ketogluconate during growth and washed cells were able to oxidize it (after an induction period) 2-ketogluconokinase activity was not detected in cell-free extracts, prepared from these organisms, namely: species of *Agrobacterium*, *Corynebacterium*, *Schwanniomycetes*, *Debaryomyces*, *Lipomyces*, *Candida*. Several moulds (chiefly Pyrenomycetes, Aspergillales and Fungi Imperfecti) displayed the same phenomena. A few strains grew weakly on the substrate; however, unadapted cells did not show uptake of oxygen. The remaining strains of bacteria, yeasts, moulds and all the actinomycetes were without activity on 2-ketogluconate.

The results in this paper substantiate the opinion that the 'direct oxidation' pathways of carbohydrate metabolism are very widespread and important among micro-organisms.

The enzymes of the pathway of carbohydrate metabolism which is called the Warburg-Dickens scheme, direct oxidation or hexose-monophosphate-oxidative route (HMP), have recently been extensively investigated. They have been detected and studied in many mammalian tissues (with exceptionally high concentrations in adrenal cortex, lactating mammary gland, lymphatic tissue and rat embryo in early stages of development); in plant tissues (various seeds, spinach, pea leaves) and in micro-organisms (yeast, *Escherichia coli* and *Aerobacter cloacae*). One of us (De Ley, 1953*a*, and unpublished results) recently showed it to be probable that this pathway is very common among bacteria.

The discovery of a new enzyme '2-ketogluconokinase' in adapted *Aerobacter cloacae* (De Ley, 1953*c*), followed by the isolation of a new phosphate ester, 2-keto-D-gluconate-6-phosphate (De Ley, 1954*a, b*) opened new possibilities to explore this field. It is the aim of the present paper to emphasize again the importance of the HMP-oxidative route in micro-organisms. We report here experiments on the O_2 uptake on, and the disappearance under aerobic conditions of, sodium 2-keto-D-gluconate by a series of bacteria, actinomycetes, yeasts and moulds, selected to give a fairly representative general view of microbial taxonomy. The strains which took up O_2 in the presence of 2-ketogluconate were examined for the presence of a 2-ketogluconokinase.

METHODS

Organisms used. We used strains from the personal collection of micro-organisms of one of us (J.deL.). This collection of bacteria is perhaps unique because it contains chiefly gluconate-consuming species. We only selected moulds which were able to use D-gluconate.

For bacteria and actinomycetes the nomenclature of *Bergey's Manual* (1948) was followed. A difficulty arose with some Enterobacteriaceae from the National Collection of Type Cultures (Colindale, London), since for these the nomenclature of the *Report of the Coliform Subcommittee* (1949) was followed, except for the strains which ferment lactose slowly, or not at all (*Paracolobactrum*; *Bergey's Manual*, 1948, p. 460). For this reason, both nomenclatures are used in Table 1. For yeasts the nomenclature of Lodder & Kreger-van Rij (1952) was followed.

2-Keto-D-gluconate. This substance was prepared by the method of Ohle & Wolter (1930) and by a microbiological method in which calcium gluconate was oxidized by a strain of *Pseudomonas putida* (Harsveldt, private communication). We prefer the latter method because of its simplicity and good yield.

Growth on and consumption of 2-keto-D-gluconate

Culture medium for bacteria. Concentrations (as %, w/v, final concentration): Difco yeast extract, 0.1; K_2HPO_4 , 0.5; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.025; $FeSO_4 \cdot 7H_2O$, 0.025. The pH value was brought to 8.5 and the solution boiled and filtered while still hot. After cooling, ammonium sulphate was added to 0.15 %. A concentrated solution of sodium 2-ketogluconate was separately prepared (from the calcium salt and sodium oxalate, with removal of calcium oxalate) and added to the above basal medium to have a final concentration of 0.9 %; the pH value was then adjusted to 7.2. The complete medium was sterilized by filtration, distributed in 5 ml. volumes and incubated for a few days at 30° to test sterility.

