Title: GLUCONIC ACID: PROPERTIES, PRODUCTION METHODS AND APPLICATIONS – AN EXCELLENT OPPORTUNITY FOR AGRO-INDUSTRIAL BY-PRODUCTS AND WASTE BIO-VALORIZATION

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Abstract

Agro-industrial by-products and wastes pose serious, widespread problems with considerable economic and environmental consequences in developed countries. However, many of the by-products contain large amounts of sugars that make them potentially excellent raw materials for the biotechnological production of added value products; in particular, by-products from perishables such as fruits can be highly useful for this aim. The growing significance and demand for gluconic acid have promoted an interest in integrating both issues as a strategy for the revalorization of these resources. The pertinence of this strategy can be better understood by examining the properties of gluconic acid and its derivatives and their uses and production methods, especially biotechnological methods, to update the existing reviews on the topic.

Future advances in this direction may be promoted by the development of genetically modified organisms for the generation of new technological processes and the optimization of existing ones. Particular attention is paid to acetic acid bacteria.

Keywords

Acetic acid bacteria, Filamentous fungi, Agro-industrial by-products, Food additives, Gluconic acid bioproduction, Revalorization.

1. Introduction

Although the production of food for human consumption has progressed markedly in the past several years, a recent report by the Food and Agriculture Organization (FAO) estimates that roughly one-third of the world food production (approximately 1300 million ton) deteriorates or is wasted along the supply chain [1]. This waste is especially true for perishable items. The origin of the losses differs markedly between underdeveloped countries, where the lack of proper production causes these wastes to occur mainly at the harvest, storage and processing stages. In industrialized countries, the supply usually exceeds the demand, such that the losses instead occur largely at the consumption stage (Table 1). Overall, the losses amount to approximately 400–500 kcal per person per day in underdeveloped regions but can rise to 1300 kcal in developed regions [2].

The situation has led to a highly contrasting map of world regions striving to eradicate hunger in the poorest areas and to regulate overproduction in the richest. Effective solutions to the problem can only come from close cooperation between governments, producers, distributors, sellers and consumers, aided by imaginative proposals. According to Galanakis [3], exploitation and revalorization of agro-industrial by-products and wastes can be useful in developed countries. In fact, agro-industrial by-products can provide excellent raw materials for obtaining products with an added value and/or new products with specific properties (especially in connection with biotransformation processes).

This review focuses on D-gluconic acid (GA), a common additive used in pharmaceutical, textile, building and, especially, food industries. GA is usually obtained through biological methods involving the partial oxidation of glucose. This acid

71 provides an excellent example of how some production wastes and surpluses with high
72 carbohydrate contents can be optimally exploited.
73 This proposal is supported by summarizing the main properties, uses and production
74 methods for GA and its derivatives (particularly its biotechnological derivatives). This
75 review, which is essentially practical in scope, updates the content of previous reviews
76 by authors such as Singh and Kumar [4], Anastassiadis and Morgunov [5],
77 Ramachandran et al. [6], Rogers et al. [7], Roehr et al. [8] and Milsom and Meers [9].
78 The review reflects deeply on what are the potentially most useful aspects of this field
79 with the hope of improving the exploitation of GA through the use of different types of
80 surpluses and production wastes as raw materials and the development of new
81 biotechnological production processes involving cellular microorganisms (fungi or
82 bacteria) or their enzymes. In the future, genetically modified microorganisms
83 (particularly acetic acid bacteria (AAB)) might allow the existing GA production
84 processes to be optimized and new, more effective ones to be developed.
85
86 **2. Gluconic acid**
87 GA ($C_6H_{12}O_7$) is an acid sugar belonging to the aldonic acid family. Chemically, GA is
88 one of the 16 stereoisomers of 2,3,4,5,6-pentahydroxyhexanoic acid and results from the
89 specific oxidation of the aldehyde group at C1 in β-D-glucose to a carboxyl group by
90 chemical, electrochemical, or catalytic means or, alternatively, through
91 biotransformation by microorganisms (fungi or bacteria) or their enzymes.
92 Because it is both an acid and an alcohol, GA can undergo 1,5 intramolecular
93 esterification. The process is favoured by an acid medium and involves the spontaneous
94 loss of a water molecule to yield intramolecular anhydride glucono-δ-lactone, which is a
95 cyclic ester [9]. In aqueous solutions, the acid is in equilibrium with its lactones (Fig.

1a). Because GA is a weak acid ($K_a = 1.99 \cdot 10^{-4}$ at 25 °C), it accounts for 55–66% of all species in solution. Because γ-lactone forms roughly one hundred times more slowly than δ-lactone, equilibrium is reached very slowly. Adding a base to the ester rapidly cleaves the aldonic ring to yield an open-chain salt [10]: a gluconate (Fig. 1b). This salt can easily bind di- and trivalent metals to form highly stable chemical compounds that can only react with strong oxidants such as nitric acid or hydrogen peroxide. Under typical conditions, the oxidation reaction yields a mixture of 2- and 5-D-ketogluconate (2-KGA and 5-KGA, respectively) – and, under extreme conditions, also 2,5-diketo-D-gluconate (2,5-DKGA) – in variable proportions [11].

GA is a weak, non-volatile, harmless (odourless, non-corrosive, non-toxic), easily biodegradable acid that is soluble in water and insoluble in non-polar solvents [6].

GA and its derivatives occur naturally in plants, fruits and other foods such as rice, honey, grapes, apples, meat, wine and vinegar [6]. Like many other organic acids, GA is involved in the metabolism of a number of living organisms.

The acid and its derivatives have gained increasing interest in food, pharmaceutical, textile and building industries over the past 50 years. At present, the production of GA is estimated to amount to approximately 100,000 ton/year and to be almost exclusively biotechnological [12], with production costs ranging from 1.20 US$/kg for GA to 8.50 US$/kg for calcium gluconate and glucono-δ-lactone [4]. Sodium gluconate is the most widely marketed GA derivative, accounting for more than 80% of the world production, according to Roehr et al. [8]. GA is commercially available as a 50% aqueous solution of a pH of 1.82 and 1.23 g/cm^3 density.

3. Current uses

Organic acids comprise a highly heterogeneous group of compounds with diverse uses that range widely depending on their particular structure. Thus, GA and its derivatives – except glucono-γ-lactone – are used mainly as additives by food, pharmaceutical, hygiene and building industries. For example, they are commonly added to dairy products and soft drinks to preserve and/or enhance their sensory properties [6,7].

In 1986, the US Food and Drug Administration granted *Generally Recognized As Safe* status to the GA derivatives, glucono-δ-lactone and sodium gluconate, and authorized their unrestricted use as food ingredients [13]. In parallel, UN's FAO and the World Health Organization have regulated the use of GA and its derivatives (glucono-δ-lactone and D-gluconates) as food additives through good manufacturing practices established in their *Codex General Standard for Food Additives*.

GA derivatives are acidity regulators (E574–E580), with raising, sequestering, hardening and flavour-enhancing properties (Table 2). In addition to improving the sensory properties of food products by imparting a bitter but refreshing taste, GA prevents clouding by binding some metals potentially present at trace levels in drinks, for instance, Ca and Fe in fruit juices. GA is also used as a food preservative for pickled foods, and glucono-δ-lactone is used as a preservative for cured meat-based sausages. In addition, some food processing plants use GA derivatives as cleaning agents for their industrial facilities [6,9]. Thus, alkaline solutions of sodium gluconate are used to clean glassware, but GA is preferred for metal (steel, alloyed) components. For example, the dairy industry uses GA derivatives to prevent the precipitation of calcium salts in processing equipment and glass storage vessels. Additionally, the bakery industry uses glucono-δ-lactone to reduce the absorption of fatty compounds and as an acidifier and chemical baker's yeast [6].

