

Title	Physiology of acetic acid bacteria and their role in vinegar and fermented beverages
Authors	Lynch, Kieran M.;Zannini, Emanuele;Wilkinson, Stuart;Daenen, Luk;Arendt, Elke K.
Publication date	2019-04-02
Original Citation	Lynch, K. M., Zannini, E., Wilkinson, S., Daenen, L. and Arendt, E. K. (2019) 'Physiology of acetic acid bacteria and their role in vinegar and fermented beverages', Comprehensive Reviews in Food Science and Food Safety. doi: 10.1111/1541-4337.12440
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://onlinelibrary.wiley.com/doi/abs/10.1111/1541-4337.12440 - 10.1111/1541-4337.12440
Rights	© 2019, Institute of Food Technologists. Published by John Wiley & Sons Inc. This is the peer reviewed version of the following article: Lynch, K. M., et al (2019) 'Physiology of acetic acid bacteria and their role in vinegar and fermented beverages', Comprehensive Reviews in Food Science and Food Safety. doi: 10.1111/1541-4337.12440, which has been published in final form at https://doi.org/10.1111/1541-4337.12440. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
Download date	2025-08-26 19:08:11
ltem downloaded from	https://hdl.handle.net/10468/7852



University College Cork, Ireland Coláiste na hOllscoile Corcaigh

1	Physiology of Acetic Acid Bacteria and Their Role in Vinegar and
2	Fermented Beverages
3	
4 5	Kieran M. Lynch ¹ , Emanuele Zannini ¹ , Stuart Wilkinson ² , Luk Daenen ² , and Elke K. Arendt ^{1,3*}
6	¹ School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
7 8	² Global Innovation & Technology Centre, Anheuser-Busch InBev nv/sa, Brouwerijplein 1, 3000 Leuven, Belgium
9	³ APC Microbiome Ireland, University College Cork, Cork, Ireland
10	
11	
12	
13	* Corresponding author:
14	Mailing address: School of Food and Nutritional Sciences, University College Cork, Cork,
15	Ireland.
16	Tel +353 21 490 2064; Fax +353 21 427 0213
17	E-mail address: <u>e.arendt@ucc.ie</u>
18	
19	
20	
21	
22	Short title: Microbiology of Acetic acid bacteria
23	
24	
25	Keywords: Acetic acid bacteria, oxidative fermentation, exopolysaccharides, vinegar, Vitamin C
20	
<i>L</i>	
28	

29 ABSTRACT

Acetic acid bacteria (AAB) have, for centuries, been important microorganisms in the 30 production of fermented foods and beverages such as vinegar, kombucha, (water) kefir and 31 lambic beer. Their unique form of metabolism, known as "oxidative" fermentation, mediates 32 the transformation of a variety of substrates into products which are of importance in the food 33 34 and beverage industry and beyond; the most well-known of which is the oxidation of ethanol into acetic acid. Here, a comprehensive review of the physiology of acetic acid bacteria is 35 presented, with particular emphasis on their importance in the production of vinegar and 36 37 fermented beverages. In addition, particular reference is addressed towards Gluconobacter oxydans due to its biotechnological applications, such as its role in Vitamin C production. The 38 production of vinegar and fermented beverages in which AAB play an important role is 39 40 discussed, followed by an examination of the literature relating to the health benefits associated with consumption of these products. Acetic acid bacteria hold great promise for future 41 exploitation, both due to increased consumer demand for traditional fermented beverages such 42 as kombucha, and for the development of new types of products. Further studies on the health 43 benefits related to the consumption of these fermented products and guidelines on assessing 44 45 the safety of AAB for use as microbial food cultures (starter cultures) are, however, necessary in order to take full advantage of this important group of microorganisms. 46

2

47 Introduction

Acetic acid bacteria (AAB), first described as "vinegar bacteria" by Louis Pasteur over 150 48 years ago, are an important and diverse group of bacteria involved in the production of 49 fermented foods and beverages, especially known for their production of acetic acid (ethanoic 50 acid) in the making of vinegar (Hutkins, 2006; Pasteur, 1864). Acetic acid bacteria are 51 52 characterized by their ability to oxidize carbohydrates, alcohols and sugar alcohols (polyhydric alcohols or polyols) into their corresponding organic acids, aldehydes or ketones, in a process 53 termed "oxidative fermentation", from which they gain energy (Taban & Saichana, 2017). This 54 55 unique property also has applications in the production of industrially-relevant compounds and has primarily been exploited in the synthesis of ascorbic acid (Vitamin C) and miglitol (an 56 antidiabetic drug used in the treatment of type II diabetes mellitus) (Shinjoh & Toyama, 2016; 57 58 Taban & Saichana, 2017). Some genera of AAB are notable for their production of a variety of exopolysaccharides (EPS), the most valuable of which are bacterial cellulose (BC) and 59 acetan. BC offers several advantages over plant-derived cellulose, particularly because it is free 60 of hemicellulose and lignin associated with cellulose (Dağbağlı & Göksungur, 2017). Acetic 61 acid bacteria are associated with, and have been isolated from, carbohydrate-rich and acidic 62 63 environments such as fruits and flowers, and are involved in the production of a variety of 64 fermented foods and beverages including vinegar, kombucha, lambic beers, kefir and nata de 65 coco (Table 1). They also play an important role in the cocoa fermentation process (Pothakos 66 et al., 2016). Unsurprisingly, AAB have also been associated with the spoilage of foods and alcoholic beverages such as beer, wine, cider and fruit juices (Taban & Saichana, 2017). The 67 purpose of this review is to provide an overview of the physiology and biochemistry of AAB 68 69 and to provide an understanding of how the unique capabilities of these microorganisms are 70 important in the production of vinegar and other fermented beverages. Other technologicallyrelevant aspects, such as EPS biosynthesis and their role in the bioconversion of products useful 71

in the food, chemical and pharmaceutical industries, such as ascorbic acid, are also considered.
While recent reviews from others such as Ho et al. (2017) and De Roos & De Vuyst (2018a)
have addressed specific topics, the aim here was to provide a broad and comprehensive review
on the physiology and application of AAB (De Roos & De Vuyst, 2018a; Ho, Lazim, Fazry,
Zaki, & Lim, 2017).

77 Characteristics of the AAB and their identification

Acetic acid bacteria are Gram-negative or Gram-variable, obligate aerobes, and are classified 78 79 in the family Acetobacteraceae. They are non-spore forming, ellipsoidal to rod-shaped cells that can occur singly, in pairs or in short chains (Malimas et al., 2017). The members of the 80 Acetobacteraceae family are separated into two groups, the acetous and the acidophilic groups, 81 82 of which AAB are included in the former (Komagata, Iino, & Yamada, 2014). Eighteen genera are currently reported in the acetous group; the most important in terms of fermented foods are 83 Acetobacter, Gluconobacter, Gluconacetobacter, and Komagataeibacter (Table 2) (Giudici, 84 85 De Vero, & Gullo, 2017). In addition, the aforementioned genera contain many species while the remaining genera consist of only one or two (Yamada, 2016). Some genera are motile, 86 having either peritrichous or polar flagella. The majority of species are catalase-positive and 87 88 oxidase-negative, however, there are exceptions. Growth in the presence of 0.35% acetic acid has been used as a criterion; however, while members of the genera Acetobacter, 89 Gluconobacter, Gluconacetobacter, and Komagataeibacter grow in the presence of 0.35% 90 acetic acid, not all genera can e.g. Asaia. Acetic acid bacteria are typically considered to be 91 92 mesophilic, with the optimum temperature for growth being around 30°C (Malimas et al., 93 2017). At higher temperatures, growth reduces significantly, with none usually occurring above 94 34°C (Saichana, Matsushita, Adachi, Frebort, & Frebortova, 2015). The mesophilic character 95 of AAB, which require strict temperature control, poses a challenge for industrial application. 96 However, thermotolerant strains that can grow at a temperature of up to 42°C have been 97 identified (Saichana et al., 2015). It is noteworthy that the genera constituting the AAB are undergoing continuous revision and taxonomic changes and the reader is directed elsewhere 98 99 for further information on this topic, such as Malimas et al. (2017). Acetic acid bacteria are 100 notable for their direct oxidation of carbohydrates and sugar alcohols from which they accumulate large amounts of the corresponding oxidation products, gleaning metabolic energy 101

from the process. This so-called "oxidative fermentation" is a key metabolic characteristic in
AAB and is carried out by membrane dehydrogenases and will be considered in greater detail
in subsequent sections (Matsushita & Matsutani, 2016).

Acetic acid bacteria have primarily been isolated from sugary, acidic and alcoholic habitats, materials such as flowers or fruits, or from the fermented foods which they inhabit, such as vinegar, beer, cider and cocoa pulp-bean mass. As AAB occur as symbionts of insects, this can be another isolation source (Mamlouk & Gullo, 2013). Typically, species of *Gluconobacter* are associated with carbohydrate-rich environments, whereas species of *Acetobacter* can be found in alcohol-enriched niches (Raspor & Goranovic, 2008).

Nutrient media for the isolation and growth of AAB can vary, mainly in the types of carbon 111 sources which are included, as strains from different environments or niches differ in their 112 113 nutritional requirements (Table 3). For example, glucose yeast extract carbonate (GYC) medium is commonly used for the isolation and growth of strains originating from 114 115 carbohydrate-rich environments. Calcium carbonate is added to buffer the acid production, with a zone of clearing being observed around colonies of AAB when grown on agar media to 116 which it has been added. For the isolation and growth of strains originating from ethanol- and 117 acetic acid-rich environments the use of media containing ethanol and acetic acid is 118 recommended (e.g. acetic acid ethanol (AE) medium) (Gullo & Giudici, 2008). When isolating 119 strains from environmental sources or matrices likely to contain other microorganisms, AAB 120 can be selected for by reducing the pH of the growth medium to pH 4.4 and/or by adding 121 antimicrobial agents such as cycloheximide for the inhibition of yeasts or penicillin to inhibit 122 lactic acid bacteria (LAB) (De Vero, Gullo, & Giudici, 2017). Media which contain alternative 123 carbon sources to glucose, such as mannitol or malt extract, are commonly used for isolating 124 AAB e.g. yeast extract peptone mannitol (YPM) medium and malt yeast extract agar (MYA) 125 medium (Mamlouk & Gullo, 2013). Deoxycholate mannitol sorbitol (DMS) agar, which 126

contains a number of carbon sources has been used for the selective isolation and enumeration
of presumptive AAB, for example, from cocoa pulp-bean mass and lambic beer. A modified
version (mDMS) containing lactic acid, acetic acid and ethanol is advisable for the isolation of
a number of AAB species (Camu et al., 2008; De Roos, Verce, Aerts, Vandamme, & De Vuyst,
2018a; Papalexandratou et al., 2013; Wieme et al., 2014).

132 The capability to accurately identify microorganisms is indisputable, not only from the perspective of obtaining a basic understanding of the microorganism(s) being applied, 133 (particularly where the properties and desired traits that different species of microorganism 134 possess, or ideally should possess, can vary), but also from a food safety perspective. Many 135 DNA-based methods have been used for the identification of AAB, both as single isolated 136 strains and as members of complex food matrices, such as fermenting wine (Gonzalez, Hierro, 137 138 Poblet, Mas, & Guillamon, 2005). More recently, as the number of described AAB species has risen taxonomically, in part as a consequence of the use of such molecular techniques, the 139 requirement for higher resolution and better differentiation between species has increased, 140 necessitating the development of, firstly, molecular targets with increasing resolving power, 141 and secondly, non-DNA-based methods that are sensitive and rapid (Andrés-Barrao et al., 142 143 2013).

Early molecular methods focused on the 16S rRNA gene and included Restriction Fragment 144 Length Polymorphism Analysis (RFLP) of the gene or PCR amplification and direct 145 sequencing (Andrés-Barrao et al., 2017). RFLP was a fingerprinting method that allowed 146 identification to the species level, but required the restriction pattern of a known species as a 147 comparison for identification. Also, direct sequencing of the gene necessitates that the 148 sequence of a known species has been deposited in a database and can be used similarly for 149 comparison. In addition, due to the sequence of the 16S rRNA gene being highly conserved 150 between some species, differentiation between such species is not always possible. Therefore, 151

RFLP of the spacer region between the 16S and 23S rRNA genes (internal transcribed spacer, 152 ITS) has been used to provide such differentiation (Gonzalez & Mas, 2011). Intergenic regions 153 are known to have higher variability than functional, protein-coding sequences, thus being able 154 to resolve closely related species (Barry, Colleran, Glennon, Dunican, & Gannon, 1991). 155 However, due to frequent variations and high divergences of intergenic sequences, even among 156 strains of the same species, direct sequencing of the ITS was not useful for identification to 157 158 species level (Ruiz, Poblet, Mas, & Guillamon, 2000). Nevertheless, the use of a polyphasic approach including the sequencing of both the 16S rRNA gene and the ITS region for use in 159 160 phylogenetic analysis has enabled differentiation of closely related strains (Gonzalez & Mas, 2011). Alternative loci such as the *adhA* gene, *recA* gene and *tuf* gene can be used for 161 polygenetic studies, showing similar results to phylogenies prepared with 16S rRNA and ITS 162 163 sequences (Greenberg et al., 2006; Trcek, 2005).

Another method of phylogenetic analysis which has gained prominence is Multilocus Sequence 164 Typing (MLST). This is based on the phylogenetic analysis of concatenated sequences from 165 single-copy, ubiquitous, protein-coding genes, typically house-keeping genes, which evolve 166 faster than rRNA. Construction of phylogenetic trees based on concatenated sequences of the 167 168 housekeeping genes dnaK, rpoB and groEL, produced similar results to those obtained with 169 the 16S rRNA gene and delineation of closely related species of the K. liquefaciens and K. 170 xylinus groups. In addition, trees based solely on individual dnaK, groEL and rpoB sequences 171 showed similar topology to that of the tree based on the concatenated sequence of the same genes (Cleenwerck, De Vos, & De Vuyst, 2010). An increasingly applied format of MLST is 172 the use of whole-genome sequences (as opposed to only a few house-keeping genes) in the 173 174 phylogenetic analysis. Here, all of the genes that are present in all isolated strains or species under investigation (i.e. the core genome) are concatenated and used to build the phylogenetic 175 tree (Matsutani, Hirakawa, Yakushi, & Matsushita, 2011). 176

177 For genotyping and identification to the strain level, methods such as Random Amplification of Polymorphic DNA (RAPD) and Amplified Length Fragment Polymorphism (ALFP), and 178 techniques based on amplification of repetitive sequences, such as Enterobacterial Repetitive 179 180 Intergenic Consensus-PCR (ERIC-PCR), Repetitive Extragenic Palindromic-PCR (REP-PCR) and (GTG)₅-PCR have been variously applied in studies, all of which are fingerprinting 181 techniques. Both ERIC-PCR and (GTG)₅-PCR have been demonstrated to be most suitable for 182 183 the differentiation of isolates to strain level, in some studies being used to monitor the population dynamics of AAB in traditional wine vinegar production at the strain level (De 184 185 Vuyst et al., 2008; Papalexandratou & De Vuyst, 2011; Vegas et al., 2010).

Alternatives to DNA-dependant molecular methods are increasingly being explored as 186 accurate, rapid and high throughput means of microbial identification. One such method is 187 188 Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) which has originally been exploited in the field of clinical microbiology for the rapid 189 identification of human pathogens, but has in recent years developed in the area of food 190 microbiology (Croxatto, Prod'hom, & Greub, 2012; De Roos, et al., 2018a; Spitaels et al., 191 2014a; Spitaels et al., 2015). MALDI is a soft ionization method used with mass spectrometry 192 193 for the analysis of large organic biomolecules. Briefly, the sample is bombarded with a high-194 energy laser beam leading to ionization of the sample in the form of cations. These ions are 195 then accelerated in an electric field to a speed that depends on the mass-to-charge (m/z) ratio 196 of each specific particle produced upon sample ionisation. The particles then enter a TOF mass analyser and travel along a field-free flight path towards the detector. The time required for 197 each particle to reach the detector is precisely measured and is dependent on its m/z ratio. The 198 199 m/z ratio of each particle is determined and a mass spectrum is generated, representing both m/z and signal intensity of the detected ions. The mass spectrum generated from a bacterium 200 corresponds to high-abundance soluble proteins, predominantly ribosomal proteins and other 201

202 abundant cytosolic proteins, and is unique to a bacterium because protein composition differs between different bacterial genera and species (Andrés-Barrao et al., 2017; Bourassa & Butler-203 Wu, 2015). MALDI-TOF is comparable to 16S rRNA gene sequencing in its ability to 204 205 differentiate to species level, as phylogenetic dendrograms produced by both methods were identical except for certain outlier strains which were positioned away from their expected 206 207 taxonomic position on the tree (Andrés-Barrao et al., 2013). MALDI-TOF is not however suitable for differentiation at the strain level due to a strong effect of the growth medium used 208 on the proteomic profile of the strains (Wieme et al., 2014). However, efforts to minimise the 209 210 effect of the growth medium, with the potential to enable inter-strain discrimination, have been applied in some studies; for instance, sub-culturing of isolates under investigation multiple 211 212 times on the same, defined agar medium prior to MALDI-TOF analysis (De Roos, et al., 2018a; 213 Spitaels, Wieme, & Vandamme 2016).