Culture medium for actinomycetes, yeasts and moulds. A modified Czapek medium was prepared consisting of (g.): $NaNO_3$, 0.2; K_2HPO_4 , 0.1; $(NH_4)_2SO_4$, 0.3; $MgSO_4 \cdot 7H_2O$, 0.05; Difco yeast extract, 0.2; KCl, 0.05; $FeSO_4 \cdot 7H_2O$, 0.001; sodium 2-ketogluconate (prepared as above), 0.9; dissolved in distilled water to 100 ml.; 1 ml. growth factor solution added; pH value adjusted to 5.6. This medium was sterilized by filtration, distributed aseptically in 5 ml. lots in test-tubes and incubated to test sterility. The growth factor solution contained (mg./100 ml. distilled water): 0.02, biotin; 4, calcium pantothenate; 20, inositol; 4, nicotinic acid; 2, *p*-aminobenzoic acid; 4, pyridoxin; 4, thiamine; 2, riboflavin.

Growth conditions. The cultures were placed on a shaking machine for maximal aeration in a constant temperature room at their optimal temperature, usually 30°, occasionally 20°, 25° or 37°. Bacteria were grown for 8 days, actinomycetes and yeasts for about 1 week and moulds for 2 weeks. Yeasts and moulds were also sometimes grown without shaking.

Determination of 2-ketogluconate consumption. The decrease of the 2-ketogluconate concentration was determined by the method of Luff & Schoorl (Schoorl, 1912). Uninoculated tubes of medium were always used as controls.

Oxidation of 2-ketogluconate by unadapted micro-organisms

Culture medium for bacteria (% w/v, final concentration): 0.5, Difco peptone; 0.25, Difco yeast extract; 1.5, agar; pH 7.2; in Roux flasks.

Culture medium for yeasts. Beer wort, 8° Balling; 1.5 % (w/v) agar; pH 5.6; in Roux flasks.

Warburg manometric experiments. The micro-organisms were grown at optimal temperature for 1–2 days, harvested, washed with physiological saline and centrifuged. They were resuspended in M/60 phosphate solution (adjusted to pH 7.2 for bacteria, 5.6 for yeasts) and shaken for 3 hr. at 30° to decrease the endogenous respiration.

The oxidation of 2-ketogluconate was studied in the Warburg respirometer at 30°. Each vessel contained: 1.4 ml. suspension of bacteria or yeasts (*c.* 25 mg. dry weight), 0.5 ml. 0.066 M-phosphate solution (adjusted to pH 7.2 for bacteria, 5.6 for yeasts); the side arm contained 0.1 ml. water or 0.1 M-sodium 2-ketogluconate. The pH value of the contents of the Warburg vessel was roughly measured with bromthymol blue after the experiments.

Preparation of cell-free extracts of adapted micro-organisms

Cultures. These were the same as those used to establish the consumption of 2-ketogluconate. Bacteria and yeast cultures were used when 1–2 days old, mould and actinomycete cultures when about 1 week old.

Cell-free preparations of 2-ketogluconokinase. (a) Grinding with alumina (McIlwain, 1948). After grinding for 3 min. in the cold, extraction proceeded with 0.05 M-Tris buffer [tris-(hydroxymethyl)-aminomethane; pH 7.4; Gomori, 1946] for 30 min. at 0°, followed by centrifugation at 0° in a Servall angle head centrifuge at 5000 g for 1 hr. The supernatant fluid was used as the enzyme preparation. This method, and the following one, had previously been used successfully to obtain soluble 2-ketogluconokinase from *Aerobacter cloacae*. We used this method with all the bacteria and yeasts, and also with *Monascus ruber* and *Aspergillus flavus*.

(b) Use of the Hughes block (Hughes, 1951). The micro-organisms were introduced into the block previously cooled to –20°. They were crushed after a few vigorous blows with the Denbigh fly press and suspended in either 0.05 M-Tris solution (pH 7.4) or 0.01 M-phosphate buffer (pH 7.4). Microscopical observation showed that nearly all cells were disrupted. Either the supernatant fluid after centrifugation, or the entire mass of debris was used. Some bacteria, yeasts, actinomycetes, *Neurospora sitophila* and *Aspergillus flavus* were subjected to this treatment.

(c) The Mickle disintegrator (Mickle, 1948). Three g. yeast or mould were mixed with 7 ml. 0.05 M-Tris buffer (pH 7.4) + 10 ml. of Ballotini glass beads and shaken in the Mickle apparatus at 0°. Every 10 min. microscopical colour

tests (Loeffler methylene blue and Gram stain) were carried out until at least 90 % of the cells appeared to be disrupted. The Ballotini were removed and the resulting suspension (including cell debris) was used as enzyme preparation.