As shown by studies on mango [14], peach [15], grape [16,17], apple [18,19] and orange [20], the presence of excessive amounts of these compounds in fruits is suggestive of a loss of quality due to infection by biological pathogens. Specifically, the presence of GA and/or its keto-acids in grapes or grape must is a sign of "noble rot" or contamination with *Botrytis cinerea*, which has an adverse impact on the physical and chemical stability of wine and on its sensory properties [21,22]. As a result, the determination of GA has become a common practice for the wine production industry as a tool for assessing grape ripeness and wine quality. According to the *Organisation Internationale de la Vigne et du Vin*, the GA content of wine should never exceed 300 mg/L.

As noted earlier, however, these compounds have additional uses. The pharmaceutical industry uses gluconates of divalent metals such as Ca^{2+}, Mg^{2+} and Fe^{2+} as mineral supplements to treat hypocalcaemia, hypomagnesaemia and anaemia, respectively. Additionally, sodium gluconate is used as a cement additive to increase hardness and resistance to extreme environmental conditions [11,23].

The uses of GA continue to expand. Thus, the excellent chelating properties of GA with Fe^{2+} and Fe^{+3} have been used to remove, at a neutral pH, hazardous chlorinated substances such as 2,4,6-trichlorophenol (TCP) and trichloroethylene from ground water through the Fenton reaction [24]. Additionally, the acid has been used to recycle exhausted Mo, Ni and Al catalysts with recoveries of 99, 46 and 14%, respectively, under optimal bioleaching conditions [25].

Finally, GA is used as a substrate for the production of derivatives such as 2-KGA and 5-KGA, through regio-selective oxidation by the dehydrogenases of some AAB, e.g. *Gluconobacter oxydans* strains. 2-KGA can be used as a building block for chemical synthesis, as in its chemical conversion to isoascorbic acid or the synthesis of

heterocycles [26,27]. 5-KGA is useful in the production of tartaric acid (an antioxidant in the food industry, a reducing agent in the textile industry and a chiral compound for chemical synthesis) [28], xylaric acid, the savoury flavour compound 4-hydroxy-5-methyl-2,3-dihydrofuranone-3 as well as for vitamin C production through the Gray method [29-31].

4. Production methods

Although GA can be obtained using chemical and biotechnological methods, the latter prevails at the industrial scale [5]. Specifically, early methods based on surface fermentation with *Penicillium* fungi [32] have been superseded by the use of submerged cultures of filamentous fungi such as *A. niger* or AAB (particularly *Gluconobacter oxydans*), which are highly selective and technically efficient. Other bacterial strains capable of metabolizing glucose to GA, including *Acetobacter diazotrophicus* [33] and *Zymomonas mobilis* [34], have also been identified. Additionally, new fermentation methods using immobilized cells [35-39] or enzymes [40,41] have recently emerged and might become competitive choices for GA production in the future.

4.1. Chemical methods

Ever since Isbell's general method for the electrochemical synthesis of calcium salts of aldonic acids by reacting sugars with calcium carbonate and bromide ion was reported [42,43], a number of authors have promoted the development of chemical (electrolytic or catalytic) oxidation methods for glucose as alternatives to the microbiological synthesis of GA.

Although glucose can be easily oxidized by heterogeneous catalysis in an aqueous solution, the efficiency of the process depends on the activity, selectivity and stability of

194 the catalyst. Most research in this area has focused on catalysts of transition metals (Pd,
195 Pt and Au) supported on TiO_2, Al_2O_3 or activated carbon, among other solids [44-48].
196 The catalytic oxidation of glucose is favoured by an alkaline pH (pH 9–10), which
197 increases the reaction rate and avoids the rapid deactivation of the catalyst. Supported
198 Pd and Pt catalysts usually promote unwanted side reactions and are rapidly deactivated
199 through the conversion of glucose into GA, which is especially efficient with activated
200 C as support [47]. Although doping with Bi has been found to increase the catalytic
201 activity, the poor stability of the resulting catalysts against leaching has hindered the use
202 of synthetic gluconate for nutritional and pharmaceutical purposes [45,49,50]. Gold-
203 based catalysts are excellent choices for glucose conversion into GA. [46]; however, the
204 fact that their activity declines after the second catalytic cycle has hindered their
205 implementation at the industrial scale [45].
206 In recent years, the focus has shifted to facilitating the industrial adaptation of the
207 previous procedures by using new raw materials and more inexpensive and
208 environmentally benign reagents. For example, aqueous hydrogen peroxide has been
209 used as an alternative reagent [51,52]. Additionally, a recent catalytic method affords
210 glucose conversions of 99% at 25 ºC using a $FeSO_4$ catalyst in combination with low-
211 frequency (20 kHz) ultrasound to accelerate the reaction [53].
212 The high costs and deactivation of the catalyst, as well as some leaching problems,
213 make chemical processes less competitive than fermentation methods when uses in
214 either the food or pharmaceutical industries are aimed [54].
215
216 **4.2. Microbiological methods**

The current importance of microbiologically based conversion methods is largely due to the ability of many biological systems to transform different substrates selectively and the economic feasibility of the ensuing processes.

4.2.1. Filamentous fungi

The oxidation of glucose to GA by filamentous fungi is an aerobic fermentation process with a high oxygen demand [55,56]. The process is catalyzed by the enzyme glucose oxidase (GOD). This enzyme is a flavoprotein that is predominantly located in the cell wall and the extracellular fluid. Roughly 80% of the total enzyme activity is found to be associated with these two locations in *Aspergillus* and *Penicillium* species [57-60]. D-glucose is converted into D-glucono-δ-lactone through a dehydrogenation reaction that also produces H_2O_2; the peroxide is subsequently decomposed into O_2 and H_2O under the action of a catalase. GA is formed by the spontaneous or catalytic hydrolysis of the lactone. Figure 2 depicts the overall process in terms of the activity of GOD from *A. niger*. Lactone undergoes spontaneous hydrolysis at a neutral or alkaline pH. The process is favoured by the enzyme lactonase, which is only required when the medium becomes acidic due to the accumulation of GA. Subsequently, GA permeates the cell walls and is internalized and metabolized through the pentose phosphate pathway. Because fungal growth and GA formation are unrelated, continuous operation is impossible. As a result, most industrial processes involving *A. niger* are performed in batches, using fermentation media containing large amounts of glucose (110–250 g/L) and small amounts of nitrogen compounds (> 20 mM N/P) at a pH of 4.5–6.5; in addition, a neutralizing agent is added as GA accumulates [6,61]. Although *A. niger* allows the near-quantitative conversion of glucose under optimal conditions, it forms mycelia that usually increase the viscosity of the medium and hinder the aeration and

homogenization of the medium as a result [62]. However, the process can be improved by controlling the seed morphology, which is dependent on the agitation speed during the inoculum preparation [63]. As an alternative, the high *A. niger* spore content in GOD, previously treated to prevent germination and facilitate permeabilization, has been found to result in very high glucose conversion rates (4.5 g/L·h) relative to the commercially available GOD (2 g/L·h) [64].

Alternative glucose feeding methods and microbial strains have also been assessed. Thus, *Penicillium variable* P16 was found to provide a peak conversion rate of 2.02 g GA/h for a short time if the substrate feed was supplied with various mineral salts and mycological peptone as the N source [65].

Anastassiadis and Rehm [66,67] have reported several continuous GA production methods using *Aureobasidium pullulans* strains with and without biomass retention capable of producing up to 375 g GA/L with 78% selectivity. This strain is a strictly aerobic yeast with a high osmotolerance that degrades hexoses preferentially through the pentose phosphate pathway [68].