214 Physiology and metabolism of AAB

• Aerobic respiration

Similar to many aerobic bacteria, AAB gain the majority of their energy by performing a type 216 of aerobic respiration (Matsushita & Matsutani, 2016). In the general process of aerobic 217 respiration, initially pyruvate is completely oxidised to carbon dioxide (CO₂) in the citric acid 218 cycle. Subsequently, the reduced electron acceptors formed in the citric acid cycle are shuttled 219 220 to the respiratory chain in the cytoplasmic membrane. Here, oxidation of the reduced electron 221 carriers by components of the respiratory chain (oxidative phosphorylation) results in the formation of water, along with exclusion of protons from the cytoplasm, producing a proton 222 223 gradient. Equalisation of this proton-motive force via transfer of protons back into the cell 224 through a transmembrane ATPase (F_1F_0 -type ATP synthase) leads to the biosynthesis of energy in the form ATP (Madigan, Martinko, Bender, Buckley, & Stahl, 2015). 225

226 The basic components of the AAB respiratory machinery consist of two periplasmic dehydrogenases: a membrane-bound proton pumping transhydrogenase, a non-proton 227 translocating NADH: ubiquinone oxidoreductase, and two terminal oxidases of the ubiquinol 228 oxidase-type. Ubiquinone (UQ) acts as the electron shuttle, in its reduced form, ubiquinol 229 (UQH₂), between these respiratory proteins. The function of the transhydrogenase and NADH: 230 ubiquinone oxidoreductase is the regeneration of NADP⁺ and NAD⁺, respectively, with the 231 concomitant exclusion of protons in the case of the transhydrogenase complex. The terminal 232 233 oxidases accept the electrons from ubiquinone, transferring them to molecular oxygen, the final electron acceptor, forming water (Figure 1). AAB have two terminal ubiquinol oxidases, 234 designated cytochrome bo₃ ubiquinol oxidase and cytochrome bd quinol oxidase. Cytochrome 235 bo3 ubiquinol oxidase catalyses a reaction which contributes to the generation of a proton-236 237 motive force while cytochrome bd quinol oxidase does not. An important function of the latter 238 terminal oxidase is believed to be the re-oxidation of ubiquinol to ubiquinone, thus rapidly regenerating ubiquinone that can contribute to further reactions in the respiratory chain or in 239 the reactions of oxidative fermentation. In addition, it has been found that, in G. oxydans, the 240 cytochrome bd quinol oxidase is particularly active at low pH (Hanke et al., 2012). Thus, it is 241 suggested that cytochrome bo_3 oxidase may serve as a major terminal oxidase at the early 242 growth phase, when the culture pH is closer to neutral, and when the pH is decreased as a result 243 244 of the production of a large amount of oxidized products, cytochrome bd quinol oxidase maintains oxidative fermentation under acidic conditions by complementing the function of 245 246 cytochrome *bo*₃ oxidase (Miura et al., 2013).

Compared to some microorganisms which obtain energy via respiration (e.g. *Escherichia coli*), 247 the energy yield, and thus biomass yield, of AAB are relatively low (Luttik, Van Spanning, 248 249 Schipper, Van Dijken, & Pronk, 1997). This can be attributed to the absence of certain key respiratory chain components in these microorganisms. For example, the genome of 250 Gluconobacter oxydans 621H lacks genes encoding cytochrome c oxidase (complex IV; 251 despite encoding genes for a cytochrome bc1 complex and for a soluble cytochrome c) and the 252 proton-translocating NADH: ubiquinone oxidoreductase (complex I; G. oxydans has a non-253 254 proton-translocating NADH: ubiquinone oxidoreductase instead) (Prust et al., 2005). Thus, G. oxydans lacks two components which would normally perform proton translocation leading to 255 256 the generation of a proton-motive force. However, not all AAB are as deficient in their 257 respiratory machinery, for example, A. pasteurianus 386B, a strain isolated from a spontaneous cocoa bean fermentation, encodes a complete proton-translocating complex I (Illeghems, De 258 Vuyst, & Weckx, 2013). 259

Therefore, due to the inadequate coupling of the electron transport with proton translocation, the proton-translocating potential and thus ability for energy transduction in *G. oxydans* is relatively limited. Thus, a low amount of energy is conserved by the microorganism, which 263 limits its growth rates, with most of the energy being lost as heat (Matsushita, Nagatani, Shinagawa, Adachi, & Ameyama, 1989). In this context, while appearing inefficient, and as 264 will be discussed further below, the presence of membrane-bound (periplasmic) 265 dehydrogenases in AAB enable rapid oxidation of substrates via "oxidative fermentation" at 266 the cell membrane level, without the need for time-consuming intracellular transport; this 267 generates the necessary proton-motive force and allows rapid energy conservation, in addition 268 to generating an unfavourable environment for competing microorganisms through the 269 production of acidic products of oxidation (Zahid, 2017). 270

271

• Oxidative fermentation

"Oxidative fermentation" is a process of incomplete oxidation of substrates which are oxidised 273 by primary dehydrogenases of a respiratory chain, with the concomitant release of oxidised 274 275 products into the surrounding medium. Bacteria capable of performing oxidative fermentation are termed "oxidative bacteria". Acetic acid bacteria are most prominent of such bacteria and 276 commonly oxidise ethanol, carbohydrates and sugar alcohols to the various corresponding 277 products such as organic acids, aldehydes and ketones (Table 4) (Matsushita & Matsutani, 278 2016). Oxidative fermentation can be considered as an "overflow metabolism", from which 279 280 lower amounts of energy are conserved compared to if the substrates were completely oxidised to CO₂ and water by aerobic respiration (Deppenmeier & Ehrenreich, 2009). Examples of 281 282 oxidative fermentation reactions include the conversion of ethanol to acetic acid (carried out by almost all genera of AAB and from where these bacteria get their name), and the conversion 283 of glucose via glucono delta-lactone to gluconic acid/gluconate (GA), amongst others. Oxygen 284 availability is of prime importance and profoundly affects the fermentation rate and 285 286 productivity (Gullo, Verzelloni, & Canonico, 2014). The high accumulation of acidic products

in their environment give AAB an advantage over competitive microorganisms (Matsutani etal., 2014).

Oxidative fermentation reactions are performed by respiratory chains in AAB that are similar 289 290 to the respiratory chains discussed above that oxidise reduced electron carriers (i.e. nicotinamide adenine dinucleotide phosphate [NAD(P)]-dependent dehydrogenases) and 291 292 reduce ubiquinone (Figure 1). That is, in the process, electrons are channelled by ubiquinone to molecular oxygen at terminal oxidases while protons are abstracted to create a proton-motive 293 force. This proton-motive force could be used by ATP synthase, or to perform some other 294 295 energetic work. However, the dehydrogenase enzymes in the respiratory chains involved in oxidative fermentations are enzymes which specifically oxidise substrates such as ethanol, 296 carbohydrates and sugar alcohols (as opposed to reduced electron carriers such as NADH). 297 298 These enzymes contain a prosthetic group and are typically either PQQ (pyrroloquinoline quinone)-dependent dehydrogenases (quinoproteins and quinoprotein-cytochrome c 299 complexes) or FAD-dependent dehydrogenases (flavoprotein-cytochrome c complexes), some 300 of which work on the same substrate but produce different oxidation products (Adachi et al., 301 2003) (Table 4). MCD (methylsuccinyl-CoA dehydrogenase)-dependent dehydrogenases 302 303 (molybdoprotein-cytochrome c complexes) have also been identified in some genera (Thurner, 304 Vela, Thöny-Meyer, Meile, & Teuber, 1997). The cytochrome subunit of these dehydrogenases 305 is responsible for the transfer of electrons to, and thus reduction of, ubiquinone to ubiquinol 306 (Matsushita, Yakushi, Toyama, Shinagawa, & Adachi, 1996). In addition, these respiratory chains are located on the periplasmic face of the cytoplasmic membrane, while those involved 307 308 in the oxidation of reduced electron carriers are located in the cytosol of the cell. The cytosolic 309 NAD(P)-dependent dehydrogenases have no role in oxidative fermentation. Indeed, it appears 310 that the cytosolic respiratory chain competes with the oxidative fermentation (periplasmic) respiratory chains regarding electron transfer and energetics and that both forms of respiration 311

occur in different growth phases (discussed further below) (Matsushita & Matsutani, 2016).
Thirty-two membrane-bound dehydrogenases have been identified in the genome of *G*. *oxydans* 621H with 11 known and 21 unknown substrate specificities (Richhardt, Luchterhand,
Bringer, Buchs, & Bott, 2013).

316

317 *Ethanol oxidation*

One of the most well-known and important oxidative fermentation reactions performed by 318 319 AAB is the production of acetic acid (vinegar) from ethanol. Both Acetobacter and Komagataeibacter species have a strong ability to produce acetic acid and both genera also 320 321 show high resistance to high ethanol and acetic acid levels, which are important traits for 322 industrial vinegar production (Taban & Saichana, 2017). Ethanol oxidation is catalysed by two membrane-bound enzymes located on the outer surface of the cytoplasmic membrane 323 (periplasmic side). Ethanol is first oxidized to acetaldehyde by a POO-dependent alcohol 324 dehydrogenase (ADH) and acetaldehyde is further oxidized to acetic acid by aldehyde 325 dehydrogenase (ALDH). The prosthetic group of ALDH has been shown to be different 326 327 between genera, being either PQQ- or MCD-dependent (Gómez-Manzo et al., 2010; Thurner et al., 1997). ADH is stable over a broad pH range of 2.3 to 8.0 and retained more than 90% 328 activity when incubated on ice for 30 minutes. ALDH, while also stable at acidic pHs (optimum 329 pH 4 - 5, but can also function at lower pH values), is more heat stable than ADH, retaining 330 more than 50% activity after 30 min at 60°C (Kanchanarach et al., 2010a). However, ALDH is 331 sensitive to the level of oxygen present; when this is either too low or too high its activity falls, 332 333 allowing acetaldehyde to accumulate in the medium (Mamlouk & Gullo, 2013; Rubio-Fernandez, Desamparados Salvador, & Fregapane, 2004). 334

15

335 The ADH activity in Acetobacter species has been found to be more stable under acid conditions than that of *Gluconobacter* species, which may partly explain why acetobacters are 336 more proficient in acetic acid production than gluconobacters and gluconacetobacters 337 338 (Matsushita, Toyama, & Adachi, 1994). In addition, when G. oxydans is grown in media supplemented with a fixed amount of ethanol at the beginning, the levels of acetic acid 339 produced by the strain are lower when glucose is present as a carbon source and GA is therefore 340 341 produced; that is, acetic acid is still produced by G. oxydans, albeit at lower levels if glucose is available for GA production (authors own observations). 342

343 Inactive forms of ADH have been identified from strains of Gluconobacter and Gluconacetobacter. Ethanol-oxidation-deficient strains of Gluconobacter have a single 344 nucleotide polymorphism that results in a truncated signal peptide and therefore incorrect 345 346 localisation of the protein in the cytoplasmic membrane. When these gluconobacters are grown in acidic or high aeration conditions, they produce a large amount of ADH protein, but ADH 347 activity remains unchanged, suggesting the presence of an inactive protein. Such inactive ADH 348 displays a tenth of the activity of the active form (Gomez-Manzo et al., 2012; Matsushita, 349 Yakushi, Takaki, Toyama, & Adachi, 1995). Certain cultivation conditions such as low pH 350 351 and/or high aeration also reduce ADH activity, such as in *Gluconobacter suboxydans*, where low aeration was shown to favour active over inactive ADH formation (Matsushita et al., 1995). 352

ADH displays a wide specificity for short-chain alcohols, except methanol. Glycerol can be a substrate, yielding glyceraldehyde, but only under high concentrations does ADH oxidise glycerol at a significant rate. Aldehydes can also be oxidised by ADH, and in some AAB, at a similar rate to alcohols. This has led to the suggestion that ADH alone (and not in concert with ALDH) can perform the acetic acid fermentation, which has been shown for the strain *Ga. diazotrophicus* Pal5 (Gomez-Manzo et al., 2015). 359 When ethanol has been completely oxidised and depleted, some genera of AAB, namely Acetobacter, Gluconacetobacter and Komagataeibacter, can assimilate acetic acid and oxidise 360 it completely to CO₂ and water using the citric acid cycle and glyoxylate shunt, which is known 361 as acetate "overoxidation" (Sievers & Swings, 2005). There also appears to be an irreversible 362 metabolic change, after which they are unable to oxidize ethanol again; this is evidently 363 unfavourable in vinegar production as it leads to lower acetic acid yields (Raspor & Goranovic, 364 365 2008). Gluconobacter and some other AAB genera do not overoxidise acetate as they lack key enzymes in the citric acid cycle and glyoxylate shunt (Deppenmeier & Ehrenreich, 2009; 366 367 Mamlouk & Gullo, 2013). Because of this difference in oxidative potential, gluconobacters are sometimes referred to as "under-oxidisers" and acetobacters (and gluconacetobacters) as "over-368 oxidisers" (Bartowsky & Henschke, 2008). Overoxidation can be avoided if a small proportion 369 370 of ethanol is maintained in the medium (Raspor & Goranovic, 2008).

371

372 Oxidation of carbohydrates and carbon metabolism in Gluconobacter oxydans

Catalysed by various periplasmic dehydrogenases involved in oxidative fermentation, AAB 373 374 oxidise a number of carbohydrates to their corresponding carboxylic acids, obtaining metabolic energy in the process through the ultimate generation of a proton-motive force, as described 375 above. As mentioned, while Acetobacter and Komagataeibacter are efficient at ethanol 376 oxidation, *Gluconobacter* species are particularly proficient in carbohydrate and sugar alcohol 377 oxidation (Matsushita & Matsutani, 2016). The production of GA and associated 378 ketogluconates from glucose by *Gluconobacter oxydans* will be described below to illustrate 379 380 carbohydrate oxidation in AAB, followed by a description of carbon metabolism in G. oxydans. Gluconic acid and its salts has wide application in various industries such as the food, 381 construction, textile and pharmaceutical sectors (discussed further in the section 382

"Biotechnological applications of *Gluconobacter oxydans* relevant to the food industry") and *G. oxydans* represents an alternative to the current use of the fungus *Aspergillus niger* as a
source of GA (García-García et al., 2017).

Acetic acid bacteria oxidise D-glucose firstly to D-glucono- δ -lactone by PQQ-dependent 386 glucose dehydrogenase (PQQ-GDH) and then to D-gluconate, either spontaneously or via a 387 gluconolactonase located in the cytoplasmic membrane (Raspor & Goranovic, 2008). D-388 gluconate can be further converted to ketogluconates by other periplasmic dehydrogenases. It 389 390 can be oxidised further to either 2-keto-D-gluconate (2KGA) by an FAD-containing gluconate (FAD-GADH) or 5-keto-D-gluconate (5KGA) by PQQ-glycerol 391 dehydrogenase dehydrogenase (PQQ-GLDH). In some AAB strains, 2KGA is later converted to 2,5-diketo-392 gluconate (2,5-diKGA) by FAD-containing 2KGA dehydrogenase (FAD-2KGADH) 393 (Shinagawa, Ano, Yakushi, Adachi, & Matsushita, 2009; Toyama et al., 2007). Conversion of 394 395 GA to its associated ketogluconates is both strain- and growth condition-dependent. Factors such as glucose concentration, pH and the level of oxygenation effect the yield and ratio of GA 396 397 to ketogluconates (García-García et al., 2017; Saichana et al., 2015). It is clear that the further 398 oxidation of GA to ketogluconates is undesirable if the production of D-gluconate is to be maximised (Mamlouk & Gullo, 2013); this will be discussed further in the section 399 "Biotechnological applications of Gluconobacter oxydans relevant to the food industry". 400

When AAB are grown on alcohols, carbohydrates or sugar alcohols two growth phases (biphasic growth, phase I and II) can be observed (Malimas et al., 2017). In both phases, the majority of energy is obtained from respiratory chains and the generation of a proton-motive force; however, in the second growth phase some of the energy is obtained through assimilation and metabolic catabolism of the oxidised products produced in the first growth phase. In the case of glucose, for example, the early, logarithmic growth phase results from the energy derived from the oxidative fermentation of glucose, which produces gluconate and a small 408 amount of 2KGA, as described above. In the process, a proton-motive force is generated which drives ATP biosynthesis. When glucose is used up, the cells enter a stationary phase. However, 409 a second growth phase is subsequently observed, which is primarily due to the further oxidation 410 of gluconate to 2KGA. In both phases I and II a small proportion of gluconate is assimilated 411 by the cells and is subsequently catabolised intracellularly in the reactions of primarily the 412 Pentose Phosphate pathway (PPP), but the Entner Doudoroff pathway (EDP) and the citric acid 413 414 cycle also have a role (Prust et al., 2005). These reactions yield additional energy primarily via the generation of reduced electron acceptors (e.g. NADH) which are subsequently oxidised by 415 416 the cytosolic respiratory chains (NAD(P)-dependent dehydrogenases), thus generating a proton-motive force. Actually, only a small proportion of the glucose (phase I) and gluconate 417 (phase II) are assimilated and catabolised intracellularly, the majority of the energy being 418 419 yielded by oxidative fermentation reactions (Bringer & Bott, 2016). In this way, the cytosolic 420 NAD(P)-dependent respiratory chains primarily have a role in the later phase of growth.

Catabolism of GA and associated ketogluconate products is possible only via the PPP and EDP
because AAB do not have the enzyme, phosphofructokinase, and thus glycolysis is not active.
In addition, while most AAB genera can completely oxidise substrates to CO₂ and H₂O in the
citric acid cycle, in *Gluconobacter* this cycle is incomplete as they lack the enzymes succinylCoA synthetase and succinate dehydrogenase (Bringer & Bott, 2016).

While the metabolism of intracellular, assimilated glucose and gluconate, primarily via 6phosphogluconate, may involve the PPP, EDP and citric acid cycle, the majority of energy gained from intracellular metabolism is derived from the oxidative PPP, whereas the EDP is dispensable. Carbon labelling of glucose has demonstrated the carbon flux during cellular metabolism of glucose in *G. oxydans*, showing that the majority of energy is derived from the PPP which operates in a cycle (Hanke et al., 2013). The key enzymes in the cycle are glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase; deletion of 6phosphogluconate dehydrogenase was observed to severely limit the growth of *G. oxydans*. In
contrast, deletion of an integral enzyme in the EDP (2-keto-3-deoxy-6-phosphogluconate
aldolase) had little effect on strain growth (Bringer & Bott, 2016; Hanke et al., 2013).