(d) Mould tissue was ground in an all-glass homogenizer; 10 min. proved sufficient to disrupt nearly all the cells; spores were hardly attacked. The ground mass was suspended in Tris or phosphate buffer.

Paper chromatographic estimation of 2-ketogluconokinase activity (De Ley 1954a, b). The reaction mixture contained in 3 ml.: 0.3 ml. enzyme preparation; 24 μ mole Mg^{++} ; 30 μ mole NaF; 15 μ mole 2-ketogluconate; 24 μ mole ATP; 0.75 ml. 0.2 M-Tris buffer (pH 7.4). Every hour, for 3 hr., a sample (80 μ l.) was spotted, after de-cationization, on pre-washed Whatman paper no. 1 and chromatographed with a mixture of methanol (6 vol.) + concentrated ammonia solution (sp.gr. 0.880; 1 vol.) + water (3 vol.) at 4°. The developed chromatogram was sprayed with the *o*-phenylenediamine-HCl spray, which revealed the 2-keto-D-gluconate-6-phosphate specifically, as a violet spot.

RESULTS

The results obtained with organisms able to metabolize 2-ketogluconate are summarized in Tables 1–3. The strains of the following micro-organisms which were tested did not show any growth or disappearance of substrate when incubated with 2-ketogluconate, nor did washed unadapted cells consume O_2 in presence of this substrate in the Warburg apparatus:

Bacteria: *Pseudomonas fluorescens*, *P. cocovenenans*, *P. geniculata*, *P. ureae*; *Protaminobacter rubrum*; *Vibrio agar liquefaciens*, *V. comma*; *Azotobacter chroococcum*; *Rhizobium leguminosarum*; *Chromobacterium violaceum*; *Micrococcus pyogenes* var. *aureus*, *M. lysodeikticus*; *Alkaligenes faecalis*; *Achromobacter hartlebii*; *Proteus morgani*; *Bacillus subtilis*, *B. cereus*, *B. brevis*, *B. laterosporus*, *B. lentus*; *Mycobacterium phlei*.

Actinomycetes: *Nocardia opaca*, *N. caviae*, *N. lutea* and two undetermined; *Streptomyces erythreus*, *S. albus*, *S. griseolus*, *S. lavendulae* and one undetermined; *Micromonospora fusca*.

Yeasts: *Endomyces decipiens*; *Schizosaccharomyces octosporus*; *Endomycopsis fibuliger*; *Saccharomyces cerevisiae*, *S. cerevisiae* var. *ellipsoideus*, *S. pasteurianus*, *S. fragilis*; *Pichia farinosa*, *P. fermentans*; *Hansenula anomala*; *Hanseniaspora valbyensis*; *Saccharomycodes ludwigii*; *Nadsonia fulvescens*; *Nematospora coryli*; *Sporobolomyces roseus*; *Bullera alba*; *Candida mycoderma*, *C. utilis*, *C. crusei*; *Trigonopsis variabilis*; *Trichosporon pullulans*; *Rhodotorula glutinis*.

Moulds: *Absidia gracilis*, *A. orchidis*; *Cunninghamella elegans*; *Chaetomium globosum*; *Chaetothyrium javanicum*; *Elsinoe mangiferae*; *Fomes pinicola*; *Fusarium solani*.

The quantitative experiments on 2-ketogluconate consumption indicated the strains which had to be investigated further. A selection of strains with high degrees of activity was made in order to study the oxygen uptake of unadapted cells with 2-ketogluconate as substrate. A survey of these results

Table 1. *Decomposition of 2-keto-D-gluconate by various bacteria*

The number in brackets after the names of organisms gives the number of strains tested.