Early genetic engineering work in this area has focused on modifying fungal strains for industrial use to improve the efficiency of the process and reduce GA production costs. ORS-4.410 is the modified *A. niger* strain that is likely most widely used to obtain GA. The natural strain, *A. niger* ORS-4, was originally isolated from sugarcane industrial wastes [69] and mutated by successive UV irradiation. In addition to exhibiting increased cell growth rates, *A. niger* ORS-4.410 has been found to outperform the wild strain as both free cells (GA yield 88% after 6 days) and immobilized cells (78% after 12 days) [38]. This strain has also excellent fermentation capabilities for sugars from agro-industrial wastes (grape and banana) in both batch [70] (Table 3) and semi-continuous processes [35]. *A. niger* ARNU-4 has proved to be similarly effective for the

fermentation of sugarcane industrial wastes, with yields within the range of 85.2% [71]. Additionally, the *A. niger* strain UV-112, isolated from onion and mutated by UV irradiation, leads to higher GA yields on glucose than its parent strain (0.66 vs. 0.25 g/g) [72].

4.2.2. Bacteria

The wide variety of bacteria capable of metabolizing glucose to GA includes various AAB genera and strains from other genera such as *Pseudomonas* and *Zymomonas*. The metabolism of most aerobic bacteria involves the thorough oxidation of organic matter to carbon dioxide and water. Only under special growth conditions involving a high nutrient availability, certain microorganisms can lead to incomplete oxidation (the so-called *overflow metabolism*).

As a rule, AAB oxidize their substrates incompletely [73]. The name of this group of strictly aerobic bacteria is derived from their ability to oxidize ethanol to acetic acid. In addition, the bacteria can metabolize a wide range of carbohydrates – glucose included – and primary or secondary alcohols, to form partially oxidized organic acids, aldehydes and ketones that tend to accumulate in the medium, either transiently or permanently. AAB have found a number of agri-food uses, the most widespread of which is probably vinegar production [74-76]. Although the optimum pH for this process is 5.0–6.0, these bacteria are acidophilic and can grow at even lower values.

The AAB belong to the family *Acetobacteraceae,* which presently comprises 31 bacterial genera and a total of approximately 130 species [77]. Among these, in particular, *Acetobacter* sp. and *Gluconobacter* sp. are of high biotechnological interest for GA production. Essentially, the two genera differ in their flagellation mechanism and oxidative metabolism (Fig. 3). Thus, *Gluconobacter* exhibits polar flagellation but

lacks the functional mechanisms of the tricarboxylic acid (TCA) cycle, such that it cannot oxidize acetate (suboxidant). By contrast, *Acetobacter* exhibits peritrichous flagellation and possesses a functional TCA cycle; thus, it can oxidize ethanol to CO_2 (peroxidant). Regardless of the bacterial genus, glucose can be oxidized at various cell sites depending on the location of the enzymatic ensemble catalyzing the process [78]. Direct oxidation in the periplasm by the quinoprotein D-glucose dehydrogenase (GDH) is the main route. Together with a pyrroloquinoline quinone (PQQ) as a prosthetic group, GDH occurs on the outer surface of the cytoplasmic membrane. Some *Gluconobacter* strains can continue the oxidative process by metabolizing gluconates to keto-acids. Thus, *G. oxydans* oxidizes gluconates to 2-KGA by the flavoprotein gluconate dehydrogenase (GADH), and 5-KGA is produced by a PPQ-dependent protein bound to the cytoplasmic membrane [80]. Finally, 2,5-DKGA is formed by the flavoprotein 2-keto-D-gluconate dehydrogenase (2KGADH). There is also an NADP-dependent protein located in the cytoplasm, which can convert 5-KGA [81]. Other strains such as *G. japonicus* can only oxidize gluconates to 2-KGA and 5-KGA [82]. The selectivity of glucose oxidation by *Gluconobacter* sp. is seemingly pH-dependent [83-85]. *G. suboxydans* IFO 12528 at a pH of 3.5–4.0 has been shown to be highly selective for the conversion to 5-KGA (87% according to Ano et al. [86]). Membrane-bound dehydrogenases are associated with the respiratory chain via the ubiquinone coenzyme Q_{10}, which is present in the cytoplasmic membrane [87]. Thus, an electron transfer forms a proton gradient across the cytoplasmic membrane, which is ultimately used to obtain energy (ATP) for cell maintenance and/or growth.

Glucose concentrations above 15 mM and pH values below 3.5 have been found to inhibit the formation of keto-acids [8,83-85]. Under optimal industrial conditions, *G.*

316 *oxydans* shows GA yields within the range of 75 to 80%, which depend markedly on the
317 pH, the glucose concentration and degree of aeration.
318 The intracellular oxidation of glucose catalyzed by a battery of $NADP^+$-dependent
319 soluble dehydrogenases (GDH and GADH) can proceed through different metabolic
320 pathways that depend on the particular acetic bacterium. *Acetobacter* degrades D-
321 glucose through the pentose phosphate pathway and the TCA cycle, whereas
322 *Gluconobacter* uses the pentose phosphate pathway and to some extent the Entner–
323 Doudoroff pathway, but lacks a complete TCA [88].
324 *Gluconobacter oxydans* 621H (DSM 2343) is very suitable for biotechnological
325 production because it possesses a high oxidative activity, even under non-growth
326 conditions, and has a poor cell yield [84,89]. The sequencing of its genome [88] has
327 enabled the characterization of its multiple metabolic pathways and cell physiology. A
328 major fraction of oxidative metabolism in *Gluconobacter* occurs in the periplasm,
329 where the enzymatic activity of the quinoprotein GDH is much greater – approximately
330 27 times greater – than that of its $NADP^+$-dependent homologue in the cytoplasm [90].
331 In fact, the superior ability of the *Gluconobacter* species to survive and proliferate in
332 mixed cultures has been ascribed to the high activity levels of their membrane-located
333 dehydrogenases. In nutrient-rich media, these enzymes can rapidly capture and remove
334 glucose and other aldoses from the medium by converting them into organic acids,
335 which are unavailable to many other microorganisms, and may thereby lower the pH of
336 the medium, making it a more adverse environment for the growth of other microbial
337 populations [83]. Some gluconate and 5-KGA are subsequently transported into the
338 cells and assimilated by being channelled into the pentose phosphate pathway. It is
339 interesting to note that the cell mass production in *G. oxydans* competes with the
340 production of GA and ketogluconates because the disruption of the membrane-bound

GDH or of both the membrane-bound and soluble GDHs results in an improved growth rate and growth yield [91].

Other prominent AAB species are *Acidomonas methanolica* (basonym *Acetobacter methanolicus*) and *Gluconacetobacter diazotrophicus* (basonym *Acetobacter diazotrophicus*). Both catalyze the oxidation of glucose to GA through the PQQ-dependent GDH enzyme bound to the cytoplasmic membrane that is also involved in an electron-transfer chain, which ultimately leads to ATP synthesis.

A. methanolica is an acidophilic facultatively methylotrophic bacterium growing on mineral media of a pH of 4.0–4.5, containing methanol, glycerol or glucose as the only carbon source [92]. Unlike other AAB, *A. methanolica* metabolizes methanol through the ribulose phosphate pathway. Although this species is closely related to the genus *Acetobacter* and is somewhat similar to *G. oxydans*, it can oxidize glucose to GA but not to keto-acids [93]. As a result, GA synthesis by *A. methanolica* is probably not subject to any of the problems arising from the formation of keto-acids.

G. diazotrophicus, which was first isolated by Gillis et al. [94], grows efficiently on mineral media containing ammonium salts as N sources. Although it affords high conversion levels to GA, it also produces 2-KGA and 2,5-DKGA as by-products of its oxidative metabolism [95]. However, this strain is unable to grow in the presence of high substrate concentrations (more than 30% glucose) [96] or too low a pH (< 3.5) [95], two desirable attributes for industrial exploitation.