The initial generation of oxidised products of carbohydrates and sugar alcohols allows AAB to rapidly deplete the availability of carbon sources for competing microorganisms, while also producing an inhibitory environment for such competitors due to the resulting low pH generated by the accumulation of the acidic products; in addition, the potential for these products to be assimilated and used partly as an energy source by AAB provides a further competitive advantage (García-García et al., 2017).

- 442
- Tolerance to acidic environments

Acetic acid is an effective antimicrobial compound, yet most species of AAB are able to 444 445 produce and tolerate high concentrations, from 6 - 10% (v/v) and some up to 15 - 20% (v/v) (Emde, 2006; Schüller, Hertel, & Hammes, 2000). Halstead et al. (2015) found that acetic acid 446 at concentrations from 5% to as low as 0.3% were capable of preventing growth of both 447 planktonic cells and biofilms formed by a range of pathogenic microorganisms that typically 448 affect burns patients, including Staphylococcus aureus, Escherichia coli, Pseudomonas 449 450 aeruginosa and Acinetobacter baumannii. Gram-negative isolates were generally found to be more susceptible than Gram-positive strains (Halstead et al., 2015). This antibacterial efficacy 451 raises the question as to how AAB can tolerate such high acetic acid levels. Different genera 452 453 of AAB display varying tolerances to acetic acid; species of Acetobacter such as A. aceti and A. pasteurianus tolerate acidities of about 6 - 10%, while Komagataeibacter are generally more 454 acid tolerant. Strains of K. xylinus and K. hansenii, for example, show resistance to 10 - 15% 455 456 acetic acid, while strains of K. europaeus and K. oboediens, have been isolated from submerged vinegar fermentation processes with acidities as high as 15 - 20% (Andrés-Barrao & Barja,
2017; Emde, 2006).

Acetic acid is a particularly effective antimicrobial, because at a relatively high pH (pH 4.7 = 459 pKa of acetic acid) it exists primarily in its undissociated form and can enter the cell 460 (Hirshfield, Terzulli, & O'Byrne, 2003). The properties of undissociated organic acids such as 461 462 fat-solubility and neutral charge enable them to passively diffuse through the cell membrane of the target microorganism; in the cytoplasm the higher intracellular pH causes the acid to 463 become dissociated, producing primarily hydrogen ions (H⁺), but also acetate ions (CH₃COO⁻ 464). These ions are toxic to the cell and interfere with cellular processes such as enzyme activity, 465 DNA replication and transcription, and protein expression, therefore effecting the normal 466 growth of the microorganism (Chen, Chen, Giudici, & Chen, 2016; Russell & Diez-Gonzalez, 467 468 1997).

AAB employ a number of strategies to resist the detrimental effects of acetic acid, which will be discussed briefly here. Readers are directed to Andrés-Barrao & Barja (2017) for a more indepth appraisal of the topic. The mechanisms which AAB use to tolerate high concentrations of acetic acid can be broadly classified as follows, 1) adaption of and protection of intracellular proteins to and against acid stress, 2) metabolism of acetic acid which enters the cell (overoxidation), 3) acetic acid efflux from the cell and 4) prevention of acetic acid from entering the cell (Figure 3).

476 Many intracellular proteins of AAB have adapted to tolerate a low cytoplasmic pH. For 477 example, certain enzymes in particularly acid tolerant AAB (e.g. *A. pasteurianus*) have 478 structural modifications, such as a higher number of inter-subunit hydrogen bonds and an 479 increased number of arginine-containing salt bridges that not only confer stability in the acidic 480 cytoplasm of AAB, but may also contribute to thermotolerance (Settembre, Chittuluru, Mill,

21

481 Kappock, & Ealick, 2004). The heat-shock systems, GroESL and DnaJK, are also important in acetic acid resistance. These chaperones and chaperonins are general stress proteins that protect 482 other proteins from denaturation and aggregation caused by heat, but also other environmental 483 484 stresses such as oxidative, acid, salt, and starvation stresses (Andrés-Barrao & Barja, 2017). Under both [sudden] acid-shock conditions, induced through the addition of 3% acetic acid to 485 a growing culture, and more continuous stress conditions in the presence of high ethanol and 486 487 increasing acetic acid levels (e.g. conditions which occur during vinegar fermentation), increased protein expression of the chaperone system consisting of GroESL, DnaKJ and GrpE 488 489 occurs (Andrés-Barrao et al., 2012; Matsushita et al., 1994). In addition, disruption of RpoH, an important RNA polymerase sigma factor in the regulation of the heat-shock response 490 proteins such as GroEL, DnaK, DnaJ, resulted in reduced expression of concomitant genes and 491 492 decreased resistance to ethanol (5%) and acetic acid (1%) stress conditions in a strain of A. pasteurianus (Okamoto-Kainuma et al., 2011). 493

As described above, when ethanol has been completely oxidised and depleted, some genera of 494 AAB assimilate acetic acid and oxidise it completely to CO₂ and water using the citric acid 495 cycle and glyoxylate shunt (acetate "overoxidation") (Sievers & Swings, 2005). Thus, 496 497 overoxidation is a mechanism by which the intracellular acetic acid level can be decreased, 498 whilst also gleaning energy from the process through the oxidative reactions of the citric acid 499 cycle. In addition, acetic acid may be assimilated from the environment causing the pH to rise. 500 In both cases, the effect is essentially a detoxification of acetic acid and reduced acid stress on the bacterium. A fully functioning citric acid cycle is necessary for acetic acid overoxidation, 501 which has been found to be the case for the most acid tolerant AAB such as Acetobacter and 502 503 Komagataeibacter; other genera such as Gluconobacter do not possess a complete citric acid cycle which maybe an important factor in explaining their comparatively lower tolerance to 504 acetic acid (Mamlouk & Gullo, 2013; Prust et al., 2005). In addition, this lower acid tolerance 505

506 may be linked to the reduced acetic acid productivity of *Gluconobacter* strains compared to those of Acetobacter and Komagataeibacter, which are commonly used in industrial vinegar 507 production. In those AAB capable of overoxidation, a number of genes involved in the citric 508 509 acid cycle and glyoxylate shunt are up-regulated during acetic acid fermentation, primarily at the end of ethanol oxidation. These include *aarA* (encoding for a citrate synthase), *aarC* 510 (encoding for a succinyl CoA:acetate CoA-transferase) and *aconitase* (isomerises citrate) 511 512 (Sakurai, Arai, Ishii, & Igarashi, 2012). It is noteworthy that during vinegar fermentation, it is desirable to maintain a low level of residual ethanol, to avoid the overoxidation of acetic acid 513 514 which would lead to productivity losses. Therefore, in this case, acetic acid cannot be detoxified by this mechanism and other strategies must be employed to maintain acetic acid tolerance 515 (Andrés-Barrao & Barja, 2017). 516

517 The export of intracellular acetate is another strategy employed by AAB to tolerate acetic acid. Two types of export systems have been identified, an efflux pump driven by a proton-motive 518 force (anti-port of H⁺ ions) and an ATP-binding cassette (ABC) transporter (Matsushita, Inoue, 519 Adachi, & Toyama, 2005; Nakano, Fukaya, & Horinouchi, 2006). This ABC transporter has 520 been found in Acetobacter and Komagataeibacter species and confers resistance to acetic acid, 521 522 in addition to other short-chain organic acids such as formic acid, propionic acid and lactic acid (Nakano & Fukaya, 2008). Export of acetic acid may play a crucial role in cell survival under 523 524 the high acidity conditions prevailing during the industrial production of vinegar, because, as 525 mentioned above, under these conditions assimilation of acetate via the citric acid cycle is prevented in order to maintain acetic acid productivity. 526

527 Another strategy used by AAB to resist acid stress is the exclusion of acetic acid and its 528 prevention from entering the cell. To this end, certain species, for example those of the genus 529 *Komagataeibacter*, are reported to have a higher content of phosphatidylcholine (PC) in their 530 lipid membranes (Nakano & Ebisuya, 2016). In addition, during acetic acid fermentation, as 531 the acid content began to rise, the ratio of PC was observed to increase and that of phosphatidylglycerol decreased, suggesting the importance of PC in acetic acid tolerance 532 (Higashide et al., 1996). The cell membranes of Komagataeibacter have also been found to 533 534 possess a high content of hopanoids, especially tetrahydoxybacteriohopane (THBH), which may have a role in acetic acid, but also ethanol, tolerance (Nakano & Ebisuya, 2016). 535 Carbohydrate polymers attached to the outer membrane may also play a role in protecting 536 against acetic acid ingress into the cell. The intracellular content of acetate was found to be 537 significantly higher in a strain of A. *pasteurianus* displaying a non-polysaccharide-producing 538 539 phenotype, compared to a polysaccharide-producing strain (Kanchanarach et al., 2010b).

540 Finally, it is noteworthy that there appears to be a link between acetic acid resistance and the yield or productivity during acetic acid fermentation. During investigations into the 541 542 mechanisms of acetic acid resistance, strains which were modified to overexpress certain genes involved in acetic acid resistance, namely, aconitase, an enzyme in the citric acid cycle 543 (Nakano, Fukaya, & Horinouchi, 2004) or AatA, the putative ABC transporter involved in 544 acetic acid export (Nakano et al., 2006), not only displayed increased resistance to acetic acid 545 but also produced higher acetic acid yields. Thus, the higher acetic acid productivity of 546 547 Komagataeibacter and Acetobacter species may partly be a consequence of their natural intrinsic tolerance to acetic acid, when compared to species such as *Gluconobacter*, which in 548 549 general, display lower acetic acid productivity.

550 Cellulose and other exopolysaccharides produced by AAB

Cellulose is the most abundant natural polymer on the planet, with high economic value. Plants 551 (e.g. wood and cotton) and ocean phytoplankton and algae are nature's largest source of 552 cellulose (Tonouchi, 2016). Certain bacteria, most notably AAB of the genus 553 Komagataeibacter (formerly Gluconacetobacter), are capable of producing large amounts of 554 555 cellulose (Lin et al., 2013). The chemical structure of plant and bacterial cellulose (BC) is identical, that is, composed of β -1,4-linked glucopyranosyl residues; however, the structure of 556 557 BC is unique as it is composed of ultrafine fibres that form a network of pure cellulose (Tonouchi, 2016). BC can also be known as bacterial nanocellulose (Klemm et al., 2011). BC 558 is a linear, unbranched, water-insoluble homoexopolysaccharide of exceptionally high purity 559 and containing no contaminating substances, in contrast to plant cellulose which contains lignin 560 and hemicellulose (arabinoxylan), which can be tightly bound and complexed with cellulose. 561 562 Therefore, high amounts of energy are required for the purification of plant cellulose, which is not the case for BC. In addition, BC exhibits high crystallinity, water absorption, tensile 563 564 strength and mechanical strength (Mohite & Patil, 2014a). These properties make it a 565 favourable alternative to plant cellulose.

Several species of AAB within the genera *Acetobacter*, *Gluconacetobacter*, *Gluconobacter* and *Komagataeibacter* produce cellulose. However, only *Komagataeibacter* produce it at commercial levels, with *K. xylinus* and *K. hansenii* being most notable (Lin et al., 2013). BCproducing strains have been isolated from a number of environments, including fruits, flowers, fermented foods (e.g. vinegar) and beverages.

571 The ecological benefit of cellulose production for AAB is not fully clear. It is commonly 572 understood that its production forms a scaffold which floats on the liquid surface, as seen 573 particularly in static cultures, allowing the bacteria access to both nutrients in the medium and 574 sufficiently high levels of oxygen in the air, required for their oxidative metabolism (Tonouchi, 2016). As described above, EPS production may contribute to acetic acid tolerance. In addition, 575 in natural environments such as rotting fruit, cellulose production may protect the bacteria from 576 predation or from the damaging effects of UV light or dissection through moisture loss. 577 Cellulose production appears to be variable in G. oxydans, with some strains capable of 578 producing it having been identified previously (Jia et al., 2004; Valera, Torija, Mas, & Mateo, 579 580 2015). In a screening of 77 different AAB strains, representing 35 different species, Valera et al. (2015) found that of the three G. oxydans strains examined, two produced cellulose on all 581 582 the six different media used to investigate its production. Both of these strains were isolates from beer (Valera et al., 2015). However, yields of cellulose from G. oxydans are much lower 583 than those of Acetobacter xylinum (presently K. xylinus) (Jia et al., 2004). This could be due to 584 585 the fact that in AAB, both cellulose production and glucose oxidation (to GA and 586 ketogluconates) are in competition for the same glucose as substrate. Thus, in G. oxydans, which has a particularly high oxidative capacity for carbohydrates, the majority of glucose may 587 be oxidised to GA, resulting in low availability for cellulose production. In contrast, K. xylinus 588 may utilise the majority of glucose for cellulose production (De Muynck et al., 2007). Indeed, 589 590 Keshk and Sameshima (2006) demonstrated that productivity of BC from Ga. xylinus (now K. *xylinus*) increased significantly in the presence of lignosulfonate, an antioxidant compound that 591 592 inhibited the formation of GA. In its presence, one strain of Ga. xylinus produced up to 16 g/L 593 BC (Keshk & Sameshima, 2006). A similar effect was also observed in the presence of Vitamin C, which is also an antioxidant compound (Keshk, 2014). 594

BC is synthesised from a variety of carbohydrates and organic acids by AAB (Serrato et al., 2013). The cellulose biosynthesis pathway can be considered as an off-shoot of the PPP. Glucose is the polymeric unit that cellulose is composed of, therefore, other substrates must be converted to glucose-6-phosphate, a key intermediate, through various intracellular metabolic 599 reactions. Cellulose biosynthesis is comparable to that of heteropolysaccharides in other bacteria, such as LAB, the building block of which are sugar nucleotides (Lynch, Zannini, 600 Coffey, & Arendt, 2018a). In the cellulose biosynthesis pathway, glucose-6-phosphate is 601 602 converted to glucose-1-phosphate, which is subsequently metabolised to uridine diphosphoglucose (UDP-glucose), the direct precursor of cellulose. Uridine diphosphoglucose 603 biosynthesis is controlled by phosphoglucomutase and UDPG-pyrophosphorylase. Following 604 605 UDP-glucose formation, glucose polymerisation to produce cellulose is mediated by cellulose synthase, a complex of proteins spanning the periplasmic space between the cytoplasmic and 606 607 outer membranes. The cellulose synthase complex facilitates the polymerisation of UDPglucose, translocation of the polymer across the membranes and assembly of the glucan chains 608 609 extracellularly (Tonouchi, 2016). The protein subunits that constitute the cellulose synthase 610 complex are encoded in an operon (the *bcs* operon), together with accessory proteins that assist cellulose biosynthesis. Following polymerisation of the individual β -1,4-glucan chains, these 611 are brought together in a hierarchical manner to form the higher-order cellulose ribbon. In this 612 way, several glucan chains form a ~1.5 nm wide protofibril, protofibrils are arranged into ~2-613 614 4 nm microfibrils; finally, microfibrils are bundled together forming a ~20-100 nm cellulose ribbon (Jozala et al., 2016). In general, a single ribbon or fibre is produced by a bacterial cell, 615 from BC synthase complexes (sometimes termed terminal complexes) that are arranged on one 616 617 side of the cell only (Kimura, Chen, Saxena, Brown, & Itoh, 2001). This ribbon is ultrafine, being about one thousandth the width of a plant cellulose fibre (Tonouchi, 2016). As mentioned 618 above, carbon sources other than glucose can be used for BC biosynthesis, however, besides 619 mannitol and arabitol, all other sources result in lower BC productivity than on glucose 620 (Khajavi, Esfahani, & Sattari, 2011). 621

Aside from the carbon source, the nitrogen source and level of other micronutrients influencethe growth of microorganisms as well as the productivity of BC (Dağbağlı & Göksungur,

2017). A number of agro-industrial waste by-products have been investigated for use in BC
production, particularly those high in sucrose, fructose, nitrogen and vitamins (Castro et al.,
2011).