Organism	Degree of growth	Amount of 2-keto-gluconate decomposed (%)	O ₂ uptake of washed cells*	Formation of 2K6P by cell-free extract†
<i>Pseudomonas aromatica</i> (1)	Excellent	75	nt	+++
<i>P. aeruginosa</i> (1)	Good	71	nt	++
<i>P. putida</i> (1)	Good	84	nt	nt
<i>P. aromatica</i> var. <i>quercito-pyrogallica</i> (1)	Good	73	ind.	++
<i>P. fluorescens</i> (2)	Good	32-73	ind.	+
<i>P. ovalis</i> (1)	Good	40	nt	+++
<i>P. convexa</i> (1)	Good	75	nt	nt
<i>P. viscosa</i> (1)	Good	77	nt	nt
<i>Xanthomonas phaseoli</i> (1)	Weak	5	nt	+
<i>X. campestris</i> (1)	Weak	3	nt	+
<i>X. pruni</i> (1)	Weak	7	nt	±
<i>Agrobacterium tumefaciens</i> (1)	Excellent	73	ind.	-
<i>Corynebacterium simplex</i> (2)	Excellent	40-69	nt	-
<i>C. helvolum</i> (1)	Excellent	73	ind.	-
<i>C. poinsettiae</i> (1)	Good	15	nt	-
<i>Flavobacterium aquatile</i> (1)	Good	17	0	-
<i>Bacterium</i> (= <i>Escherichia</i>)				
<i>B. coli</i> I (3)	Weak-moderate	5-18	0	nt
<i>B. coli</i> I (1)	Excellent	90	nt	nt
<i>Bacterium coli</i> II (3)	Excellent	59-100	nt	++
<i>B. coli-anaerogenes</i> (1)	Weak	6	nt	nt
<i>B. intermedium</i> I (4)	Excellent	100	ind.	+++
<i>B. intermedium</i> II (1)	Excellent	100	nt	nt
<i>Bacterium</i> (<i>Aerobacter</i>)				
<i>B. aerogenes</i> I (8)	Excellent	100	ind.	+++
<i>B. aerogenes</i> II (1)	Weak		nt	nt
<i>B. aerogenes</i> II (2)	Excellent	100	nt	nt
<i>B. cloacae</i> (4)	Excellent	100	ind.	+++
<i>Paracolonobacterium aerogenoides</i> (1)	Excellent	100	ind.	+++
<i>Bacterium</i> (<i>Klebsiella</i>)				
<i>B. pneumoniae</i> (3)	Good	27-100	nt	nt
<i>B. rhinoscleromatis</i> (1)	Moderate	24	nt	nt
<i>B. ozaenae</i> (1)	Good	65	nt	nt
<i>Serratia plymouihicum</i> (2)	Good	69	ind.	++
<i>Proteus vulgaris</i> (1)	Weak	13	0	-
<i>Erwinia carotovora</i> (1)	Moderate	11	0	+
<i>Bacillus subtilis</i> (1)	Weak	20	0	-
<i>B. cereus</i> var. <i>mycoides</i> (1)	Weak	17	nt	nt
<i>B. megaterium</i> (4)	Moderate-good	26-95	ind.	++
<i>B. circulans</i> (1)	Weak	31	nt	nt
<i>B. mesentericus</i> (1)	Moderate	33	0	-

* Oxygen uptake of washed cells: ind.=only after induction period; 0=no uptake; nt=not tested.

† Intensity of the spot of 2-keto-D-gluconate-6-phosphate-quinoxaline, after 3 hr. enzyme activity of cell-free extract: +++=very intense, nearly complete phosphorylation; ++=intense, excellent phosphorylation; +=weak phosphorylation; ±=very weak but certain enzyme activity; -=no kinase activity detectable (after 3 hr.).

Table 2. *Decomposition of 2-keto-D-gluconate by certain yeasts*

The number in brackets after the names of organisms gives the number of strains tested.

Organism	Degree of growth	Amount of 2-keto-gluconate decomposed (%)	O ₂ uptake of washed cells*	Formation of 2K6P by cell-free extract†
<i>Schizosaccharomyces pombe</i> (2)	Moderate	0-21	nt	nt
<i>Endomycopsis capsularis</i> (1)	Good	39	0	nt
<i>Saccharomyces delbrueckii</i> (1)	Good	14	nt	nt
<i>Schwanniomyces occidentalis</i> (2)	Excellent	64-91	ind.	—
<i>Debaryomyces hansenii</i> (1)	Excellent	74	ind.	—
<i>Hanseniaspora apiculata</i> (1)	Good	37	0	nt
<i>Lipomyces lipoferus</i> (1)	Excellent	81	ind.	—
<i>Torulopsis holmii</i> (1)	Good	13	0	nt
<i>Brettanomyces bruxellensis</i> (1)	Good	10	nt	nt
<i>Candida albicans</i> (1)	Excellent	100	ind.	—
<i>Kloeckera apiculata</i> (2)	Moderate-good	18-40	0	nt

* Oxygen uptake of washed cells: *ind.* = only after induction period; 0 = no uptake; nt = not tested.