There have been some preliminary studies on the microbiological synthesis of GA by *Pseudomonads*, a group of strictly aerobic bacteria with polar flagellation that grow efficiently at a near-neutral pH. Most use oxygen as their final electron acceptor. Some, however, can use the nitrate ion and grow under anaerobic conditions. Like most AAB, *P. aeruginosa* can use two different metabolic pathways for the breakdown of glucose,

namely, direct oxidation and oxidation by cytoplasmic enzymes, which comprise the Entner–Doudoroff pathway and the TCA cycle. The direct pathway involves two consecutive oxidation reactions in the periplasm, through which glucose is converted into gluconate under the action of GDH and the gluconate is further oxidized to 2-KGA by a GADH. Although both pathways operate simultaneously in *Pseudomonas* sp., the oxidative pathway is favoured by aerobic conditions and the cytoplasmic pathway by anaerobic conditions [97,98]. Additionally, in glucose-limited media, the oxidative metabolism of *P. aeruginosa* occurs largely through the cytoplasmic pathway due to the effects of the specific transfer system for gluconate and 2-KGA repression [99]. Other *Pseudomonas* strains such as *P. putida* have proved to be highly competitive for glucose by oxidizing this sugar to GA, which then is accumulated in the fermentation broth prior to its catabolization. As a result, *P. putida* starts to grow only after all the glucose has been removed from the culture medium [100]. In recent years, *P. fluorescens* [101-103] has been increasingly used for the production of 2-KGA in preference over GA. Additionally, another *Pseudomonas* strain, *P. taetrolens*, has also been used for the simultaneous production of lactobionic acid and GA by a co-fermentation system combining cheese whey (Table 3) and glucose [104]. Finally, it should be highlighted that *Pseudomonas* strains can also further metabolize GA for growth and maintenance purposes through the Entner–Doudoroff pathway; from the point of view of GA production, this is a drawback also suffered by AAB, see Fig. 1.

Zymomonas mobilis, which is known to produce ethanol, also possesses a high biotechnological potential. Although its taxonomic classification is troublesome, it is currently classified under the family *Sphingomonadaceae*. Its biological and physiological properties are comprehensively reviewed by Swings and De Ley [105]. It uses only glucose, fructose and sucrose as carbon sources for growth. *Z. mobilis* is a

facultatively anaerobic bacterium that metabolizes its substrates through the Entner–Doudoroff pathway, which is the typical pathway for strictly aerobic microorganisms such as *Pseudomonas*. In the presence of high concentrations of substrate, the bacterium simultaneously oxidizes glucose to glucono-δ-lactone and fructose to sorbitol by 1-D-glucose-2-D-fructose-oxidoreductase, which is $NADP^+$-dependent [106]. Additionally, the enzyme glucono-δ-lactonase ensures rapid hydrolysis of the lactone to GA. Unlike the previous bacteria, *Z. mobilis* possesses a stereospecific system of facilitated diffusion for glucose. Even so, it is the phosphorylation rate of glucose rather than its transfer rate to the inside of the cell that governs its oxidative metabolism.

5. Gluconic acid and its derivatives: health-promoting effects

As noted earlier, GA and its major metabolites have been classified as "harmless to health" among agri-food products. Mammalian metabolism includes functional mechanisms for the degradation of GA to water and oxygen, which ultimately provide the energy needed for anabolic and catabolic reactions. However, GA and its metabolites have also been ascribed potential benefits not directly related to health. Thus, GA and, by extension, its δ-lactone possess prebiotic properties with a favourable impact on a small number of bacterial strains in the colon growth and/or metabolic activity of which is selectively boosted as a result. Overall, intestinal microbiota consists of beneficial bacteria and, to a lesser extent, toxic and/or harmful bacteria engaging in mutualistic and commensalistic symbiotic relationships. In the human colon, GA is fermented mainly by *Lactobacillus* sp. and *Bifidobacterium adolescentis*, and the formed products (lactate and acetate) are used to form short-chain fatty acids by acid-utilizing bacteria [107]; these not only increase the acidity of the medium but are also absorbed by the large intestinal mucosa to deliver energy to epithelial cells [108].

Ever since the prominent role of bifidobacteria in intestinal transit was discovered, the agri-food industry has incorporated them into various foods and drinks as both dietary supplements and starter cultures (in dairy products). However, these bacteria are being increasingly replaced with prebiotic and probiotic agents. GA and its derivatives may be potentially effective as intestinal control agents, inhibitors of intestinal putrefaction, faecal deodorants or even anti-binding agents for both animals and humans [108]. For example, GA has for several years been widely used as a dietary supplement for animals (particularly weaned individuals) in the replacement of traditional antibiotics [109,110]. Recent research in this area has focused on the antioxidant properties of D-glucono-δ-lactone and sodium D-gluconate. On the basis of the existing results, both might be useful as dietary supplements and/or nutraceuticals for the prevention of cardiovascular disorders. According to Saluk-Juszczak [111], the antioxidant power of these glucose derivatives may result from reactions involving their hydroxyl groups, as may their ability to sequester metal cofactors involved in platelet aggregation. In this way, excessive platelet activation, potentially leading to arterial disorders, can be avoided. An in vivo study of the antioxidant activity in D-glucono-δ-lactone and sodium D-gluconate in the plasma revealed their ability to protect proteins and lipids against strong oxidants such as the peroxonitrite ion [112].

6. Gluconic acid as a food additive: use of agri-food resources as raw materials for its production

Unsurprisingly, given the interest of GA as a food additive, there has been a search for new raw materials – industrial by-products included – for the more economical obtainment of GA products with an added value. Table 3 lists the main agro-industrial

by-products and fermentation techniques used for GA production, the sample pretreatments involved and the yields obtained in each case.

As shown, the traditional substrates consisting of glucose or sucrose either have been or are being successfully replaced with agro-industrial by-products, such as starch, sugarcane molasses, fig, banana, grape, pear, whey, paper waste or hydrolysates from lignocellulosic biomass, as natural sources of carbohydrates that allow processing with environmental-friendly technologies and the sustainable exploitation of natural resources.

The use of starch-based fermentation media yields better results than lignocellulosic-based ones. The heavy conditions (high temperature and pressure, low pH) needed for the previous hydrolysis of lignocellulosic materials yield a wide range of compounds (carboxylic acids, furan aldehydes and aromatic compounds) which are toxic/inhibitory to microorganisms [115, 123-125]. For instance, Zhang et al. [115], working with corn stover hydrolysate, studied the inhibitor tolerance on cell growth, gluconic acid production and GOD activity of *A. niger* SIIM M276 of several inhibitors normally found in these media: furan derivatives, organic acids and phenolic compounds. The most toxic effect on gluconic fermentation was found to be for furfural: concentrations higher than 1.0 g/L almost stopped the metabolism of the fungus.

At the same time as conventional gluconic fermentation using submerged cultures of free cells, new production processes based on immobilized cells facilitating cell reuse, continuous operation and foaming control could be developed [35,40]. Materials such as polyurethane foam, calcium alginate [35] and chemically reduced graphene oxide (CRGO) [40] have been explored, among others, as immobilization supports in recent years.