Apart from the substrate material affecting yield, the method of production (e.g. static vs. 627 stirred culture) can influence the structure and fibre network arrangement of the BC produced, 628 629 thus having an effect on its physical and mechanical properties. Under static conditions, bacterial cellulose is produced in a gelatinous form. However, under agitation, shear stress 630 seems to inhibit gel formation, resulting in the production of small aggregates, or in some cases, 631 distinctive spheres of BC (Mohite & Patil, 2014b). In addition, in agitated cultures the fibrous 632 network of BC is more disordered due to the physical effects of agitation (Watanabe, Tabuchi, 633 Morinaga, & Yoshinaga, 1998). A negative effect of agitation is that it can induce mutations 634 635 in certain strains, resulting in decreased BC production (Krystynowicz et al., 2002). Therefore, both the nature of the substrate material (carbon, nitrogen, micronutrient composition and 636 concentration) and the method of production (static or stirred culture, type of bioreactor) are 637 factors which contribute to the productivity and yield of BC. 638

Due to physicochemical properties such as structure-forming potential, high purity and bio-639 compatibility, BC has numerous potential applications, for example in the medical (e.g. as an 640 artificial skin) and food industries (Tonouchi, 2016). Due to the scope of this review, only those 641 applications relevant to the food industry will be described briefly below. Many current 642 applications continue to employ plant-derived cellulose and it is used in food as a thickening, 643 gelling, stabilising or water-binding agent (Shi, Zhang, Phillips, & Yang, 2014). For example, 644 cellulose and its derivatives (known in the food industry as cellulosics) such as 645 carboxymethylcellulose (CMC) and hydroxypropylmethylcellulose (HPMC) are important in 646 the ice-cream and bakery industries, being used as a stabilising agent and a texture-modifying 647 hydrocolloid, respectively (Murray, 2009). In particular, in gluten-free bread making, the use 648

649 of HPMC can increase moisture retention, increase loaf volume and decrease crumb hardness and the rate of staling (Mir, Shah, Naik, & Zargar, 2016). BC has functionality in areas where 650 plant cellulose is limited. This is particularly attributed to the high purity, crystalline structure 651 and high water-binding capacity of BC. Thus, it is recommended where low use levels, foam 652 stabilisation, and stability over a wide range of pH, temperature and freeze-thaw conditions are 653 desirable. Such products in which it has potential use include pourable and spoonable dressings, 654 655 whipped toppings and aerated desserts, fermented and frozen dairy products, and sauces (Park & Khan, 2009). As a raw material, BC is a distinct constituent of Nata and kombucha tea. Nata 656 657 is a BC gel, consumed as a desert in the Philippines, but becoming more popular worldwide. It is produced by fermenting coconut water (Nata de Coco) or pineapple juice (Nata de Piña) as 658 a substrate with cellulose-producing AAB. The cellulose gel produced is cut into pieces and 659 660 consumed, having the flavour of the original substrate (Shi et al., 2014). Kombucha tea is 661 produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY) or "tea fungus", of which cellulose-producing AAB constitute important members and produce 662 a characteristic cellulose pellicle on the surface of the medium (De Roos & De Vuyst, 2018a). 663 This pellicle harbours the community of bacteria and yeast which ferment the tea, producing a 664 mildly acidic beverage. Kombucha tea is discussed further below. 665

666

667 Acetan

Acetan is formed by cellulose-producing AAB and is composed of a cellulose backbone, substituted on alternate glucose residues with a charged pentasaccharide side chain. This side chain is composed of D-glucose, D-mannose, L-rhamnose and D-glucuronic acid (Dağbağlı & Göksungur, 2017). Acetan biosynthesis is similar to that of heteropolysaccharides in other bacteria, consisting of formation of a lipid-linked oligosaccharide intracellularly followed by polymerisation in the periplasmic space and export and release extracellularly (Tonouchi,
2016). Production of acetan appears to affect cellulose biosynthesis as mutant acetan-nonproducing strains produce a cellulose that differs in its structure and crystallinity (Watanabe et
al., 1998). Acetan has reportedly been widely used in various industries as a viscosifier and
emulsifier; however, it is not listed in the current European regulation on food additives
(European Commission, 2008; Ishida, Sugano, & Shoda, 2002).

679

680 *Levan*

Levan is a homoexopolysaccharide composed of fructose units (i.e. a fructan) linked via β -2,6 681 glycosidic bonds. It is one of two types of fructans, the other being inulin (a β -2,1 linked 682 fructan) (Monsan et al., 2001). Among the AAB, strains from the genera Gluconobacter, 683 Komagataeibacter, Kozakia and Neoasaia are known to produce levan (Dağbağlı & 684 Göksungur, 2017; Jakob et al., 2013). Levan is biosynthesised by an extracellular enzyme, 685 known as levansucrase (a fructosyltransferase) secreted by the bacterium and using sucrose as 686 a substrate (Srikanth, Reddy, Siddartha, Ramaiah, & Uppuluri, 2015). Jakob et al. (2012) 687 screened 21 strains of AAB, representing 5 different genera for their ability to produce EPS on 688 sucrose-based media. Of the strains investigated, only those in the genera Gluconobacter, 689 Neoasaia, and Kozakia were shown to produce EPS of the levan type. A yield of 12 g/L was 690 obtained from a strain of *Gluconobacter frateurii* (Jakob, Steger, & Vogel, 2012). Levan, due 691 to its hydrocolloid properties, has potential applications in the food industry, for example, as 692 an emulsifier, stabiliser, texture modifier or fat substitute. EPSs, including levan, have 693 particularly been exploited in the bakery industry, and, through their application with 694 sourdough technology, represent a natural alternative to commercial hydrocolloids for the 695 enhancement of both conventional wheat-containing baked goods and gluten-free products 696

697 (Lynch, Coffey, & Arendt, 2018b). To date, EPS-producing strains of LAB have mainly been investigated for such application, but, in recent years AAB have become of increasing interest 698 in this area (Jakob et al., 2012). Application by Jakob et al. (2012) of purified levan (1 - 2% 699 700 w/w) from AAB to a wheat bread system increased the bread volume, softened the crumb and retarded staling (Jakob et al., 2012). Furthermore, in sucrose-supplemented spelt dough, a strain 701 702 of Kozakia baliensis has been shown to produce up to 49 g/kg (of flour) EPS, followed by 33 703 g/kg in whole wheat, and 32–36 g/kg in wheat and rye doughs (Hermann, Petermeier, & Vogel, 2015). Ua-Arak et al. (2016) also demonstrated their potential for in-situ application and ability 704 705 to grow and produce EPS in a gluten-free (buckwheat) sourdough system, with levan productivity of between 16 and 20 g/kg, in molasses-supplemented flour (Ua-Arak, Jakob, & 706 707 Vogel, 2016). In a later study, the application of this sourdough improved the bread sensory 708 and quality parameters, which included increasing specific volume and increasing crumb 709 softness. However, the positive effects of sourdough application were masked by the impact of the natural acidification during fermentation. Thus, while the ability of AAB to produce a large 710 711 quantity of high molecular mass levan is attractive and shows potential in bakery applications, a challenge remains due to the necessity for high levels of oxygen for growth during in-situ 712 sourdough fermentation, coupled with the strong acidification (Ua-Arak, Jakob, & Vogel, 713 2017). 714

715

716 Dextran

⁷¹⁷ Dextran is a homoexopolysaccharide composed of glucose units (i.e. a glucan) linked via α -⁷¹⁸ 1,6 glycosidic bonds (at least 50%) and variable amounts of α -1,4, α -1,3 and α -1,2 linkages, ⁷¹⁹ with or without branching. Dextrans are principally produced by species of LAB such as ⁷²⁰ *Leuconostoc*, which use sucrose as a substrate (Monsan et al., 2001). The enzyme which 721 mediates the production of dextran in LAB is known as a dextransucrase (Lynch et al. 2018a). Many strains of G. oxydans produce dextran using a similar enzyme, known as dextran 722 723 dextrinase. However, instead of sucrose, dextran dextrinase uses maltodextrins (α -1,4 glucan) as the substrate for dextran formation. Dextran cannot be produced from un-hydrolysed starch 724 however. This has led to the suggestion that dextran dextrinase can act on the non-reducing 725 ends of linear α -1,4 chain structures, but not on structures close to branch points in starch 726 727 (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). G. oxydans appears to produce both an intracellullar and extracellullular dextran dextrinase, the extracellular form of which is 728 particularly high in the presence of hydrolysed starch and maltodextrins. However, it was 729 previously unclear whether these two forms of the enzyme were in fact the same enzyme, and 730 whether the strain is stimulated to simply secrete the intracellular dextran dextrinase when 731 732 hydrolysed starch and maltodextrins are present in the environment (Naessens et al., 2005). Recently, both forms of the enzyme were indeed shown to be identical (Sadahiro, Mori, Saburi, 733 Okuyama, & Kimura, 2015). Compared to commercial dextran of a similar molecular mass 734 735 produced by Leuconostoc mesenteroides, dextran produced by G. oxydans has a higher degree of branching and displays lower viscosity. Therefore, the latter may be more suitable for food 736 applications not requiring a thickening functionality such as use as a dietary fibre or as a low-737 738 calorie bulking agent (Naessens et al., 2005).

739 Vinegar fermentation

740 *Legal definition of vinegar*

The definition of vinegar itself varies from country to country and production is regulated by different laws (Table 5). "Vinegar" primarily is defined as a liquid product produced by the fermentation of carbohydrate sources and must contain a minimum of 3.75 to 5% (w/v) acetic acid. Based on the different laws on vinegar, the unifying parameters for its classification are the acidity, level of residual ethanol and their ratio to one another.

746

747 *History of vinegar*

The history of vinegar production, which dates back more than 10,000 years (Johnston & Gaas, 748 2006) represents a keystone example of microbial biotransformation. Vinegar, from the French 749 word, vinaigre, meaning "sour wine" that in turn came from the Latin, vinum acetum, "wine 750 751 vinegar" has always been considered the "poor relation" among fermented foods: it is not 752 considered a "food", nor does it have high nutritional value, and it is made through the biotransformation of richer, more nutritive products. Vinegar can be produced using almost 753 754 anything that is a source of fermentable carbohydrate, including wine, molasses, beer, various fruits, honey or whey (Johnston & Gaas, 2006). Hippocrates of Kos (460-377 BC), father of 755 756 modern medicine, recommended vinegar as a treatment for a number of diseases including the common cold and cough (Food: A Culinary History, 2000) and for the treatment of sores 757 (Johnston & Gaas, 2006). The great military leader, Hannibal of Carthage (c. 200 BC) 758 759 reportedly used vinegar to dissolve rocks that blocked his army's path over the Alps. Pliny the 760 Elder (AD 23-79), recounts that the Egyptian Queen, Cleopatra (c. 50 BC) made a bet with the 761 Roman general Mark Anthony that she could host the most expensive banquet costing ten 762 million sesterces. He laughed at her, but during the meal, for the desert, she had a bowl of 763 strong vinegar brought in and dissolved a pearl of inestimable value in it, and then drank the 764 resulting potion (Mazza & Murooka, 2009). In the 8th century, Samurai warriors of Japan used vinegar as a tonic, believing that it gave them power and strength (Ho et al., 2017). The study 765 766 of its medical properties and its use was widespread in the Middle Ages, becoming a common remedy. During the Great Plague in the fourteenth century in France, vinegar was used to offer 767 protection against the bubonic plague. In England, to prevent the spread of plague during the 768 769 1660s money was deposited in troughs of vinegar in order to disinfect the coins (Solieri & 770 Giudici, 2009). A chronicle recounts that a concoction made from vinegar, garlic, mint and 771 other herbs, supposedly invented by thieves, allowed them to rob the houses of plague victims without contracting the disease themselves. Fittingly, the mixture was called "Four Thieves 772 773 Vinegar" and is still manufactured today, mainly in France (Mazza & Murooka, 2009). 774 Through the centuries, from the beginnings of agriculture until today, vinegar has been 775 employed by every culture in some form; as a condiment, as a pickling or preserving agent, as a disinfectant and cleansing agent and as a beverage. 776

777

778 Fermentative processes in vinegar production

Following the international definition of vinegar, in this review only vinegars derived from 779 alcoholic and subsequent acetous fermentation processes of agriculturally-produced raw 780 781 materials are considered. In Table 6 a list of vinegars is presented, but cannot be considered exhaustive because of the countless varieties produced worldwide. The diversity of raw 782 materials used in the production of vinegar is very broad, ranging from by-products and 783 784 agricultural surpluses to high quality substrates for the most prized vinegars, such as Aceto Balsamico Tradizionale (Italy) and Vinagre de Jerez (Spain). In Mediterranean countries, wine 785 vinegar is unquestionably the most common type of vinegar. In general, fruit vinegars are 786

common in Europe while cereal vinegars are more common in China and Japan (Lu et al.,
2017). However, worldwide, most of the vinegar produced is "white" vinegar, made from
diluted alcohol (Table 6).

790 Even though the raw materials and end products of vinegar fermentation are diverse, the actual production processes are similar. In general, there are two fermentation steps involved in the 791 792 vinegar production, which are alcoholic and acetous fermentation, as depicted in Figure 3. During production, ethanol formed by yeast, during fermentation under anaerobic conditions, 793 normally by strains of Saccharomyces cerevisiae, is converted to acetic acid (acetification) by 794 795 AAB, mainly members of the genus Acetobacter and Gluconacetobacter (now mainly Komagataeibacter) under aerobic conditions (Budak, Aykin, Seydim, Greene, & Guzel-796 Seydim, 2014). However, other microorganisms, such as fungi and LAB, may be involved in 797 798 certain vinegar types. Besides S. cerevisiae, other yeast species ubiquitously found on fruit and vegetables may play a role in vinegars produced from these substrates; the lactose-fermenting 799 yeast, Kluyveromyces marxianus, is responsible for whey fermentation (Parrondo, Garcia, & 800 Diaz, 2009). A complex association of yeasts, AAB and LAB is involved in the fermentation 801 of kombucha and cocoa pulp-bean mass. It is probable that numerous AAB species involved 802 803 in vinegar fermentation have yet to be described because of the difficulties associated with their 804 cultivation on laboratory media (Cleenwerck & De Vos, 2008; Rainieri & Zambonelli, 2009). 805 Depending on the rate of acetic acid formation during vinegar production, the acetous 806 fermentation can be separated into two types of process: The Orléans method (slow) and the submerged and generator methods (quick). 807

The Orléans method, also called "mother of vinegar system" or French method is one of the oldest techniques for producing vinegar, and is an example of a surface fermentation method. Historically, Orléans was a big port on the longest French river, the Loire. Wines arriving there from all regions of France often suffered during transportation, turning sour en route. These

35
wines were sold to the vinegar brewers of Orléans, instead of being sent to Paris, their 812 destination (Bourgeois & Barja, 2009). In the Orléans process, acetification of ethanol into 813 acetic acid is started by "seed vinegar", or "mother of vinegar", an undefined starter culture 814 815 obtained from the previous vinegar fermentation. This process is called backslopping. This procedure promotes the initial number of desirable microorganisms (yeasts and AAB) over the 816 indigenous population (De Vuyst, 2000). The acetification is performed by a static culture of 817 818 AAB that grow at the interface between the liquid and air where the oxygen concentration is high (Sengur, 2015). In order to facilitate the oxidation of the ethanol and to leave an air 819 820 chamber, barrels are filled to approximately two-thirds their capacity and side holes enable circulation. A funnel extends to the base allowing wine to be added at the bottom of the barrel, 821 and preventing disruption of the "mother of vinegar" biofilm of AAB that forms on the liquid 822 823 surface (Mas, Torija, García-Parrilla, & Troncoso, 2014). During the first week acetous 824 fermentation is activated, after which the liquid is transferred to another vessel. Acetous fermentation is slow, occurring primarily on the surface of the liquid, where there is sufficient 825 oxygen to facilitate the conversion of ethanol to acetic acid. As the substrate ferments, the 826 changed environmental conditions (e.g. reduced pH) favour the most competitive indigenous 827 microbiota, and the more stringent the growth conditions, the greater the selective pressure. 828 Acetous fermentation continues for between 8 and 14 weeks depending on various factors, 829 830 including the initial composition of the substrate and alcoholic solution, the nature of the 831 microorganisms present, the sufficiency of oxygen supplied and the fermentation temperature (Dabija & Hatnean, 2014). This slow fermentation is difficult to control, with a high risk of 832 spoilage, and is now suitable only for small-scale production. A microbial succession occurs, 833 834 as in many spontaneous fermentations, in which LAB and yeasts often dominate initially. These consume carbohydrates and produce lactic acid and ethanol, respectively, which inhibit many 835 836 spoilage microorganisms, extending the shelf life of the resulting product. Acidity reaches a maximum level after approximately three months. Vinegars produced by this slow, traditional method are considered of high quality due to their organoleptic complexity. The complexity and the resulting product quality are strongly influenced by (i) the substrate raw material and its preparation, (ii) the metabolism of the AAB, which produce additional products of oxidation and some ester compounds besides acetic acid, (iii) the interaction between the vinegar and the wood of the barrels, and (iv) the aging process, which incorporates all the aforementioned characteristics (Mas et al., 2014).

Other surface fermentation methods such as the trickling (or quick vinegar) generator processes 844 (of which the Schutzenbach system or German rapid acetification method is an early example) 845 and the submerged process were developed to reduce the acetification time while maintaining 846 or increasing the quality. The rationale of the trickling generator processes is to increase the 847 848 surface area for oxygen contact with the fermenting vinegar. Thereby, alcoholic substrates are circulated and trickled through vessels or vats containing an inert, non-compacting material, 849 such as wood shavings or charcoal, on which a film of bacteria (AAB) is present. As the 850 alcoholic substrate trickles downward through the material, contact with AAB and oxygen 851 results in efficient oxidation to acetic acid. Once the substrate reaches the bottom it is re-852 853 circulated over the bed to promote increased transformation to acetic acid (Hutkins, 2006). 854 Therefore, the acidity successively increases. The trickling generator system has undergone a 855 number of developments over the years. The Schutzenbach system, originating in 1823, 856 developed the use of the solid packing material as a support for the bacteria and on which the vinegar was spread. Ham, in 1924, further developed this system, including forced aeration 857 through the bed (counter-current to the downward flow of substrate) and the pumping and re-858 859 distribution of the substrate over the bed by means of a sparger (Adams, 1998; Bourgeois & Barja, 2009). The Frings generator used wooden chips as the inert packing material. Today, 860 while surface fermentations are still used for the production of low volume, high quality 861

vinegars such as the Orléans method for the production of Aceto Balsamico di Modena, most
of the world's vinegar is produced at large volume in submerged fermentations, described
below (Emde, 2014).

Submerged culture systems provide a much faster alternative and involve the suspension of 865 AAB in the acetifying medium and application of stringent aeration to meet the high oxygen 866 demand. The oxidative process occurs at the liquid-air interface of the bubbles, where the AAB 867 convert ethanol into acetic acid, with limited production of other metabolites. The most 868 successful, commonly applied submerged culture system is the Acetator[®], built and sold by 869 870 Heinrich Frings GmbH & Co. (Bonn, Germany). A significant design feature is its self-priming aerator, which provides for highly efficient dispersion of air in the liquid substrate (Adams, 871 872 1998).