† As indicated by intensity of chromatogram spot for 2-keto-D-gluconate-6-phosphate-quinoxaline: — = no detectable kinase activity up to 3 hr.; nt = not tested. Compare Table 1.

Table 3. *Decomposition of 2-keto-D-gluconate by certain moulds*

Only 1 strain of each organism was tested

Organism	Degree of growth	Amount of 2-keto-gluconate decomposition (%)	Formation of 2K6P by cell-free extract*	Method of disintegrating cells and buffer used
ASCOMYCETEAEE				
<i>Neurospora sitophila</i>	Good	75	—	Mickle; Tris Hughes; phosphate
<i>Nectria cinnabarina</i>	Moderate	100	nt	nt
<i>Monascus ruber</i>	Good	76	—	Alumina; phosphate Mickle; Tris
<i>Aspergillus nidulans</i>	Good	82	nt	nt
<i>A. fumigatus</i>	Good	89	—	Homogenizer; Tris
<i>A. flavus</i>	Good	92	—	Alumina; Tris Hughes; Tris
<i>A. niger</i>	Good	88	nt	nt
<i>Penicillium chrysogenum</i>	Good	92	—	Homogenizer; Tris
<i>Penicillium</i> sp.	Good	90	—	Homogenizer; Tris
<i>Penicillium</i> sp.	Good	94	nt	nt
FUNGI IMPERFECTI				
<i>Cercospora beticola</i>	Average	74	nt	nt
<i>Helminthosporium sativum</i>	Good	92	nt	nt
<i>Monilia brunnea</i>	Weak	27	nt	nt
<i>Phoma betae</i>	Good	82	—	Homogenizer; Tris
<i>Dematium pullulans</i>	Weak	16	nt	nt

* As indicated by intensity of chromatogram spot for 2-keto-D-gluconate-6-phosphate-quinoxaline: — = no detectable kinase activity up to 3 hr.; nt = not tested. Compare Tables 1 and 2.

showed that the unadapted strains which were able to grow on 2-ketogluconate could be divided into two distinct groups with respect to oxygen uptake.

(i) Strains which consumed oxygen when 2-ketogluconate was substrate always did so after an induction period. The 2-ketogluconokinase of *Aerobacter cloacae* K3 is an adaptive enzyme (De Ley, 1953c). Figs. 1 and 2 illustrate the kind of oxygen-uptake curve which was obtained.

(ii) The second group of organisms contained those which, although able to grow poorly or moderately on 2-ketogluconate and partially to decompose it, did not show an oxygen uptake in Warburg experiments with 2-ketogluconate as substrate. The moderate growth in these cases appeared to be due to the selection of mutants during growth.

Fig. 1.

Fig. 2.

Fig. 1. The respiration of *Serratia plymuthicum* in presence of sodium 2-keto-D-gluconate (curve 2K); curve E: the endogenous respiration. For contents of the Warburg vessels, see text.

Fig. 2. The respiration of the yeast *Lipomyces lipoferus* in presence of sodium 2-keto-D-gluconate (curve 2K); curve E: endogenous respiration. For contents of the Warburg vessels see text.

The problem was investigated further by the study of the distribution of 2-ketogluconokinase in a carefully selected set of strains. A survey of these results shows that this soluble enzyme was present only in some of the bacteria examined; its absence from some 2-ketogluconate-metabolizing bacteria, yeasts and moulds was striking. Since we worked only with centrifuged extracts of alumina-ground cells in Tris buffer, it was possible that the enzyme in these strains was inactivated by this treatment or was bound to particles. We therefore used other disintegration and extraction methods (see Methods). Certain micro-organisms were disrupted by more than one method: *Corynebacterium helvolum* (alumina, Hughes block), *Candida albicans* (alumina, Hughes block, Mickle), moulds (see Table 3). We used both crude suspensions and the supernatant fluid obtained after centrifugation. We never observed

even the slightest spot of 2-keto-D-gluconate-6-phosphate on the chromatograms. This showed that the enzyme was not bound to particles. Since it is known that several enzymes require inorganic phosphate for normal activity, we substituted phosphate buffer for Tris, again however without result.