464 Singh [35], working in repeated batches of solid state surface fermentation, obtained
465 GA production rates as high as 22.5 g/L·d (i.e., 2.5 times greater than those of a typical
466 fermentation cycle), with 95–98% yield, by using *A. niger* ORS-4.410 cells
467 immobilized on polyurethane foam. In the same work, the behaviour of Ca-alginate
468 immobilized cells of *A. niger* in submerged fermentations was also studied; as used to
469 be for similar highly aerobic systems [126], the rate of oxygen transfer in the
470 fermentation medium governed the process. The oxygen mass transfer limitations
471 required vigorous agitation, leading to important shear stresses, which sharply affected
472 the rate of GA production [35].
473 Some of the apparent advantages of immobilized cell systems are high reactor
474 productivity, biomass reuse, high cell concentrations and continuous operation
475 [54,126,127]. Yet the development of successful processes using immobilized cells has
476 to deal with difficult problems concerning the cell physiology. Operational stability and
477 catalytic activity will strongly depend on the pre-cultivation stage, immobilization
478 procedure and process operating conditions. A key issue is to understand how the cell
479 physiology is affected by the microenvironmental conditions: physico-chemical
480 gradients, oxygen supply problems, removal of products, changes in growth and
481 metabolism and other unknown issues [128]. Obviously, a multiplicity of factors
482 influence the choice of a reactor for immobilized cell systems; whilst the most common
483 reactor for free cells is the completely mixed stirred tank reactor, for immobilized cells,
484 it is the fixed bed, fluidized and gas mixed reactors. It is important to bear in mind that
485 any harsh treatment, because of the high rate of shear, to the support particles should be
486 avoided [129].
487 To simplify the process and to reduce the side reactions simultaneously with higher
488 yields for the product of interest, the possibility of immobilizing enzymes instead of

whole cells is also increasingly studied. Nevertheless, the issue is quite complex; the decision of whether immobilized enzymes or immobilized cells should be chosen depends on the characteristics and requirement of each specific system. In general, immobilized enzymes could be a good option for simple, single-step reactions if the enzyme is readily available at an acceptable cost and retains its activity after immobilization [130]; otherwise, immobilized cells are better when the enzymes are intracellular and unstable during and after immobilization, the microorganisms have no interfering enzymes and the substrates and products are low molecular weight compounds [127].

Regarding GA production, there are many studies for the development of an efficient enzymatic commercial process; nevertheless, enzymatic production of GA seems to be uneconomical at the industrial scale because of the instability of the enzymes and the high resulting costs. In most of the attempts, GOD was used, which is rapidly inactivated after a single batch if free dissolved GOD is utilized or after a short period of time when the enzyme has been immobilized [5]. The problem might become more complex if a multi-enzyme system is necessary [5,40,131]. For instance, the co-immobilization of glucoamylase and GOD from *A. niger* on either graphene oxide (GO) or CRGO has been considered for obtaining a biocatalyst in the one-pot conversion of starch to GA; the results were quite different depending on the carrier: 85% and 10% of the original multi-enzyme activity could be retained after four cycles when CRGO and GO were used, respectively [40].

Additionally, a multi-enzyme system for GA production using sucrose from sugarcane as raw material and soluble enzymes (invertase, GOD and catalase) in an airlift reactor has been studied [131]; in comparison with other alternatives, a quite high GA productivity per gram of glucose was obtained (0.266 g_{GA} g_G^{-1} h^{-1}). Interestingly, the

514 stabilities of the enzymes were evaluated; finding revealed that their half-lives (time
515 required for loss of 50% of the initial enzymatic activity) were quite sensitive to
516 temperature and pH; the best conditions for GA production appeared to be 40 ºC and a
517 pH of 6.0, with around 100 h being the half-life of the enzymes, but at 50 ºC and a pH
518 of 5.0, quite lower values for half-lives were found: 4, 24 and 1 h for invertase, GOD
519 and catalase, respectively.
520 Although the use of enzymes/multi-enzyme systems has attracted considerable attention
521 in the recent years, the optimization of their operational stability and the efficient
522 control of these systems remain a challenge [131,132].
523 The revalorization of agro-industrial by-products as fermentation substrates can be
524 especially interesting with regards to perishables, for which fermentation significantly
525 extends their useful life. An example is the case of fruits and garden vegetables, which
526 suffer worldwide losses amounting to approximately 50% of their production [1]. Very
527 often, these products are hygienically and nutritionally acceptable, but are discarded
528 based on aesthetic grounds (viz., size, weight and/or appearance) because they fail to
529 meet quality regulations for marketing or simply because of their low market value.
530 The use of fruit surpluses poses the problem of working with complex matrices that
531 may imply many restrictions on the fermentation alternatives. For instance, strawberry
532 surpluses have been used to obtain new non-alcoholic fermented beverages containing
533 GA as a major ingredient, which also retain the nutritional and sensory properties of the
534 fruit as much as possible; in particular, strawberry purée was used to produce a naturally
535 sweet beverage containing no glucose by the conversion of glucose into GA while
536 maintaining the original fructose of the purée [120-122,133,134]. An AAB was used, *G.*
537 *japonicus* CECT 8443, on the grounds of its high selectivity towards glucose relative to
538 fructose [120,122,133]. The need to preserve the original properties of the fruit as much

as possible led to the use of pasteurization instead of sterilization as the only thermal pretreatment of the strawberry purée; thus, the inoculation procedure had to be carefully studied to guarantee the prevalence of the *Gluconobacter* strain over unwanted microorganisms (yeasts, mainly) unavoidably present in industrially pasteurized strawberry purée. A two-stage procedure, including a pre-cultivation in sterilized strawberry purée, yields a quite active inoculum of *G. japonicus* that can transform the glucose content before the yeast activity started to be significant [122]. Additionally, keeping in mind the complexity of the *Gluconobacter* metabolism, another problem to be solved was the need to preserve the formed GA in the fermented end-product, avoiding its conversion into keto-gluconates; the pH seems to be the key variable in this regard, which should be allowed to evolve freely throughout [120]. In any case, although problems such as those previously commented can be solved, a batch cultivation mode is imposed in practice.

7. Future potential of genetically modified acid acetic bacteria for GA production

Because of their importance in food industry [75,135,136], only AAB will be considered in this section.

Traditionally, improvements in industrial fermentation processes have relied heavily on the use of fast-growing starter cultures. However, the inception of genetic engineering has brought about dramatic changes in production strategies and techniques. For example, DNA control and transfer methods have started a technological revolution, enabling the development of microorganisms with modified properties.

Regarding fermentation, innovations have enabled the design of GMO for enzyme production and/or new microbial strains to improve the efficiency and safety of the production process. In most cases, the genetic modification involves exposure to

physical or chemical mutagens by insertion or gene overexpression (i.e., by incorporating genes, either cloned or otherwise, into the genome of a microorganism with the aid of appropriate plasmids and promoters and gene deletion or deactivation to suppress the expression of a specific gene by replacing it with a modified, non-functional version).

Rather than GA production, however, genetically modified bacteria have been preferentially used to obtain 5-KGA, which is an excellent precursor for L-(+)-tartaric acid. Thus, gene deletion and plasmid-localized gene introduction in *G. oxydans* 621H to suppress the gene (*ga-2-dh*) coding the enzyme 2KGADH [137] and overexpress the genes coding GDH [138,139] and GA5DH [140,141] have allowed the efficiency of the process to be increased by 60%. However, all strains resulted in transient accumulation of GA, which remains an unresolved problem [141-143].

Genetic modifications of *G. oxydans* bacteria similar to those aimed at boosting production of 5-KGA might be used to obtain GA in the future. This approach would be especially useful for nutritional purposes, as it might allow improvement in food processability and sensory properties and increase in food nutritional quality. To this end, new genetic tools for genome modification in AAB have recently been developed, which allow the efficient, markerless, in-frame deletion of genes from the genomes of these bacteria without leaving foreign DNA, such as selection markers, at the modification site [144,145]. These tools are also suitable for the directed insertion of genes into the AAB genomes.