873 This submerged method is more straightforward than the traditional method, consisting of application of the raw material and inoculum to the fermenter, the actual fermentation step, and 874 final unloading of the bioprocessed product (Figure 4). A portion of the product is left in the 875 876 vessel to act as starter culture for the next cycle (Tesfaye, Morales, García-Parrilla, & Troncoso, 2002). This method was introduced for the production of vinegar at the beginning 877 of 20th century and is today employed for the production of most commercial vinegars of high 878 consumption (Tesfaye et al., 2002). A disadvantage of this rapid method is that the high airflow 879 880 leads to significant stripping of the volatile components from the original substrate, producing 881 a more organoleptically limited product. Despite this, the rapidity of the process (vinegar can be produced in 24-hour cycles) and the high acidity achievable (acetic acid levels of up to 23-882 25%, compared to 6–13% with traditional systems) are key advantages. Higher acidity makes 883 884 transportation more cost-effective by reducing water transport.

Role of AAB in other fermented beverages

886 Kombucha tea

Kombucha tea is traditionally prepared with water, tea, sugar and a kombucha culture ("tea 887 fungus") in open vessels at room temperature for 1–3 weeks. This non-alcoholic, fermented 888 beverage has a sharp acidity and specific flavour (De Roos & De Vuyst, 2018a). It is consumed 889 890 traditionally in Eastern Europe and Asia. It is noteworthy that in Japan, the term "konbu-cha" can also refer to an unfermented beverage prepared with brown algae (Laminaria kelp). 891 Consumption of kombucha was first recorded in 220 BC in Manchuria. More than one thousand 892 893 years ago "tea fungus" was already consumed in Japan, China and India; then in Russia, Poland and the Baltic States starting in about 1915; in the Balkans, Germany and Eastern Europe 894 around 1925; and in Spain, Italy, France and Switzerland in about 1955 (Kraft, 1959). Similar 895 896 to other traditional beverages, the popularity of kombucha increased due to its purported beneficial health effects and its ease of preparation. To prepare a basic kombucha ferment, tea 897 leaves are steeped in boiling water, 5 - 15% w/v of sucrose is added, and the mixture is brought 898 to room temperature. A "mother" kombucha pellicle, produced from a previous kombucha 899 900 fermentation, is placed into the tea along with liquid from a previous ferment (10 - 20% v/v). 901 This pellicle is sometimes referred to as a symbiotic culture of bacteria and yeasts (SCOBY), 902 and is comprised of primarily AAB and ethanol-forming yeasts in a thick cellulose pellicle 903 (Figure 5). The pellicle floats on the liquid surface, at the air interface and grows vertically, 904 increasing biomass with cellulose striations as the fermentation matures. After 1 - 3 weeks incubation at 20 - 30°C, the tea becomes a sweet and sour, naturally carbonated beverage due 905 to microbial activity (Chen & Liu, 2000; Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 906 907 2014; Malbaša et al., 2006).

908

909 The primary metabolites in a kombucha fermentation are sugars and organic acids. Yeasts convert sucrose to glucose and fructose, and produce ethanol, preferentially from the fructose 910 (Blanc, 1996, Dufresne & Farnworth, 2000). Acetic acid bacteria then convert this ethanol into 911 acetic acid (which gives kombucha its sour flavour) and glucose to GA. The pH of the medium 912 decreases to around 2.6, signifying the maturation of the beverage, ready for consumption 913 (Malbaša et al., 2006). If fermentation is not halted or slowed, the concentrations of acetic acid 914 and GA can increase to levels of greater than 4 g/100 mL, but the beverage is then unsuitable 915 for consumption due to a strong vinegar flavour (Chen & Liu, 2000). 916

917 The microbial composition of kombucha varies depending on the source of the inoculum. The basic requirements for a kombucha ferment are at least one cellulose-producing AAB, and at 918 least one yeast that can split sucrose into glucose and fructose. Multiple microorganisms can 919 920 be present in a kombucha ferment, performing these essential roles along with producing 921 additional secondary metabolites that contribute to the final beverage (Jarrell, Cal, & Bennett, 2000; Mayser, Fromme, Leitzmann, & Gründer, 1995; Roussin, 1996). The most frequently 922 isolated genera of bacteria from kombucha are Acetobacter, Komagataeibacter, 923 Gluconacetobacter and Lactobacillus (Chen & Liu, 2000; Trovatti, Serafim, Freire, Silvestre, 924 & Neto, 2011). The predominant AAB found in kombucha ferments are K. xylinus, A. 925 pasteurianus, A. aceti, and G. oxydans (Liu, Hsu, Lee, & Liao, 1996; Marsh, O'Sullivan, Hill, 926 927 Ross, & Cotter, 2014). K. xylinus – previously known as Ga. xylinus (Yamada et al., 2012), 928 and A xylinum (Yamada, Hoshino, & Ishikawa, 1997) – are the most abundant (80 - 99%) prokaryotes in kombucha (Chen & Liu, 2000; Marsh et al., 2014) and are responsible for the 929 formation of the cellulosic pellicle (Chen & Liu, 2000; Zhu, Li, Zhou, Lin, & Zhang, 2014). 930 931 Other AAB species have potential for both cellulose production and nitrogen fixation (Cleenwerck, De Wachter, González, De Vuyst, & De Vos, 2009; Dutta & Gachhui, 2007; Tan, 932 933 Ren, Cao, Chen, & Tang, 2012).

934

Water kefir is a fermented, low-alcohol beverage with acidic and fruity flavours (De Roos & 936 937 De Vuyst, 2018a). It is produced via spontaneous fermentation of a water solution containing approximately 8% (w/v) sucrose, (dried) fruits (e.g., figs), and water kefir grains (these 938 "grains" are essentially a polysaccharide mass encapsulating a complex microbial association 939 940 of bacteria and yeasts which serve as a starter culture) in a closed container at room temperature for 2–4 days (Figure 6) (Fiorda et al., 2017; Laureys & De Vuyst, 2014). Currently the main 941 942 market for this beverage is in the USA, Japan, France, and Brazil, where it is consumed for its reported functional properties (Fiorda et al., 2017). The water kefir grain inoculum, and the 943 944 nature of the substrate (such as the type of fruit used) determines the grain growth, the microbial 945 species diversity, the metabolites formed and their concentrations (Laureys, Aerts, Vandamme, 946 & De Vuyst, 2018; Laureys & De Vuyst, 2017; Laureys, Van Jean, Dumont, & De Vuyst, 2017). In general, the microbiota of water kefir is known to be a stable consortium of different 947 LAB (such as Lactobacillus, Leuconostoc), AAB (Acetobacter, Gluconacetobacter, and 948 Gluconobacter) and yeasts (Kluyveromyces, Brettanomyces, Pichia, and Saccharomyces). A 949 strong symbiosis between these three microbial groups is documented (Fiorda et al., 2017). 950 Yeast metabolism promotes the growth of acidophilic bacterial species such as LAB and AAB. 951 952 Glucose and fructose are made available for LAB growth through the action of yeast invertase 953 on sucrose. Ethanol produced by yeasts may be metabolised to acetic acid by any viable AAB present (Magalhaes, Pereira, Dias, & Schwan, 2010). Growth of AAB particularly takes place 954 under aerobic conditions, leading to increased acetic acid content, which may be unwanted 955 956 (Gulitz, Stadie, Ehrmann, Ludwig, & Vogel, 2013; Laureys et al., 2017; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013). Under anaerobic conditions they remain in a viable but non-957 958 culturable (VBNC) state, being metabolically dormant, but can start to grow when oxygen becomes accessible (Laureys et al., 2017). While several species from the genera *Acetobacter*, *Gluconacetobacter*, and *Gluconobacter* have been recovered from water kefir fermentation
processes, *Acetobacter* species seem to be best adapted to this ecosystem (Laureys & De Vuyst,
2014; Laureys et al., 2017; Magalhaes et al., 2010; Marsh et al., 2013).

963

964 Lambic beer

Lambic sour beers are among the oldest types of refreshing, alcoholic, acidic beers still brewed and which have become increasingly popular worldwide (De Roos & De Vuyst, 2018a, 2018b; Pothakos et al., 2016). In particular, sour beers are now attracting interest in the USA. In the American craft-brewing sector, American coolship ales, for instance, mimic the lambic beer production method (Bokulich, Bamforth, & Mills, 2012). Such beers were once a seasonal product from craft breweries, but today some produce solely sour beers, much like traditional Belgian lambic breweries.

Lambic beer is the result of a spontaneous fermentation process of a barley and unmalted wheat 972 extract (wort) that continues for 1 - 3 years (De Keersmaecker, 1996). The fermentation process 973 974 is not driven by yeasts or bacteria applied as starter cultures, but by a spontaneous inoculum 975 from the environment. Microbial growth begins during the cooling of the boiled wort which occurs overnight in a shallow open vessel, known as a cooling tun or coolship. These beers are 976 977 traditionally brewed close to the Senne river valley, near Brussels, Belgium (Spitaels et al., 2014a). The following morning the cooled wort is assumed to have been inoculated with the 978 979 specific air microbiota of this region and is transferred into wooden casks which are stored at cellar or ambient temperatures, i.e., typically between 15 and 25°C. The wort then ferments 980 981 and matures in these casks. In addition to inoculation from the environment during the coolship 982 step, microorganisms present on the interior surfaces of the casks also contribute to the fermenting wort, helping to establish a stable microbial community (De Roos, Van der Veken, 983 & De Vuyst, 2018). The end product is a non-carbonated sour beer that mainly serves as a 984

985 base for gueuze or fruit lambic beers. Several studies have shown a microbial succession of Enterobacteriaceae and wild (oxidative) yeasts, including a yeast fermentation phase with 986 Saccharomyces cerevisiae and/or Saccharomyces pastorianus, an acidification phase with 987 988 Pediococcus damnosus and/or Lactobacillus brevis, and a maturation phase with Dekkera (Brettanomyces) bruxellensis (De Roos & De Vuyst, 2018b). AAB are only occasionally 989 isolated during the lambic beer fermentation and maturation process, probably due to their 990 VBNC state (Spitaels et al., 2014a; Spitaels et al., 2015). However, two new AAB species have 991 been described that seem to be characteristic for lambic beers, namely Acetobacter lambici 992 993 (Spitaels et al., 2014b) and *Gluconobacter cerevisiae* (Spitaels et al., 2014c). It is possible that the AAB, being obligate aerobes, are concentrated at the wort/air interface and, hence, are 994 995 missed during submerged sampling of the casks. Indeed, this has recently been proven to be 996 the case by De Roos et al. (2018a), who showed that the liquid nearest the interface was 997 characterized by higher AAB counts and higher concentrations of their metabolites (De Roos et al., 2018a). 998

999 Biotechnological applications of *Gluconobacter oxydans* relevant to the food industry

Apart from their historical and key role in the production of fermented foods such as vinegar, AAB are also important for the production of useful compounds that find application in the food industry. The formation of GA by *Gluconobacter oxydans*, and their role in the process of Vitamin C synthesis are discussed here.

1004

1005 *Gluconic acid production and regulation*

1006 The applications of GA in the food industry include use as an acidity regulator (E574–E580), 1007 with raising, sequestering and flavour-enhancing properties. Gluconic acid enhances the sensory characteristics of foods by imparting a bitter but refreshing taste. Gluconic acid and 1008 1009 its derivative glucono- δ -lactone are also used as food preservatives. Its ketogluconate, 5-KGA, 1010 also has important uses, including as a precursor in the production of tartaric acid, xylaric acid, 1011 as well as for Vitamin C production (Cañete-Rodríguez et al., 2016). Two methods are commonly employed for the biotechnological production of GA, use of the fungus Aspergillus 1012 1013 niger, or use of an AAB strain, primarily G. oxydans. The biochemistry of the production of GA and associated ketogluconates has been described above. This section will examine the 1014 1015 factors influencing GA production in G. oxydans. This subject was reviewed recently by 1016 García-García et al. (2017) and will be discussed briefly below (García-García et al., 2017). 1017 The main factors which affect the production of GA include, in decreasing order of importance, 1018 pH, initial concentration of glucose in the medium, concentration of calcium carbonate added to the medium and the dissolved oxygen level. 1019

Using *G. oxydans* 621H it was found that below pH 3.5 - 4 uptake and assimilation of GA into
the PPP is almost completely inhibited (Olijve & Kok, 1979). This is likely to be related to the
pH optima for the various enzymes involved in these processes – the periplasmic

1023 dehydrogenases involved in oxidative fermentation have pH optima in the acidic range of pH 1024 3 - 6, while those cytoplasmic (NAD(P)-dependent) dehydrogenases have optima in the alkaline range of pH 8 – 11 (García-García et al., 2017). Therefore, the production of GA and 1025 1026 associated pH drop may promote its own production and accumulation as the periplasmic dehydrogenases become more active. As outlined above, GA can be further oxidised to 1027 1028 ketogluconates such as 2KGA and 5KGA, thus reducing the GA yield. Olijve and Kok (1979) 1029 and Weenk et al. (1984) found glucose to be rapidly oxidized virtually quantitatively to GA without formation of any ketogluconates if the pH of the fermentation was uncontrolled (which 1030 1031 lead to a rapid pH drop), or if the pH was adjusted to 2.5 at the beginning of the fermentation (Olijve & Kok, 1979; Weenk, Olijve, & Harder, 1984). However, control of the pH at 5.5, or 1032 1033 the addition of calcium carbonate to the medium, lead to 2KGA and 5KGA formation once all 1034 initial glucose had been utilised (Weenk et al., 1984). The presence of calcium carbonate in the 1035 medium promotes ketogluconate production (Beschkov, Velizarov, & Peeva, 1995). It has been postulated that, in the absence of pH control, the formation of ketogluconates may be 1036 1037 completely inhibited (Beschkov et al., 1995; Velizarov & Beschkov, 1994).

1038 The initial glucose concentration also strongly influences the production of GA and 1039 ketogluconates. Olijve and Kok (1979) found that high glucose concentrations (0.9 - 2.7 g/L)led to rapid GA accumulation, while at lower concentrations the assimilation of glucose and 1040 1041 metabolism in the PPP was favoured (Olijve & Kok, 1979). This is understandable from an 1042 ecological viewpoint when considering the level of glucose in the environment. If the concentration is low, AAB will preferentially assimilate the glucose for biomass production; 1043 1044 in contrast, in the case of high glucose concentrations, the majority of it will be oxidised to 1045 gluconate and ketogluconates, thus making it unavailable for competing microorganisms, while 1046 also lowering the pH of the environment (García-García et al., 2017). Because GA is produced from the oxidation of glucose and the amount which is formed is directly proportional to the 1047

initial glucose concentration, it is logical that above a certain concentration of glucose, the
amount of GA formed will become inhibitory due to the resultant low pH, thus preventing
further production. Thus, a glucose concentration above 90 g/L led to a reduced rate and yield
of GA compared to a lower glucose concentration. Long lag phases were observed due to the
combined effect of high glucose concentration and the low pH due to GA formation (Velizarov
& Beschkov, 1994, 1998). High glucose concentrations (~90 g/L) also favoured GA production
over ketogluconate formation (Beschkov et al., 1995; García-García et al., 2017).

Compared to the effects of pH and glucose concentration, dissolved oxygen (DO) has a less 1055 1056 important role on the production of GA. However, Buse et al. (1992) found that DO control had a significant impact on the formation of 2,5-diKGA in G. oxydans ATCC 9937 (formerly 1057 1058 Gluconobacter oxydans subsp. melanogenum). This was related to changes in the activity of 1059 the enzyme gluconate dehydrogenase (the first enzyme in the conversion of GA to 1060 ketogluconates) at different oxygenation levels. Low oxygen (<30%) delayed the production of this enzyme (Buse, Qazi, & Onken, 1992). Thus, low DO levels appear to inhibit GA 1061 conversion to ketogluconates and it is conceivable that relatively low DO levels may have a 1062 1063 similar effect in G. oxydans.

1064

1065 Vitamin C production and potential for direct formation by G. oxydans

Since 1934 and until the late 1990s, the "Reichstein process" has been used as the main process for the production of Vitamin C, also known as L-ascorbic acid (Asc), of which more than 110,000 tones are produced annually (Bremus, Herrmann, Bringer-Meyer, & Sahm, 2006). This process, although refined and improved over the years, contains a number of chemical steps, and only a single microbially-catalysed step, and is highly energy intensive. Therefore, it has been replaced, particularly in China, by the so-called (Classical) Two-Step Fermentation Process which is less costly and more environmentally friendly (Yang & Xu, 2016). Figure 7
shows the various routes for Asc production; while different processes have been studied, the
Two-Step Fermentation process is today the primary method used for industrial Asc production
(Wang et al., 2018), with China supplying 80% of the global demand. Royal DSM remains the
sole Western Vitamin C producer (Pappenberger & Hohmann, 2014). Each of the routes to
Asc, particularly with reference to the microorganism involved and especially *G. oxydans*, will
be discussed further below.

D-glucose, D-sorbitol or D-sorbose can be considered as common starting materials for the
process; however, glucose must be chemically converted to sorbitol which is typically via
hydrogenation (Yang & Xu, 2016). Central in each process is the production of the precursor,
2-keto-L-gulonic acid (2-KLGA). For a detailed treatment of this topic, readers are also
referred to the recent review by Wang et al. (2018) (Wang et al., 2018).