DISCUSSION

Enterobacteriaceae. Nearly all the members of this family are able to grow on 2-ketogluconate, to oxidize it and to form adaptively a 2-ketogluconokinase. All the Enterobacteriaceae tested grow well on gluconate (De Ley, 1953*a, c*). *Proteus vulgaris* seems unable to form this enzyme. Among the *Erwinia* the enzyme activity is very small, which explains the absence of O₂ uptake. Although the formation of 2-keto-D-gluconate-6-phosphate by adapted *Klebsiella* spp. was not investigated (because of its high virulence) the presence of this enzyme here may also be taken for granted. It has been shown that *Escherichia coli* (McNair Scott & Cohen, 1951) and *Aerobacter cloacae* (De Ley, 1953*a-c*) possess the enzyme system for the HMP-oxidative route. From the present results it may fairly be generalized that nearly all, if not all, the members of the family Enterobacteriaceae possess this metabolic pathway (with the possible exception of *Proteus*, *Salmonella* and *Shigella*; the last two are not yet investigated).

Aerobic Bacillaceae. We observed previously that many organisms of this family are able to use gluconate (De Ley, 1953*a*). Although several species are able to use 2-keto-gluconate, *Bacillus megaterium* is the only one which shows adaptive formation of a 2-ketogluconokinase. In a preliminary note De Donder (1952) showed that both *B. subtilis* and *B. megaterium* possess a TPN-linked gluconate-6-phosphate dehydrogenase. All these facts are in favour of the view that in some members of this family also an HMP-oxidative route is present, or at least a very similar one.

Pseudomonas and Xanthomonas. It was interesting to find in the Bacillaceae 2-ketogluconokinase, although it was irregularly distributed among the different species. Some *Pseudomonas* spp. contain a hexokinase (Entner & Doudoroff, 1952; Klein, 1953), whereas others do not (Wood & Schwert, 1953). Our results show that many *Pseudomonas* and *Xanthomonas* spp. have an enzyme system for phosphorylative carbohydrate metabolism, at least after the stage of the 2-ketogluconate formation. In this connexion it must be remembered that several authors agree (Entner & Stanier, 1951; Stokes & Campbell, 1951; Wood & Schwert, 1953, 1954) that the transformation: glucose → gluconate → 2-ketogluconate in *Pseudomonas* spp. occurs without the intervention of phosphorylated intermediates. It is now clear that *P. fluorescens* and *P. saccharophila* have yet another route of gluconate-6-phosphate decomposition, namely by splitting into triose-phosphate and pyruvate (Entner & Doudoroff, 1952; Wood & Schwert, 1954) presumably after the initial formation of 2-keto-3-deoxygluconate-6-phosphate. Paper chromatography revealed that both *Pseudomonas* and *Xanthomonas* formed an unidentified reducing substance during 2-ketogluconate metabolism.

Bacteria which consume 2-ketogluconate but do not possess a soluble 2-ketogluconokinase. One strain of *Agrobacterium tumefaciens* and four *Corynebacteria* spp. occurred in this group. The mechanism of 2-ketogluconate metabolism by these strains is obscure and is discussed later.

Yeasts and moulds. Table 2 shows that only four yeasts from the collection tested were able to consume O_2 when 2-ketogluconate was the substrate. This oxidation occurred only after the formation of some adaptive enzyme which was not the normal soluble 2-ketogluconokinase. This same property is also common among moulds; it seems to be found chiefly among members of the pyrenomycetes, Aspergillales and Fungi Imperfecti. It is now clear that these yeasts, moulds and the above-mentioned bacteria do not possess a normal soluble kinase, activated by ATP and Mg^{++} . The exact mechanism is still obscure. Several explanations are possible: e.g. the presence of a kinase which requires a different activator or coenzyme, or an entirely new and unsuspected mechanism. The suggestion that some coenzyme or activator is lacking seems justified when it is remembered that these intact adapted micro-organisms rapidly decompose 2-keto-gluconate, whereas the same cells, when disintegrated, leave it completely unattacked. This problem requires a separate investigation. It will be interesting to explore this problem further, since it is well known that brewer's and baker's yeasts possess the prototype system of the HMP-oxidative route, which also seems to be present in some moulds (Koffler, 1953).

Micro-organisms unable to decompose 2-ketogluconate. The remaining yeasts, moulds, bacteria and all the actinomycetes occur in this group, as summarized at the beginning of the section headed Results. The inability to demonstrate decomposition of 2-ketogluconate by these micro-organisms does not preclude the possible presence of an HMP-oxidative route.

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