Whole-cell biocatalysts can have significant advantages over in vitro enzyme-catalyzed reactions. This advantage is observed for complex multi-step conversions or for redox reactions that require coenzyme regeneration. On the other hand, a major limitation of whole-cell biocatalysts is that due to the involvement of an entire organism with its

589 entire enzyme complement, unwanted reactions can also occur, such as by-product
590 formation via branches in metabolic pathways, cross-specificity of different enzymes
591 for the same substrate, degradation of the desired product or (partial) conversion of the
592 desired primary product into unwanted secondary products. A good example is the
593 membrane-bound oxidation system of *G. oxydans* for the oxidation of glucose to
594 gluconate by GDH and the further oxidation of gluconate to ketogluconates by different
595 GADH enzymes (see above).
596 To reduce/avoid these problems, several alternatives might be considered. On the one
597 hand, permeabilized cells can be used in which some pathways do not become
598 functional after the permeabilization treatment; an example could be the use of toluene-
599 treated permeabilized cells of *Z. mobilis* for the simultaneous production of sorbitol and
600 gluconic acid [146]. But, on the other hand, as outlined above, Merfort et al. [139] have
601 demonstrated that the *G. oxydans* strain 621H, which was engineered through the
602 disruption of GA2DH activity to avoid 2-KGA production, in combination with the
603 plasmid-driven overexpression of GA5DH activity, demonstrates a significantly
604 enhanced 5-KGA accumulation. Finally, strains of *G. oxydans* 621H have been
605 constructed that lack virtually all major membrane-bound dehydrogenases [147]. Such
606 multiple deletion strains provide an excellent foundation for the construction of *G.*
607 *oxydans* strains that contain merely one specific membrane dehydrogenase and avoid
608 competing oxidation reactions or cascades of membrane-bound DH oxidations if not
609 desired. In the end, strain engineering must not remain restricted to the membrane-
610 bound dehydrogenases themselves. Because these enzymes are merely entry points for
611 electron delivery to the respiratory chain, it is conceivable that other components of the
612 electron transport chain may become limiting for the substrate oxidation process.

However, the genetic tools now available for AAB allow further optimization of their membrane-bound oxidative metabolism.

8. Conclusions

Gluconic acid (GA) is a functional additive with many uses in food, pharmaceutical, textile and building industries. Traditionally, GA has been obtained by technological means involving fungi or, to a lesser extent, AAB. However, recent research has revealed new potentially favourable effects of this acid on human and animal health, which have boosted its use as a prebiotic in food production. The need to improve the efficiency and profitability of existing biotechnological processes, together with the increasing amounts of agro-industrial residues produced worldwide, has fostered the use of wastes with a high content of sugars as raw materials for GA production with improved methods. The promising prospects in this area may be furthered by advances in genetic engineering and the application of new molecular biology methods in the future.

9. Acknowledgements

The authors are grateful to Spain's Ministry of Science and Innovation for partially funding this study within the framework of the project AGL2010-22152-CO3-03 and to University of Córdoba within the framework of Programa Propio Mod.4-1.

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Figure captions

Fig. 1. Gluconic acid (GA) and its derivatives. (a) Chemical equilibrium of GA and its lactones in aqueous solution. (b) Chemical oxidation of GA by a strong oxidant.

Fig. 2. Glucose metabolism in *Aspergillus niger*. Released and membrane-associated glucose oxidase is mainly responsible for D-gluconate formation, whereas the enzymes in the cytosol play a role in D-glucose and D-gluconate assimilation. D-glucono-δ-lactone can be converted into D-gluconate spontaneously or by an extracellular gluconolactonase.
GOD, FAD-dependent glucose oxidase (EC 1.1.3.4); GNL, gluconolactonase (EC 3.1.1.17); CAT, catalase (EC 1.11.1.6); GK, hexokinase (EC 2.7.1.1)/glucokinase (EC 2.7.1.2); GNK; gluconokinase (EC 2.7.1.12); G6PDH, NADP-dependent glucose-6-phosphate dehydrogenase (EC 1.1.1.49); 6PGNL, 6-phosphogluconolactonase (EC 3.1.1.31); 6PGNDH, NADP-dependent 6-phosphogluconate dehydrogenase (EC 1.1.1.44); R5PI, ribose-5-phosphate isomerase EC 5.3.1.6).

Fig. 3. Glucose metabolism in *Gluconobacter* (adapted from Rogers et al. [7] and Matsushita et al. [78]). Membrane-bound primary dehydrogenases and some soluble enzymes are known to be involved in the production and assimilation of D-gluconate; D-glucono-δ-lactone can be converted into D-gluconate spontaneously or by a membrane-bound gluconolactonase (not shown here). The quinoproteins GDH and D-gluconate 5-dehydrogense and the flavoproteins D-gluconate dehydrogenase and 2-keto-D-gluconate dehydrogenase are proteins that are integrated into the cytoplasmic membrane with their active site facing towards the periplasm. Electrons from substrate

oxidation are transferred to ubiquinone (Q_{10}). For *G. oxydans*, it was shown that ubiquinol ($Q_{10}H_2$) is oxidized by a cytochrome *bo*$_3$ oxidase, or a low-oxygen affinity cytochrome *bd* oxidase, to generate a proton gradient [79]. NADP-dependent D-glucose dehydrogenase and NADP-dependent 2-keto-D-gluconate reductase are located in the cytoplasm.

GDH, PQQ-dependent D-glucose dehydrogenase (in the cytoplasmic membrane, EC 1.1.5.2) and NADP-dependent D-glucose dehydrogenase (in the cytoplasm EC 1.1.1.47); GADH: FAD-dependent D-gluconate 2-dehydrogenase (EC 1.1.99.3); 2KGADH: FAD-dependent 2-keto-D-gluconate dehydrogenase (EC 1.1.99.4); GA5DH, PPQ-dependent D-gluconate 5-dehydrogenase (in cytoplasmic membrane) and NADP-dependent D-gluconate 5-dehydrogenase (in cytoplasm EC 1.1.1.69); 2KGR: 2-keto-D-gluconate reductase (EC 1.1.99.3); 5KGR: 5-keto-D-gluconate reductase (EC 1.1.1.69); GK, Glucokinase (EC 2.7.1.2); GNK, gluconokinase; G6PD, Glucose 6-phosphate dehydrogenase (EC 1.1.1.49); 6PGDD, 6-Phosphogluconate dehydratase (EC 4.2.1.12); 6PGDH, 6-Phosphogluconate dehydrogenase (EC 1.1.1.44).

Table 1.The main origins of food loss along the supply chain [1].

UC: underdeveloped countries; DC: developed countries.

Table 2.Gluconic acid derivatives used as food additives included in the *Codex Alimentarius* (revised 2014) according to the specifications of the FAO/WHO Joint Expert Committee on Food Additives (JECFA).

Table 3.Main agro-industrial wastes used as substrates for the microbiological production of gluconic acid.

Table 1

Origin	**Steps in the food supply chain**				
	Agricultural production	**Post-harvest handling and storage**	**Processing**	**Distribution**	**Consumption**
Premature harvest	DC				
Difficulty in assuring product quality	DC and UC	DC and UC	DC and UC	DC and UC	UC
Poor facilities and infrastructure	UC	UC	UC	UC	UC
Overly strict quality control			DC	DC	
An excessive variety of products and brands				DC	
Consumer attitudes					DC

1121 **Table 2**

Additive	INS code	Description	Last revision year	Technological function	Foods containing the additive
Gluconic acid	E-574	Colourless to light yellow, clear syrup liquid (aqueous solution of gluconic acid (55–60%) and glucono-δ-lactone)	-	Acidity regulator Raising agent	Fruits and vegetables (Natural origin) Milk products
Glucono-δ-lactone	E-575	White, odourless or nearly odourless crystals or crystalline powder	1998	Acidity regulator Leavening agent Sequestering agent	Naturally fermented milk (Natural origin) Whey protein cheese Fresh pasta and noodles and related products Supplemental foods for lactating babies and infants
Sodium gluconate	E-576	White to tan, granular to fine crystalline powder	1998	Sequestering agent Stabilizer Thickener	Products containing fermented garden vegetables or seaweed Dehydrated pasta and noodles and related products Salt substitutes Coffee and its substitutes, tea, herbal infusions and other, cereal-based hot drinks
Potassium gluconate	E-577	Odourless, free flowing to yellowish white granules or crystalline powder	1998	Acidity regulator Yeast nutrient Nutritional supplement	Mozzarella cheese Biscuit and sponge cake Cold meat
Calcium gluconate	E-578	Odourless, white crystalline granules or powder	1998	Acidity regulator Hardener Sequestering agent Nutritional supplement	
Ferrous gluconate	E-579	Greenish yellow granules, pale grey granules or a crystalline powder with a slight burnt sugar-like odour	1999	Colour preservative Stabilizer Nutritional supplement	Garden vegetables and seaweed in vinegar, oil, brine or soy sauce
Magnesium D-gluconate	E-580	White to off-white, odourless fine powder	1999	Acidity regulator Firming agent Yeast nutrient Nutrient supplement	Bread-making products Water