The Reichstein process: This process consists mainly of chemical reactions for Asc synthesis, 1084 with only one microbially-catalysed step. This is the oxidation of D-sorbitol to L-sorbose and 1085 1086 which is performed by AAB due to their very efficient oxidation of carbohydrates and sugar alcohols. Originally this bioconversion was performed by Acetobacter aceti subsp. xylinum 1087 1088 (now Komagataeibacter xylinus); subsequently Gluconobacter suboxydans (now G. oxydans) was introduced due to its greater capacity for oxidation and has been employed to date, with 1089 1090 an almost 100% conversion rate on an industrial scale (Pappenberger & Hohmann, 2014; Yang & Xu, 2016). This is a key reaction, common to each route to Asc production, as can be seen 1091 1092 in Figure 7. The key enzyme mediating the conversion of D-sorbitol to L-sorbose is glycerol dehydrogenase (GLDH), a PQQ-dependent membrane protein. This enzyme is also known as 1093 1094 D-sorbitol dehydrogenase (SLDH), but it is a major polyol dehydrogenase in G. oxydans with 1095 broad substrate specificity for other sugar alcohols besides sorbitol, such as D-mannitol, D-1096 arabitol, meso-erythritol, D-adonitol and glycerol (Figure 1) (Matsushita et al., 2003; Sugisawa 4 Hoshino, 2002). A second membrane-bound sorbitol oxidising enzyme, which is specific for
sorbitol, has been found in *G. oxydans* and other *Gluconobacter* species but is FAD-dependent
i.e. FAD-SLDH (Shinjoh & Toyama, 2016). Genome analysis of the industrial strain *G. oxydans* H24 identified three sorbitol oxidising enzymes, two membrane bound and one
cytoplasmic, namely PQQ-SLDH (PQQ-GLDH), FAD-SLDH and NADP-SLDH, respectively
(Ge et al., 2013). Among these, PQQ-GLDH is believed to play the primary role in converting
D-sorbitol to L-sorbose (Matsushita et al., 2003).

Two-Step Fermentation process: This process can be considered as an improvement on the 1104 1105 Reichstein process. Two fermentations are performed in this process; the first, the 1106 bioconversion of D-sorbitol to L-sorbose is the same as that of the Reichstein process and is 1107 performed by G. oxydans. The second fermentation involves the conversion of sorbose to the 1108 Asc precursor, 2-KLGA. The subsequent transformation of 2-KLGA into Asc is performed through a number of chemical steps, as for the Reichstein process. Therefore, it is the second 1109 fermentation that differentiates the Two-Step Fermentation process from the Reichstein 1110 process (Yang & Xu, 2016). This fermentation is performed by a two-strain co-culture system, 1111 1112 neither of which is actually an AAB. This dual culture consists of the 2-KLGA-producing strain 1113 and a "helper" or companion strain which promotes the growth of the 2-KLGA producer. Early studies identified the 2-KLGA producer as G. oxydans, but it was later renamed 1114 1115 Ketogulonicigenium vulgare, a Gram-negative, facultatively anaerobic, chemoheterotrophic 1116 soil microorganism (Urbance, Bratina, Stoddard, & Schmidt, 2001). The companion strain, 1117 typically a species of *Bacillus*, is considered to stimulate K. vulgare growth and 2-KLGA 1118 accumulation by releasing particular metabolites (Feng, Zhang, & Zhang, 2000). B. 1119 megaterium and B. cereus were the primary companion strains applied in industrial Asc 1120 fermentation, and while many spore-forming strains have been found to be suitable as companion strains, only K. vulgare has been used in industrial fermentation so far (Feng et al., 1121

1122 2000; Jiao, Zhang, Xie, Yuan, & Chen, 2002; Urbance et al., 2001). Two enzymes in K. vulgare 1123 are key in the oxidation of L-sorbose to 2-KLGA. These are L-sorbose/L-sorbosone dehydrogenase (SSDH) and L-sorbosone dehydrogenase (SNDH). SSDH is a unique PQQ-1124 1125 dependent membrane dehydrogenase, with dual catalytic ability, catalysing not only the conversion of L-sorbose to L-sorbosone but also that of L-sorbosone to 2-KLGA (Asakura & 1126 Hoshino, 1999). In addition, the SNDH of K. vulgare has been found to catalyse the direct 1127 1128 conversion of L-sorbosone to Asc (Miyazaki, Sugisawa, & Hoshino, 2006). Thus, via the action 1129 of SSDH and SNDH it is possible for K. vulgare to directly produce Asc from L-sorbose and/or 1130 L-sorbosone (Sugisawa, Miyazaki, & Hoshino, 2005). Genome sequencing of the industrial strain, K. vulgare Y25, found that it contained four genes encoding SSDH and one plasmid-1131 1132 encoded gene for SNDH (Liu et al., 2011). The absence of genes or operons for the biosynthesis 1133 of many amino acids, nucleotides and cofactors may explain its dependence on a companion 1134 strain. As stated, the primary microorganism in the Two-Step Fermentation process is not an AAB sensu stricto; however, homologous enzymes to those employed by K. vulgare have been 1135 1136 found in G. oxydans (Shinjoh & Toyama, 2016), and the potential of this bacterium for the direct production of 2-KLGA and Asc is described next. 1137

1138 Microbial production of 2-KLGA and Asc by G. oxydans: There has been interest in the further 1139 exploitation of G. oxydans in Asc production, beyond its use for sorbitol oxidation. This has 1140 been supported by the observation in recent decades of enzymes in certain strains of G. oxydans 1141 which perform similar bioconversions to those in K. vulgare, strengthening the possibility of, in the future, using a single microorganism for direct 2-KLGA or Asc production. Thus, strains 1142 1143 of G. oxydans have been demonstrated to produce 2-KLGA from both D-sorbitol and L-1144 sorbose, albeit at very low yields with wild-type strains. For example, G. oxydans NBRC3292 1145 (formerly G. oxydans IFO3292 and G. melanogenus ATCC15163) was shown to produce 6.5g/L 2-KLGA from 50 g/L sorbitol over a 150 h fermentation time (Motizuki et al., 1962). 1146

A similar strain, G. oxydans NBRC3293 (formerly G. oxydans IFO3293 and G. melanogenus 1147 IFO3293) produced 2.8 g/L 2-KLGA from 25 g/L L-sorbose over 168 h fermentation 1148 (Sugisawa et al., 1990). A progenitor strain of IFO3293, designated SPO1, was isolated and 1149 1150 produced 13g/L of 2-KLGA from 50 g/L L-sorbose. Subsequent strain improvement studies with strain SPO1 using mutagens such as UV irradiation resulted in the isolation of genetically 1151 1152 modified strains producing 50 to 60 g/L 2-KLGA from 100g/L D-sorbitol or 100 g/L L-sorbose 1153 over 80 to 100 h. Particular mutant isolates arising from the above strain improvement studies, 1154 which have been subsequently extensively studied regarding Asc production by G. oxydans, 1155 are strains UV10 and N44-1 (Pappenberger & Hohmann, 2014). L-sorbose dehydrogenase (SDH) was identified in the mutant strain UV10. The enzyme was membrane bound and FAD-1156 1157 dependent with a high activity for L-sorbose (L-sorbosone as product). In addition, L-1158 sorbosone itself was also identified as a substrate for SDH, with 2-KLGA as the product. Thus, 1159 this SDH in G. oxydans UV10 has very similar activity to the SSDH of K. vulgare described above. Similarly, a SNDH was identified in G. oxydans UV10, this enzyme being found in the 1160 1161 cytosolic fraction and being NAD(P)-dependent, with 2-KLGA as the product (Hoshino, Sugisawa, & Fujiwara, 1991; Pappenberger & Hohmann, 2014). A second strain of G. oxydans, 1162 1163 T-100, was subsequently shown to contain the same dehydrogenases (Saito et al., 1997). Based on genome analysis, homologs of the associated genes are present in many *Gluconobacter* sp. 1164 1165 (Gao, Zhou, Liu, Du, & Chen, 2012; Wang et al., 2018). Thus, the formation of 2-KLGA in G. 1166 oxydans is mediated by either of two enzymes: the membrane bound SDH i.e. from L-sorbose or L-sorbosone, or, the cytosolic SNDH, from L-sorbosone. The more recent observation of 1167 small amounts of Asc production by G. oxydans NBRC3293 when provided with L-sorbosone 1168 1169 has led to the identification of a second type of L-sorbosone dehydrogenase in G. oxydans. In contrast to the FAD-dependent cytosolic SNDH of G. oxydans UV10, this enzyme was 1170 1171 membrane bound and PQQ-dependent and was designated SNDHai (Berry, Lee, Mayer, &

Shinjoh, 2003). A homologous enzyme, designated SNDHak was identified in K. vulgare 1172 1173 (Miyazaki et al., 2006). Although SNDHai uses L-sorbosone as a substrate, which it converts directly to Asc, it displays much higher activity for the cyclic polyol, myo-inositol; thus, it has 1174 1175 been suggested that L-sorbosone oxidation by SNDHai may be a case of fortuitous crossreactivity with the non-physiological L-sorbosone (Pappenberger & Hohmann, 2014). As 1176 1177 shown in Figure 7(d), as little as three periplasmic oxidation steps are required to convert sorbitol to Vitamin C – mediated by GLDH, SDH and SNDHai – all of which are endogenous 1178 1179 G. oxydans enzymes (Pappenberger & Hohmann, 2016). Despite low yields of Asc, the 1180 identification of SNDHai has, nevertheless, increased the possibility that G. oxydans may, at some future point, be used as the sole microorganism for the direct production of Vitamin C. 1181 1182 With respect to the yield of Asc, there are three challenges which need to be overcome. Firstly, 1183 the low affinity of SNDHai for L-sorbosone, as described above. Secondly, the presence of the 1184 cytoplasmic SNDH in G. oxydans means that a certain proportion of the L-sorbosone will be converted to 2-KLGA and therefore not to Asc. Thirdly, and most critically, is the stability of 1185 1186 Asc once it is produced. This is primarily due to the reductive properties of Asc and its propensity to become oxidised, forming L-dehydroascorbic acid. If L-dehydroascorbic acid is 1187 not rapidly reduced to again form Asc, it spontaneously and irreversibly degrades with a half-1188 life of minutes. In this way, Asc is oxidised by molecular oxygen, and this reaction is 1189 1190 accelerated at above neutral pH and in the presence of trace amounts of transition metal ions. 1191 The challenge is that such trace metals are required as a growth factor and, moreover, the strictly oxidative metabolism of AAB means that the presence of molecular oxygen is clearly 1192 unavoidable (Pappenberger & Hohmann, 2016). Attempts to overcome such barriers have 1193 1194 included the use of resting cells in media in the absence of detrimental substances or with the use of engineered strains. Using the latter approach, a near 90% substrate conversion and yields 1195 1196 of 10 g/L Asc were achieved using a genetically engineered strain of G. oxydans that was overexpressing genes for SDH and SNDHai and also had a gene knockout for cytoplasmic SNDH
(Pappenberger & Hohmann, 2016). Thus, it appears that at the current state of the science and
knowledge, significant yields of Asc using (wild-type) *G. oxydans* alone are not yet possible
without the use of engineered strains.

1201 It is noteworthy that G. oxydans has also the potential to be used for the biosynthesis of 2-1202 KLGA via a different mechanism to the D-sorbitol pathway, that is, via the biosynthesis of GA and its associated ketogluconates - the 2,5-diKGA pathway (Figure 7(e)). In this system the 1203 bioconversion of glucose as substrate into 2-KLGA is performed by cell preparations 1204 1205 containing the necessary complement of enzymes such as glucose dehydrogenase, D-gluconate 1206 and 2-keto-D-gluconate dehydrogenase, including cytochrome C as a co-factor. A final 1207 enzyme, 2,5-diKGA reductase, performs the conversion of 2,5-diKGA into 2-KLGA (Hancock 1208 & Viola, 2002). As has been discussed above, specific G. oxydans strains have the potential to produce 2,5-diKGA from glucose oxidation via GA. Indeed, resting cells of G. oxydans have 1209 been used as a source of gluconate dehydrogenase and 2-keto-D-gluconate dehydrogenase for 1210 2-KLGA formation via the 2,5-diKGA pathway (Hancock & Viola, 2002; Ji & Gao, 2001). 1211 However, most research into this pathway have used species of Erwinia and Corynebacterium 1212 1213 as the enzyme sources (Wang et al., 2018).

1214 Beneficial effects of AAB-fermented products

1215 Health benefits associated with vinegar consumption

1216 Vinegar is today primarily used as a condiment or seasoning alone, or as a seasoning and preservative agent in salad dressings, mayonnaise, ketchup and similar condiment sauces for 1217 its desirable organoleptic properties. However, in historical times it was used medicinally, as 1218 described above (Budak et al., 2014). A number of beneficial effects on health have been 1219 claimed to be associated with the consumption of vinegar, and while these are numerous, only 1220 few are based on clear evidence (Mas, Troncoso, García-Parrilla, & Torija, 2016). Health 1221 benefits associated with vinegar include antimicrobial activity, antioxidant activity, modulation 1222 of the glycaemic response, positive effects on cardiovascular health, such as cholesterol-1223 1224 lowering and antihypertensive action, positive effects in weight loss, improvement of appetite, 1225 reduction of fatigue and anticancer activity (Chen et al., 2016). Organic acids, primarily acetic acid, and polyphenols have been attributed as the main functional compounds in vinegar and 1226 1227 are present in all varieties at varying levels (Chen et al., 2016). Other bioactive compounds, 1228 their presence and concentration which can vary depending on the type of vinegar and substrate material, may also contribute to the functionality e.g. tryptophol as an anticancer compound in 1229 1230 Japanese black soybean vinegar (Inagaki et al., 2007). Certain factors influence the chemical composition of vinegar and thus its functional properties, including the raw material, the 1231 production process (acetification method) and the amount of time spent aging in wood 1232 (Guerreiro, de Oliveira, Ferreira, & Catharino, 2014). 1233

The antibacterial effects of vinegar have mainly been investigated in the context of in-vitro application to food products, such as fresh fruits and vegetables, for the inhibition of pathogenic bacteria. The antibacterial mechanism of vinegar is primarily due to its acetic acid content. When the bactericidal effects of a number of organic acids, including lactic acid, acetic acid,

citric acid, and malic acid on Escherichia coli O157:H7 were investigated, acetic acid was 1238 found to be most effective, followed by lactic acid, citric acid, and malic acid. Other pathogens 1239 inhibited by acetic acid included Salmonella enterica subsp. enterica, 1240 Vibrio parahaemolyticus, Staphylococcus aureus, Aeromonas hydrophila, and Bacillus cereus 1241 (Entani, Asai, Tsujihata, Tsukamoto, & Ohta, 1998). Chang and Fang (2007) observed a 3-log 1242 reduction in numbers of E. coli O157:H7 when rice vinegar containing 5% acetic acid was 1243 applied to lettuce for 5 min at 25°C. Use of lower acetic acid levels led to less of a reduction 1244 within the same time (Chang & Fang, 2007). The antimicrobial activity of organic acids is 1245 1246 influenced by the target bacterial strain(s), temperature, pH, acid concentration, and ionic strength (Budak et al., 2014). The properties of undissociated organic acids such as fat-1247 1248 solubility and neutral charge enable them to diffuse through the cell membrane of the target 1249 microorganism and enter the cell; in the cytoplasm, the higher intracellular pH causes the acid 1250 to become dissociated, producing hydrogen ions. Hydrogen ion production thus reduces the intracellular pH and interferes with cellular processes, such as enzyme activity, DNA 1251 1252 replication and transcription, and protein expression, therefore effecting the normal growth of the microorganism (Chen et al., 2016). Acetic acid is a particularly effective antimicrobial 1253 1254 because at a relatively high pH (pH 4.7 = pKa of acetic acid) it exists primarily in its undissociated form and can enter the cell. Other organic acids, for example lactic acid, are not 1255 1256 as effective; lactic acid cannot easily enter cells at a pH>3.8 (its pKa) as it exists primarily in 1257 its dissociated form.

A high oxidant and low antioxidant level in the human body is associated with the development of chronic, inflammatory diseases, such as cancer and cardiovascular disease (Srdic-Rajic & Konic Ristic, 2016). Intake of dietary antioxidants inhibit the formation of peroxides and their absorption in the gastrointestinal tract (Verzelloni, Tagliazucchi, & Conte, 2007). Vinegar exhibits antioxidant capacity which has been associated with the presence of polyphenols and 1263 derived phenolic compounds, such as, gallic acid, caffeic acid, p-coumaric acid and ferulic acid amongst many others (Garcia-Parrilla, Torija, Mas, Cerezo, & Troncoso, 2017). In addition, 1264 carotenoids, phytosterols and vitamins, such as Vitamins C and E, and melanoidins also 1265 1266 contribute to the antioxidant capacity (Ho et al., 2017; Tagliazucchi, Verzelloni, & Conte, 2010). As stated above, the processes used in vinegar production can influence its chemical 1267 composition, and it is noteworthy that, in the case of the phenolic composition, contact with 1268 1269 wood can influence the phenolic content, due to polyphenol release via alcoholysis of wood 1270 lignin (Tesfaye et al., 2002).

1271 It has been demonstrated in-vitro that the antioxidant capacity of Traditional Balsamic Vinegar 1272 (TBV) was equal to that of a 0.2% Vitamin C solution (Chen et al., 2016). Tagliazucchi et al. (2008) showed, in-vitro, that TBV had antioxidant activity equal to or higher than that of red 1273 1274 wine, with 45% of the antioxidant activity due to the total polyphenolic fraction, primarily tannins, and 45% due to melanoidins and other lower molecular mass Maillard reaction 1275 products (Tagliazucchi, Verzelloni, & Conte, 2008). TBV melanoidins were responsible for 1276 preventing the pro-oxidant and cytotoxic effects of heme during simulated gastric digestion of 1277 1278 meat (Verzelloni, Tagliazucchi, & Conte, 2010). Kurosu, a Japanese vinegar produced from 1279 unpolished rice, has been reported to have a high antioxidant activity. An ethyl acetate extract 1280 of kurosu inhibited myeloperoxidase activity, hydrogen peroxide generation and lipid 1281 peroxidation in mouse skin cells, and had the highest antioxidant activity compared to ethyl 1282 acetate extracts of grain vinegar, apple vinegar and wine vinegar (Nishidai et al., 2000).