1122

1123 **Table 3**

Carbon source	Microorganism or enzyme used	Culturing method	Substrate pretreatment	GA yield [(*)] (%)	Reference
Cornstarch	*A. niger* ORS-4	Batch culture/surface fermentation [a]	None	24,39	[113]
			Hydrolysis	35,92	
	Immobilized *A. niger* JCM 5549	Batch culture/submerged fermentation [a] Nonwoven fabric [b]	Hydrolysis	84,91	[114]
Starch	Immobilized glucoamylase and glucose oxidase (from *A. niger*)	Batch culture/submerged fermentation [a] Chemically reduced graphene oxide (CRGO) [b]	Hydrolysis	82,00	[40]
Lignocellulosic biomass	*A. niger* SIIM M276	Batch culture/submerged fermentation [a]	Hydrolysis	94,83	[115]
Sugarcane molasses	*A. niger* ORS-4	Batch culture/surface fermentation [a]	None	8,27	[113]
			Clarification	38,47	
	A. niger ORS-4410 (mutant strain)	Batch culture/submerged fermentation [a]	None	15,30	[70]
			Clarification	61,30	
	A. niger ARNU-4 (mutant strain)	Batch culture/solid state fermentation [a] Tea waste [b]	None	85,20	[71]
Whey	*A. niger* NCIM 548	Batch culture/submerged fermentation [a]	Deproteination	69,00	[116]
	Immobilized *A. niger* NCIM 548	Batch culture/submerged fermentation [a] Polyurethane foam [b]		92,00	
Cheese whey/glucose	*Pseudomonas taetrolens* LMG 2336	Fed-batch culture/submerged fermentation [a,c]	Sweetening	-	[104]
Waste paper	*A. niger* IAM 2094	Batch culture/submerged fermentation [a]	Hydrolysis	84,84	[117]

Fig	*A. niger* ATCC 1057	Batch culture/solid state fermentation [a,d]	Sterilization	63,00	[118] 1124
		Batch culture/surface fermentation [a,d]	Extraction and sterilization	79,70	[119]
Banana must	*A. niger* ORS-4	Batch culture/surface fermentation [a]	Clarification	40,08	[113]
	A. niger ORS-4410 (mutant strain)	Batch culture/submerged fermentation [a]	None	51,70	[70]
			Rectification	72,40	
Grape must	*A. niger* ORS-4410 (mutant strain)	Batch culture/Submerged fermentation [a]	None	60,40	
			Rectification	80,60	
	Immobilized *A. niger* ORS-4410 (mutant strain)	Semi-continuous culture/submerged fermentation [a] Ca-alginate [b]	Rectification	89,50	[35]
		Semi-continuous culture/submerged fermentation [a] Polyurethane-sponge [b]		98,60	
Strawberry purée	*Gluconobacter japonicus* CECT 8443	Batch culture/submerged fermentation [a]	Pasteurization	95,00	[120-122]

1125 [(*)] Calculated on utilized glucose

1126 [a] Cultivation method

1127 [b] Support used to immobilize the cells/enzymes or to grow the cells.

1128 [c] Simultaneous co-production of lactobionic and gluconic acid

1129 [d] Simultaneous co-production of citric and gluconic acid.

1130 **Figure 1**

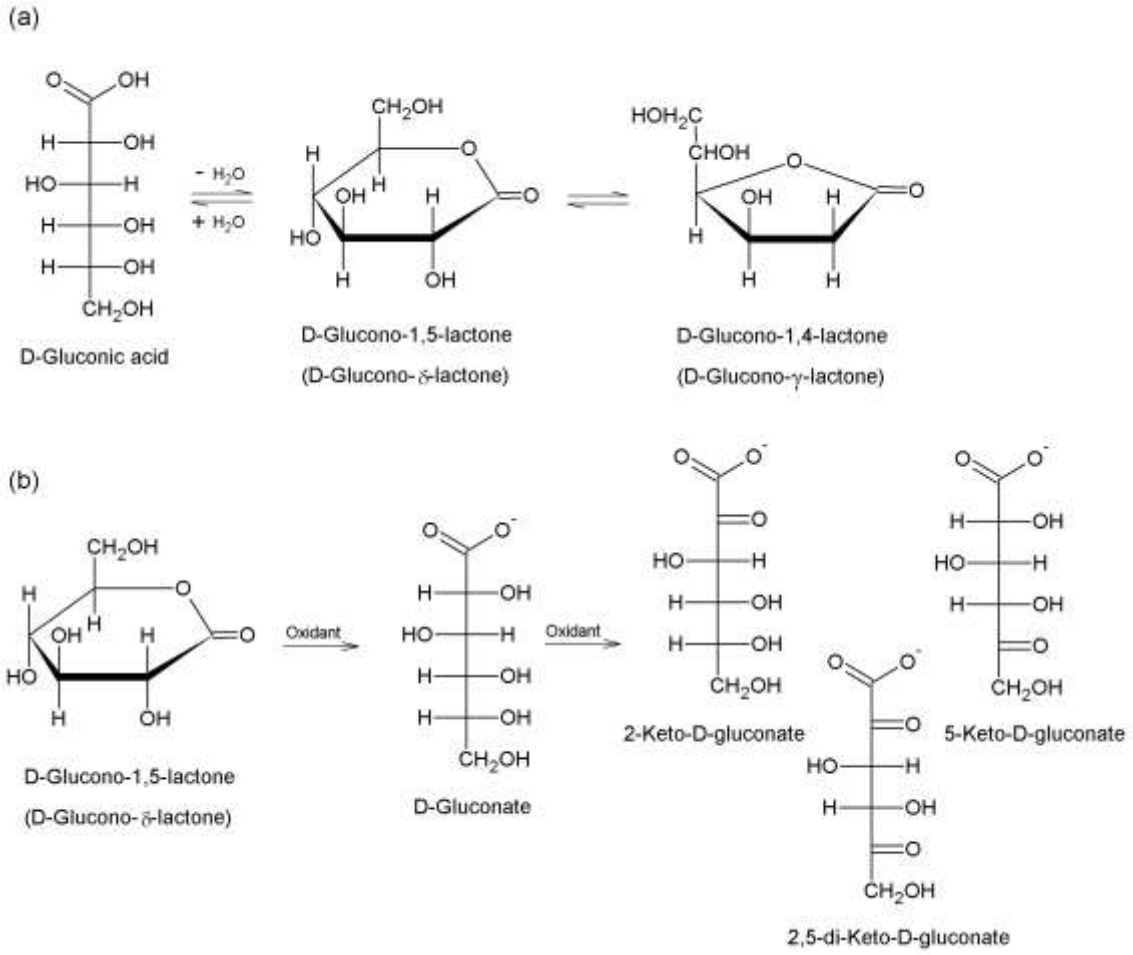

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Figure 2

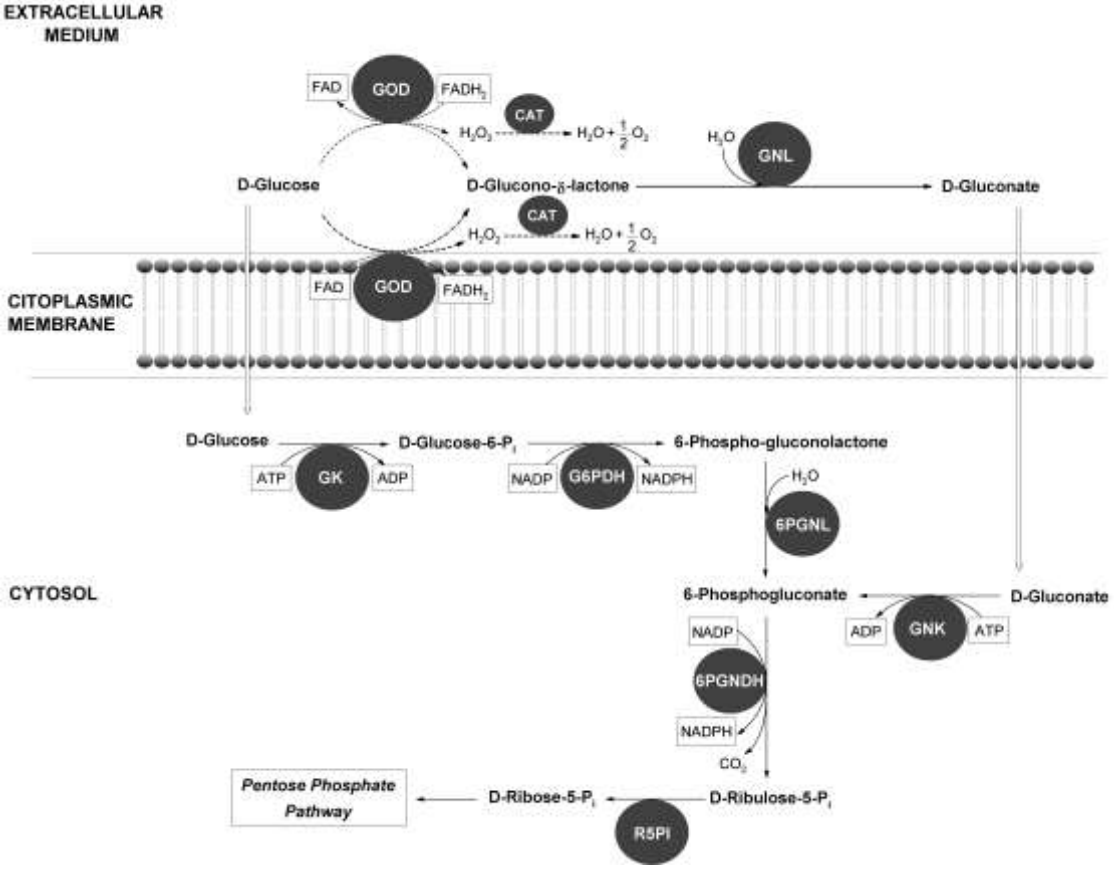
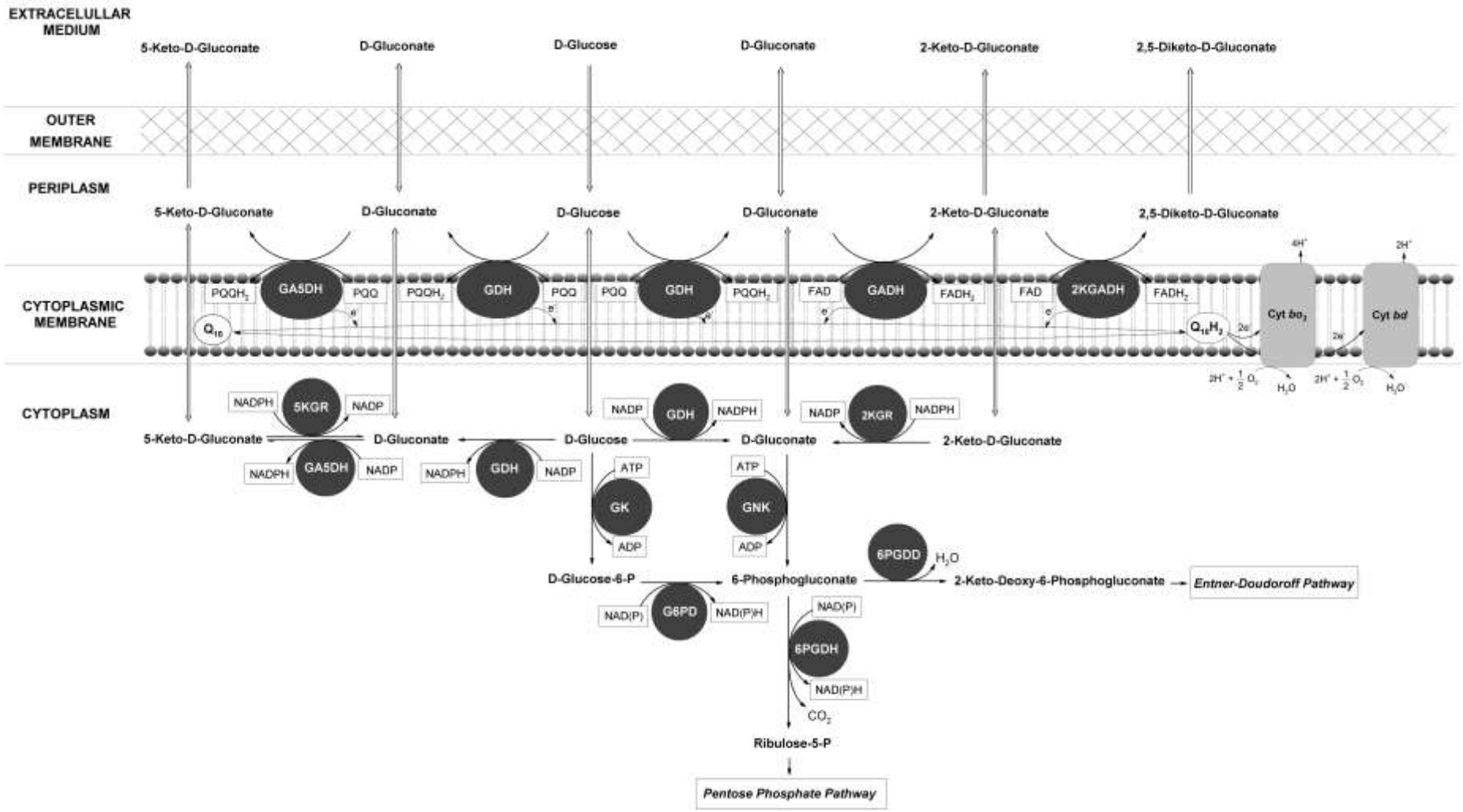

EXTRACELLULAR MEDIUM
FAD
GOD
FADH2
CAT
H2O2
H2O + 1/2 O2
H2O
GNL
D-Glucose
D-Glucono-δ-lactone
D-Gluconate
CITOPLASMIC MEMBRANE
D-Glucose
D-Glucose-6-P1
6-Phospho-gluconolactone
ATP
GK
ADP
NADP
G6PDH
NADPH
6PGNL
CYTOSOL
6-Phosphogluconate
D-Gluconate
ADP
GNK
ATP
NADP
6PGNDH
NADPH
CO2
Pentose Phosphate Pathway
D-Ribose-5-P1
D-Ribulose-5-P1
R5PI

Figure 3


EXTRACELULLAR MEDIUM
5-Keto-D-Gluconate
D-Gluconate
D-Glucose
D-Gluconate
2-Keto-D-Gluconate
2,5-Diketo-D-Gluconate
OUTER MEMBRANE
PERIPLASM
CYTOPLASMIC MEMBRANE
GA5DH
GDH
GADH
2KGADH
PQQ
FAD
Cyt bo3
Cyt bd
CYTOPLASM
5KGR
2KGR
NADPH
NADP
ATP
ADP
GK
GNK
6PGDD
G6PD
6PGDH
D-Glucose-6-P
6-Phosphogluconate
2-Keto-Deoxy-6-Phosphogluconate
Entner-Doudoroff Pathway
Ribulose-5-P
Pentose Phosphate Pathway