Vinegar has been described to have an antiglycaemic effect and to improve blood glucose control and insulin resistance. Indeed, before the advent of pharmacological hypoglycaemic agents, vinegar "teas" were consumed by diabetics to help manage their condition (Johnston & Gaas, 2006). Many types of vinegars including apple cider vinegar, ginsam vinegar (an Asian vinegar produced from *Panax ginseng*), and tomato vinegar are capable of reducing 1288 postprandial blood glucose and alleviating insulin resistance as well as promoting insulin production. This antiglycaemic effect of vinegar was first reported by Ebihara and Nakajima 1289 (1988). The authors found that co-administration of 2% acetic acid with a high glycaemic load 1290 1291 meal consisting of 10% corn starch, significantly reduced the blood glucose response in rats (Ebihara & Nakajima, 1988). In human subjects, the consumption of sucrose accompanied by 1292 vinegar decreased the area under the insulin response curve by 20% (Johnston & Gaas, 2006). 1293 1294 Administration of 20 mL white vinegar (equivalent to 5% acetic acid) as a salad dressing with a mixed meal consisting of white bread (50 g carbohydrate) and lettuce reduced the glycaemic 1295 1296 response by over 30% in healthy individuals. Neutralisation of the acetic acid with sodium bicarbonate destroyed this antiglycaemic effect (Brighenti et al., 1995). The substitution of 1297 1298 pickled cucumber for fresh cucumber in a meal consisting of bread, butter and yogurt reduced 1299 the glycaemic index (GI) by over 30% in healthy subjects (Östman, Liljeberg Elmståhl, & 1300 Björck, 2001). Similar results were observed by Johnston et al. (2010), who demonstrated that the postprandial glucose response was reduced by 23% in healthy individuals fed a meal of a 1301 1302 bagel and juice containing 10 g apple cider vinegar (5% acidity), but not by the same meal containing neutralised vinegar. It was concluded that the neutralised salt of acetic acid (acetate) 1303 does not appear to possess antiglycaemic properties. In addition, ingestion of vinegar five hours 1304 before the meal reduced the antiglycaemic effect compared to consumption of vinegar with the 1305 1306 meal (Johnston, Steplewska, Long, Harris, & Ryals, 2010).

Vinegar has also been shown to modulate the glucose response and insulin sensitivity in diabetic individuals. In patients with type 2 diabetes, dietary consumption of acetic acid significantly reduced the level of glycated haemoglobin (0.16%) during a 12-week experiment (Johnston, White, & Kent, 2009). Furthermore, in healthy individuals at risk of developing type II diabetes mellitus, ingestion of 0.75 g acetic acid as a vinegar drink twice daily at mealtime, for 12 weeks, reduced fasting blood glucose levels, and to a greater extent than diabetic pharmaceutical medications (Johnston, Quagliano, & White, 2013). This is in agreement with
an earlier study by Johnston et al. (2004) which showed that ingestion of a vinegar drink (20 g
vinegar, 40 g water, 1 tablespoon saccharine) by individuals with insulin resistance (prediabetic) had a marked reduction in postprandial glycaemia (64%) and improved insulin
sensitivity (34%) (Johnston, Kim, & Buller, 2004).

1318 It has been reported that the reduction of postprandial glucose affected by vinegar in high GI meals is not observed for low GI meals (Liatis et al., 2010). However, vinegar ingestion with 1319 both high- and even low-GI meals does improve insulin sensitivity, independent of blood 1320 1321 glucose level, as glucose uptake was enhanced after both meal types when vinegar was also ingested (Mitrou et al., 2015). Indeed, even the improvement by vinegar consumption of insulin 1322 sensitivity alone is noteworthy, particularly in insulin-resistant (pre-diabetic) subjects, as trials 1323 1324 have demonstrated that slowing the progression to diabetes in high-risk individuals and improving their insulin sensitivity may increase the probability that such individuals may revert 1325 to a normal, glucose-tolerant state over time (Johnston & Gaas, 2006). 1326

Postprandial blood glucose levels are primarily determined by 1) the rate that glucose enters 1327 the blood and 2) the rate at which it is consumed in-vivo. The rate of gastric emptying, 1328 1329 digestion, and absorption in the small intestine determine the rate that glucose enters the blood (Chen et al., 2016). It is not yet fully understood how vinegar modulates glucose metabolism. 1330 Acetic acid in vinegar may supress carbohydrate absorption in the gut, more specifically 1331 through reducing disaccharidase activity and decreasing the digestion of disaccharides and 1332 oligosaccharides. Thus, monosaccharide absorption in the gut is reduced, lowering the blood 1333 glucose level (Johnston et al., 2013). Ogawa et al. (2000) demonstrated that acetic acid 1334 significantly inhibited the disaccharidase (e.g. sucrase, lactase) activity of Caco-2 cells, an 1335 effect not seen with other organic acids such as lactic acid or citric acid (Ogawa et al., 2000). 1336 In addition, acetic acid may regulate glucose metabolism by promoting uptake of glucose by 1337

1338 the liver and skeletal muscle and its conversion to glycogen stores (Hlebowicz, Darwiche, Bjorgell, & Almer, 2007). In this case, the mechanism by which glucose metabolism is 1339 regulated is believed to be through activation of the adenosine monophosphate-activated 1340 protein kinase (AMPK) pathway. Acetic acid is a building block for the biosynthesis of acetyl-1341 1342 coenzyme A (acetyl-CoA). During acetyl-CoA biosynthesis, adenosine triphosphate (ATP) is consumed and adenosine monophosphate (AMP) is produced, increasing the AMP/ATP ratio 1343 1344 and leading to activation of the AMPK pathway. This causes a reduction in blood glucose levels and concomitant glycogen biosynthesis (Sakakibara, Yamauchi, Oshima, Tsukamoto, & 1345 1346 Kadowaki, 2006). Another mechanism by which vinegar may reduce the postprandial blood glucose level is via effecting delayed gastric emptying. Hlebowicz et al. (2007) demonstrated 1347 1348 that consumption of 30 mL of apple vinegar decreased the postprandial gastric emptying rate 1349 by 10% (Hlebowicz et al., 2007). The stabilisation of the postprandial blood glucose level 1350 through the mechanism described above may also increase postprandial satiety, thus reducing dietary intake and, as such, a further increase in blood glucose (Chen et al., 2016). 1351

There is evidence that vinegar consumption can also affect lipid metabolism and, by 1352 1353 association, promote weight loss. Studies, in particular in animal models, have highlighted that 1354 vinegar consumption can increase the concentration of high-density lipoprotein (HDL) 1355 cholesterol while reducing triglycerides, total cholesterol, and low-density lipoprotein (LDL) 1356 cholesterol (Fushimi et al., 2006). Similar effects on lipid levels were observed in human 1357 subjects displaying hyperlipidaemia and in obese individuals following consumption of apple cider vinegar (Beheshti et al., 2012; Kondo, Kishi, Fushimi, Ugajin, & Kaga, 2009a). Obese 1358 individuals, following long term apple vinegar consumption, also displayed significantly 1359 1360 reduced body weight and body mass index (Kondo et al., 2009a). Regulation of lipid 1361 metabolism by vinegar intake is understood to be due to acetic acid, and, in a similar mechanism to glycaemic control, via activation of the AMPK pathway. Activation of this 1362

58

1363 pathway decreases the biosynthesis of lipids, specifically through inhibition of genes related to fatty acid biosynthesis, and also increases their breakdown and excretion. It is suggested that 1364 lipid oxidation, due to acetic acid for example, stimulates expression of certain oxidase 1365 enzymes, resulting in lipolysis (Samad, Azlan, & Ismail, 2016). The effect of acetic acid on 1366 postprandial satiety, as mentioned above, may also have a role in simply decreasing food, and 1367 thus fat, intake (Chen et al., 2016). Acetic acid, and thus vinegar, may have a role in reducing 1368 1369 hypertension (blood pressure) through inhibiting angiotensin-converting enzyme (ACE) which reduces plasma levels of the strong vasoconstrictive, angiotensin II (Samad et al., 2016). 1370

1371 Few studies on the anticancer properties of vinegar or acetic acid are available. The effect of kurosu, a Japanese rice vinegar on the proliferation of a number of human cancer cell lines, 1372 1373 including colon adenocarcinoma, lung carcinoma, breast adenocarcinoma, bladder carcinoma, 1374 and prostate carcinoma cells has been studied. It was reported to up-regulate the expression of enzymes involved in DNA repair and cell apoptosis in cells, and to inhibit the growth of all 1375 tested cell lines in a dose-dependent manner (Nanda et al., 2004). Kurosu was also shown by 1376 Baba et al. (2013) to inhibit the proliferation of human squamous cell carcinoma cells via 1377 programmed necrosis (Baba, Higashi, & Kanekura, 2013). The antioxidative nature of certain 1378 1379 types of vinegar is also postulated to have a role in anticancer activity. Kibizu, a Japanese 1380 vinegar made from sugar cane, inhibited the growth of human leukaemia cells due to its high 1381 radical-scavenging capacity (Mimura et al., 2004).

While there is significant evidence linking vinegar consumption to the modulation of blood glucose levels, scientific studies linking vinegar intake to other health benefits such as control of lipid levels and anticancer effects remain equivocal (Johnston & Gaas, 2006). As shown in Table 7, the majority of studies have been performed in animal models; therefore, more human studies, including large-scale and long-term clinical trials are required before any definitive health claims can be made (Karabiyikli & Sengun, 2017).

1388

1389 Health benefits associated with kombucha

1390 As reported by Dufresne and Farnworth (2000), a myriad of health benefits have been attributed to the consumption of kombucha, from protecting against diabetes to counteracting ageing and 1391 1392 improving eyesight, mostly based on drinkers testimonials, and few of which have been investigated scientifically (Dufresne & Farnworth, 2000). Those health benefits that have been 1393 studied have primarily been performed in animal models, with very little data related to studies 1394 on humans (Jayabalan et al., 2014). Many of the claimed beneficial effects of kombucha have 1395 been correlated with its antioxidant activity. This has been mainly attributed to the tea substrate 1396 and the presence of tea polyphenols, Vitamin C and D-saccharic acid-1,4-lactone (DSL). 1397 1398 Kombucha tea has a higher polyphenol content than un-fermented tea which is hypothesised to 1399 be due to structural modification of tea polyphenols by enzymes produced during fermentation by bacteria and yeast (Jayabalan et al., 2014). The polyphenol content of kombucha, and thus 1400 1401 its antioxidant capacity can be dependent on a number of factors, such as, the fermentation time 1402 and conditions, variety of tea substrate, and the kombucha culture microbiota, which itself can vary depending of the substrate and fermentation conditions, and which, in turn determines the 1403 1404 nature of the metabolites produced and present in the final beverage. In kombucha prepared with different types of tea, reducing power, hydroxyl radical scavenging ability, and anti-lipid 1405 1406 peroxidation were decreased, while total phenolic compounds and scavenging activity against DPPH and the superoxide radical increased with a prolonged fermentation time (Jayabalan, 1407 1408 Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008). Preparation of kombucha with different starter cultures of mixed AAB and a single yeast species on green tea had lower 1409 1410 antioxidant capacity compared to a native (complex culture) kombucha (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011). In a recent study, the polyphenol content of kombucha 1411 was shown to be dependent on fermentation temperature, with a higher level at low $(20^{\circ}C)$ 1412

1413 compared to high (30°C) temperatures. This was linked to the varying species dominance at
1414 the different temperatures (De Filippis, Troise, Vitaglione, & Ercolini, 2018).

Investigations in cell lines animal models found that kombucha can protect against 1415 1416 hepatotoxicity caused by several toxicants such as paracetamol, carbon-tetrachloride, aflatoxin B1 and acetaminophen (Jayabalan, Baskaran, Marimuthu, Swaminathan, & Yun, 2010; Pauline 1417 1418 et al., 2001; Wang et al., 2014). Bellassoued et al. (2015) demonstrated that the high thiobarbituric acid reactive substances (TBARS) concentration was significantly reduced in the 1419 1420 liver and kidney of rats fed with cholesterol-rich diets after the treatment with fermented tea 1421 (Bellassoued et al., 2015). The anti-toxigenic effect of kombucha has been attributed to its 1422 antioxidant activity and the hepatoprotective effects against acetaminophen were primarily attributed to the presence of DSL (Wang et al., 2014). Early investigations of the potential 1423 1424 health effects and detoxifying capacity of kombucha had primarily attributed the effects to its acidic composition; in particular, the ability of glucuronic acid to bind toxic components 1425 (glucuronidation) and to increase their excretion from the body was cited. However, the 1426 1427 possibility that what was actually being measured in those early studies as glucuronic acid was 1428 in fact 2KGA, has been debated (Dufresne & Farnworth, 2000). Indeed, the concentration of 1429 glucuronic acid in kombucha may be as much as ten thousand times lower than that of GA 1430 (Lončar, Petrovič, Malbača, & Verac, 2000).

Given that acetic acid is a primary metabolite of the microbial consortia in kombucha, it could be hypothesised that those health benefits attributed to the presence of acetic acid, as observed in vinegar, may also contribute to the potential health effects of kombucha, notwithstanding the difference in acetic acid concentration between both fermented products. Srihari et al. (2013a) observed that the daily administration for 45 days, of a kombucha extract, reduced glycated haemoglobin and increased the plasma insulin level, thus demonstrating an antiglycaemic effect. However, the authors attributed the effect mainly to the polyphenolic 1438 component of kombucha and non-specifically to the presence of organic acids and B-complex
1439 vitamins (Srihari, Karthikesan, Ashokkumar, & Satyanarayana, 2013a).

1440 Kombucha has for many decades been claimed by drinkers to have anticancer effects, even in 1441 a population study conducted in 1951 in Russia by the "Central Oncological Research Unit" and the Russian Academy of Sciences in Moscow. The antiproliferative activity of kombucha 1442 1443 produced from black tea has been demonstrated against a number of cancer cell lines, such as HeLa (cervix epithelial carcinoma) and HT-29 (colon adenocarcinoma) cells (Cetojevic-Simin, 1444 Bogdanovic, Cvetkovic, & Velicanski, 2008). In addition, an ethyl acetate extract of black tea 1445 1446 kombucha caused cytotoxic effects on 786-O (human renal carcinoma) and U2OS (human 1447 osteosarcoma) cells and significantly reduced cell invasion and motility of these cells in addition to A549 (human lung carcinoma) cells (Jayabalan et al., 2011). Srihari et al. (2013b) 1448 1449 demonstrated that a lyophilized extract of kombucha significantly reduced the survival of prostate cancer cells via down regulation of angiogenesis stimulators (Srihari, Arunkumar, 1450 Arunakaran, & Satyanarayana, 2013b). Again, the presence of polyphenols and their phenolic 1451 degradation products, as a consequence of fermentation, have been generally attributed as the 1452 1453 anticancer bioactive compounds in kombucha.

Similar to those studies on the health effects of vinegar, many of those investigating similar benefits associated with the consumption of kombucha tea have relied on in vitro and animal model investigations. Therefore, clinical trials and further in vivo evaluations are necessary in order to confirm the claimed health benefits of kombucha tea. In particular, it is questionable, and data is necessary to support such claims, as to whether the efficacious concentrations for the positive effects observed through in-vitro studies would be achievable in-vivo in humans.

62

1460 Regulatory aspects on the use of AAB as food cultures

The application of microbial food cultures in the production of fermented foods, used in both 1461 traditional backslopping practices and in recent decades as defined starter cultures, has an 1462 1463 important role in ensuring the quality and safety of these products, in addition to imparting desirable flavour, aroma and textural properties (Bourdichon et al., 2012a). Today, with the 1464 1465 large and every expanding variety of fermented foods and beverages that exists, and the possibility of applying new strains and species in foods, where they previously may not have 1466 been used, ensuring consumer safety is of prime importance. Following a brief description of 1467 1468 the regulations pertinent to microbial food cultures in the European Union (EU) and the United 1469 States of America (USA), aspects related to the application of AAB will be considered.

1470

1471 Microbial Food Cultures and Regulation in the European Union

1472 In the EU, the European Food Safety Authority (EFSA) operates the Qualified Presumption of 1473 Safety (QPS) approach (European Food Safety Authority, 2007). Hereby, a taxonomic unit 1474 (usually species) that is notified to the EFSA is pre-assessed for its safety based on aspects such 1475 as the associated body of knowledge, a history of apparent safe use in food, scientific literature, clinical aspects and industrial application. In this approach, familiarity is a critical aspect to 1476 support evidence of a microbial presumption of safety (Russo, Spano, & Capozzi, 2017). 1477 1478 Familiarity, as a concept, is "taken to include practical experience of use of the organism(s) including its history of use for particular purposes and any body of literature on the biology of 1479 1480 the taxonomic unit" (European Food Safety Authority, 2005), which could be translated to the body of knowledge supporting evidence for the historical use or consumption of such 1481 microorganisms in fermented foods, with no apparent adverse effects. For those 1482 microorganisms which have not traditionally been significantly consumed within the EU (prior 1483

to May 1997), or, are not generally associated with foods, the concept of a "novel food" has
been devised and is regulated separately (European Commission, 2015).

If a species is deemed safe by the EFSA Panel on Biological Hazards it is placed on the QPS 1486 1487 list which is published by EFSA (Ricci et al., 2017a). However, individual strains must still satisfy certain criteria, or qualifications, before being applied in food (and beverages) or feed. 1488 1489 As an example, a generic qualification for all bacterial taxonomic units on the OPS list is that strains should not harbour any acquired antimicrobial resistance genes to clinically relevant 1490 antimicrobials (Ricci et al., 2017a). In addition, as an alternative to exclusion from the list, 1491 1492 certain bacterial species may have specific qualifications placed on them, which give them QPS 1493 status, but only when used for a defined application (Leuschner et al., 2010). Thus, for those species that are on the QPS list, they may be permitted for use in food or feed once the strain(s) 1494 1495 being applied satisfy the attached qualifications. All microorganisms not on the QPS list remain subject to a full safety assessment (European Food Safety Authority, 2007). However, it is not 1496 entirely clear, at least from documents and literature from EFSA, what constitutes a full safety 1497 1498 assessment. To this end, Pariza et al. (2015) proposed a decision tree that could be used for the safety evaluation of both non-QPS and QPS strains (Pariza, Gillies, Kraak-Ripple, Leyer, & 1499 1500 Smith, 2015) (Figure 8). This will be discussed further below with relevance to AAB. In 1501 addition, microbial cultures assessed under the novel foods regulation (and not on the QPS list) 1502 must undergo a full safety assessment (Laulund, Wind, Derkx, & Zuliani, 2017).

The absence of a species from the QPS list does not necessarily imply a risk associated with its use. Individual strains may be safe, but the body of knowledge may not be sufficient to exclude any potential risk. In addition, the EFSA may not have been requested to date to evaluate the taxonomic unit in question (Bourdichon et al., 2012a). It is also possible that, for some microorganisms on the QPS list, EFSA may have been requested to evaluate a taxonomic unit for use in a defined application, and the scope of the evaluation may have been narrow, to exclude only the potential risks associated with the application for which the microorganism
was notified. Such microorganisms are included on the list, with a qualification of a specific
use (e.g. QPS applies only when used for vitamin production).

There remains some ambiguity and uncertainty around the QPS approach and regulation, as 1512 outlined by Laulund et al. (2017) (Laulund et al., 2017). It appears that microbial food cultures 1513 1514 with a history of safe use in food are considered as traditional food ingredients and are legally permitted for use in food in the EU without pre-market authorisation. On the other hand, those 1515 1516 with no history of use in foods would be considered as novel food, and require full safety assessment, as mentioned above. This seems to suggest that it would be possible to place a 1517 product on the market that uses microbial cultures that have not undergone any safety 1518 assessment, if the manufacture considers that such cultures have a historical safe use in food. 1519 1520 It also raises two questions. Firstly, what exactly defines microbial food cultures? Given the use of the wording "history of safe" and "traditional food ingredients", does this suggest 1521 complex, multi-strain cultures? What about single strain starter cultures? Secondly, how is a 1522 history of safe use defined and how can it be proven? To address these questions and the gaps 1523 in the regulation, the European Food and Feed Cultures Association (EFFCA) have proposed 1524 1525 a definition of food cultures, which outlines what constitutes a microbial food culture. Their 2015 definition defines microbial food cultures as "safe live bacteria, yeasts or moulds used in 1526 1527 food production, and they are in themselves a characteristic food ingredient. FC [food culture] preparations are formulations, consisting of concentrates (> 10^8 CFU per g or mL) of one or 1528 more live and active microbial species and/or strains, including unavoidable media components 1529 1530 carried over from the fermentation and components, which are necessary for their survival, 1531 storage and to facilitate their application in the food production process, and are in some cases 1532 standardised to a low count with carriers" (European Food and Feed Cultures Association, 2018). In addition, to address the question of cultures with a history of safe use in food, EFFCA 1533

1534 have, in association with the International Dairy Federation (IDF), compiled an "Inventory of Microorganisms with a documented history of use in food", the first inventory of FC with a 1535 documented significant use in food production before 1997 (Laulund et al., 2017). The first 1536 1537 IDF/EFFCA Inventory, published in 2002, and primarily addressing cultures used in the dairy fermentation industry, was updated in 2012 with an expanded scope to include microbial 1538 cultures used in a wider range of food products (including meat, vegetable, cereals, beverages 1539 and vinegar) (Bourdichon et al., 2012b). One of the main criteria for inclusion in the inventory 1540 1541 is a documented presence in fermented foods, and not just an incidental isolate (Bourdichon et 1542 al., 2012c).

Therefore, it appears that a food producer could conceivably place a product on the market that uses a single microbial culture or a number of cultures, which have an associated history of safe use in food, proven due to their inclusion in the IDF/EFFCA Inventory, and therefore without the need for pre-authorisation or safety assessment of the employed strains. However, it is likely that food producers would preferably opt to assess the safety of any strains employed as much as possible, primarily to ensure the safety of consumers, but secondly, to minimise liability, especially if the species is not on the QPS list.

1550

1551 Microbial Food Cultures and Regulation in the United States of America

In the USA, the Food and Drug Administration (FDA) operates the Generally Recognised as Safe (GRAS) system. This system is applied to a wide range of ingredients and is not specific to microbial FCs in the way that the EU QPS system is. Substances (including microbial cultures) added to food can be considered either as additives or GRAS substances. If they are considered as additives, then a pre-market authorisation by the FDA is required. However, if their use pre-dates 1958, or they have GRAS status, then no pre-market approval is required 1558 (Russo et al., 2017). Achieving GRAS status is, similar to the QPS system, built on evidence of safety; in the case of GRAS, this must be guaranteed, with reasonable certainty, through 1559 examination of the body of knowledge (typically scientific publications) and the assessment 1560 1561 and consensus of a panel of experts as to the safety of the substance or FC under its conditions 1562 for use (Bourdichon et al., 2012a). In contrast to QPS, the GRAS designation encompasses the substance / microbial culture and its specific application or usage (e.g. in a particular type of 1563 1564 product), rather than applying solely to the microorganism itself; thus, while for QPS the evaluation is at the level of the taxonomic unit (species), and applies to that unit independent 1565 1566 of application, GRAS designation can be at the species or strain level because the specific application (including formulation, dosages etc.) is considered (Ricci et al., 2017b). 1567

1568 From a legal standpoint, with a GRAS determination the onus and liability are placed on the 1569 food company, as a GRAS designation is issued based on evidence evaluated by the food manufacturer and the panel of experts engaged by that company. This is in contrast to the food 1570 additive designation, where the onus is placed to a greater degree on the FDA, where a full 1571 safety assessment by the authority is required. However, by law, neither a GRAS determination 1572 nor the notification to the FDA of the new use of a microbial culture is mandatory for a food 1573 1574 company (Russo et al., 2017). Nevertheless, performance of a safety evaluation and the 1575 attainment of GRAS status would serve to reduce the liability on the food manufacturer.

1576

1577 Safety aspects of AAB, with emphasis on G. oxydans

Unlike LAB which are today primarily applied as single, defined starter cultures, the application of AAB is still via undefined, mixed or complex cultures, where the mode of transfer of the microorganisms to the next fermentation is through the traditional process of "backslopping". This is, for example, the case for both traditional and industrial methods of 1582 acetous vinegar fermentation. This has been described to be, firstly, due to the nutritionally fastidious nature of AAB, which are difficult to preserve as a dried starter; and, secondly, 1583 vinegar is generally viewed as an inexpensive commodity and its production has therefore not 1584 warranted the development or use of expensive starter cultures (Solieri & Giudici, 2009). This 1585 1586 is also the case for other fermented foods in which AAB play a dominant role; thus, as their use has been seen as traditional and viewed as safe through a long history of use, this group of 1587 1588 microorganisms have not been considered to a large extent with regard to the regulations surrounding microbial FCs. However, with the increasing consumer interest in fermented food 1589 1590 products it is foreseeable that the industry will require the future development of defined AAB starter cultures, which will necessitate the safety evaluation of such strains. 1591

1592 The only AAB (and one of only two Gram-negative bacteria) listed on the QPS list is 1593 Gluconobacter oxydans (EFSA Panel on Biological Hazards, 2018). G. oxydans was assessed by the EFSA Panel on Biological Hazards for the first time in 2013 and recommended for the 1594 QPS list. The species was subject to a qualification, however; QPS only applies when the 1595 species is used for vitamin production (EFSA Panel on Biological Hazards, 2013). It is not 1596 stated in the Scientific Opinion exactly why the qualification was put in place; the panel noted 1597 1598 the general non-pathogenic nature of the genus and species to humans and animals (De Muynck 1599 et al., 2007), and a review of over 5,000 references raised no human or animal safety concerns. 1600 The possible pathogenic effect of [unidentified or unstated] Gluconobacter species was 1601 mentioned by two studies, however, these cases involved individuals with compromised immune systems (Alauzet et al., 2010; Bassetti et al., 2013). While the panel noted the reported 1602 rare occurrence of infections, as well as colonization with AAB in patients with underlying 1603 1604 chronic diseases and/or indwelling devices, and the potential of some Gluconobacter species 1605 (although not G. oxydans) to be opportunistic pathogens (Alauzet et al., 2010), no article reported safety concerns related to consumption of foods and feed. The potential for 1606

1607 monobactam antibiotic production by strains of G. oxydans, as reported in one study in 1982 1608 was noted (Wells et al., 1982), with a decision to follow this aspect in future QPS reviews. Nevertheless, the Panel placed G. oxydans on the QPS list with a qualification that it applies 1609 1610 only when the species is used for vitamin production. While it was not stated why the panel placed this qualification on the taxonomic unit, it was noted that the qualification was "relevant 1611 for the intended use for which the species was notified". Whether the Panel placed this 1612 1613 qualification on the taxonomic unit because of unanswered questions around the safety of the 1614 species, or, considered the safety of the species only in the context of the notified (narrow) 1615 application is not known (EFSA Panel on Biological Hazards, 2013). In the intervening time period to the present (August 2018), no new safety concerns were raised by EFSA and G. 1616 oxydans has been maintained on the QPS list (EFSA Panel on Biological Hazards, 2018). In 1617 1618 the USA, to date, no products using *Gluconobacter* species have been submitted to the FDA 1619 for GRAS designation (Food and Drug Administration, 2018).

The 2012 IDF/EFFCA Inventory lists 3 genera of AAB, encompassing 18 species. Species 1620 from the genera Acetobacter, Gluconacetobacter (some of which would now be 1621 Komagataeibacter) and Gluconobacter are included for their roles in vinegar, vegetable, coffee 1622 1623 and cocoa fermentations (Bourdichon et al., 2012b). Thus, the historic and safe use of these 1624 species, with relative certainty, is unquestionable. With regard to any future safety assessment 1625 of these species, the following paragraphs will outline some important considerations, using 1626 the decision tree outlined in Figure 8 to guide the discussion, and with emphasis on G. oxydans. 1627 Strain characterisation and genome sequencing: It is generally accepted today that bacterial isolates are identifiable to species level using molecular approaches such as 16S rRNA gene 1628 sequencing (Weisburg, Barns, Pelletier, & Lane, 1991). In addition, whole genome sequencing 1629 of strains is now commonly performed and can prove indispensable for the safety assessment, 1630

1631 providing additional insights into the genetic basis of strain safety. Different bioinformatics

1632 tools and databases can be used to screen for the presence of virulence factors and for antibiotic 1633 resistance genes e.g. microbial virulence factors can be screened for using the MvirDb database (Zhou et al., 2006), while the Comprehensive Antibiotic Resistance Database (McArthur et al., 1634 1635 2013) and ResFinder (Zankari et al., 2012) can be used to search for antibiotic resistance determinants (Laulund et al., 2017). Currently, in the National Center for Biotechnology 1636 Information (NCBI) database there are approximately 170 sequenced genomes of AAB strains 1637 1638 of the genera Acetobacter, Gluconacetobacter, Gluconobacter and Komagataeibacter, with 14 G. oxydans genomes, 5 of which are complete (NCBI, 2018). 1639

1640 Screening for undesirable attributes and metabolites: With regard to the AAB strain producing virulence factors or toxins, a whole genome sequence can be used to screen for genetic elements 1641 encoding such traits, as described above. Besides those studies detailed in the 2013 QPS update, 1642 1643 which highlighted the rare occurrence of AAB as potential opportunistic pathogens, such cases related to individuals with underlying chronic diseases and/or indwelling devices (EFSA Panel 1644 on Biological Hazards, 2013). In the case of G. oxydans, no further studies in the intervening 1645 time period raised any new concerns (EFSA Panel on Biological Hazards, 2018). In addition, 1646 their long history of safe use and the presence of many AAB species on the 2012 IDF/EFFCA 1647 1648 Inventory is testament, within the limits of reasonable certainty, to their safety when consumed 1649 by healthy individuals. Unlike the LAB, some species of which are biogenic amine producers, 1650 AAB have not been found to produce these toxigenic compounds (Landete, Ferrer, & Pardo, 1651 2007). However, in-vitro tests can be performed on strains to identify biogenic amine producers; in addition, the genome sequence of strains can be screened for putative responsible 1652 amino acid decarboxylase genes (or their homologues). The question of antibiotic resistance is 1653 1654 fundamental in assessing the safety of a strain. Chiefly, the strain must be free of functional 1655 and transferable antibiotic resistance genes. Proven intrinsic (natural) resistance is generally acceptable; the resistance determinant must not be transferable - this is to prevent the 1656

1657 horizontal or lateral transfer of (acquired) resistance to antimicrobials of human and veterinary 1658 importance from FC microorganisms to (potentially pathogenic) commensal microorganisms in the gut (Pariza et al., 2015). Unlike LAB, for which guidelines and procedures have been 1659 1660 developed by the regulatory authorities to inform the testing and interpretation of antibiotic susceptibilities of this microbial group (EFSA FEEDAP, 2012; International Organization for 1661 Standardization, 2010), no such distinct guidelines are in place for AAB. In this case, it is 1662 1663 recommended that methods described by the Clinical and Laboratory Standard Institute be 1664 used. Again, the genome sequence can also be useful to search for genes conferring antibiotic 1665 resistance, with particular focus on genes associated with mobile genetic elements (plasmids, conjugative transposons) that would be potentially transferrable (Laulund et al., 2017). In the 1666 2013 QPS update when G. oxydans was admitted to the QPS list, the Panel reported that no 1667 1668 evidence of resistance to antibiotics was found in any of the papers screened. According to only one publication, strains of some *Gluconobacter* species, although not *G. oxydans*, may be 1669 multi-resistant to some antimicrobial agents. In addition, such strains were isolated from 1670 1671 hospitalised patients and the nature of the resistance (intrinsic or acquired) was not investigated (Alauzet et al., 2010). When performing antibiotic susceptibility testing of strains, it is 1672 important to differentiate between intrinsic and acquired antibiotic resistance and have 1673 1674 knowledge of the potential for intrinsic resistance in the strains being assessed; for example, 1675 bacteria differ in their susceptibility to penicillin G; Gram-positive bacteria are generally 1676 sensitive, while most Gram-negative bacteria are naturally resistant because this compound 1677 cannot penetrate the outer cell membrane (Madigan et al., 2015). Gram-negative bacteria are also resistant to glycopeptide antibiotics (such as vancomycin) for the same reason. Although 1678 1679 arguable, for food products where the microbial culture has been killed or inactivated (through an intense pasteurisation, for example), it appears, at least in the past, that the presence of 1680 acquired microbial resistance was not considered to be an issue and was acceptable (Russo et 1681
al., 2017). However, if these dead cells are damaged or lysed in some way, either because of
food processing steps or passage through the gastrointestinal tract, it should be considered
whether mobile genetic elements carrying antibiotic resistance genes could still be released
into the gut environment and taken up, for example, via transformation by other
microorganisms present (European Food Safety Authority, 2008).

The ability of microbial food cultures to produce antimicrobial agents is also appreciable because such agents could select for resistance in the host bacterial population (Bourdichon et al., 2012a). As mentioned above, the potential for monobactam antibiotic production by strains of *G. oxydans* was reported in a 1982 study (EFSA Panel on Biological Hazards, 2013; Wells et al., 1982). The Panel stated the decision to follow this aspect in future QPS reviews; however, up to and including the latest QPS update, no further information has emerged.

In general, there is only a very small body of knowledge and few studies related to such safety aspects of AAB - antimicrobial susceptibility and potential pathogenicity. More research is required, research which will also inform regulatory authorities and enable the development of guidelines which should 1) guide academia and industry on the safety testing of AAB strains and 2) allow streamlining of the pre-market approval process for the use of such strains as starter cultures in both traditional and novel food products.

<u>Genetic modification</u>: If a strain has been isolated from a natural environment or system (e.g. a fermented food product produced by traditional methods), it is unlikely to have been genetically modified. If the strain has been genetically modified, regulatory approval and an assessment of the safety of the expressed product is required in many countries (Pariza, 2007).

<u>Strain origin</u>: Consideration of the origin of the microbial FC focuses on its isolation from a
food with a demonstrated history of safe consumption and its significant role in the production
and characteristics of that food. As outlined in previous sections in this review, AAB, in

particular members of the genera Acetobacter, Gluconobacter, Gluconacetobacter and 1706 1707 Komagataeibacter, are key members in fermented food products such as vinegar and kombucha (Table 1), which are often sources for the isolation of new strains. Such fermented 1708 1709 foods have a long history of safe consumption, particularly in Eastern Europe, Russia and Asia. Therefore, the long history of safe consumption of members of the AAB is self-evident. 1710 Nonetheless, non-food products such as flowers can also be sources of new AAB strains; the 1711 1712 acceptability of the use of such strains, not strictly satisfying the guidelines of having been 1713 isolated from food, could be an important topic for discussion.

1714 The safety of a strain can only be assessed based on the existing body of scientific knowledge. 1715 In addition, this knowledge should be assessed by an authoritative group and/or a group of qualified scientific experts (Pariza et al., 2015). While G. oxydans is on the QPS list, with 1716 1717 qualifications, no other AAB has yet been placed on the list. However, as outlined above, 8 species, including G. oxydans, are included in the 2012 IDF/EFFCA Inventory (Bourdichon et 1718 al., 2012b). Thus, the IDF/EFFCA Panel would be considered as an authoritative group of 1719 scientific experts, and their inclusion of these AAB in the Inventory should be considered as 1720 1721 an affirmation, with reasonable certainty, of their safety. Nevertheless, such expert evaluations 1722 should not be taken alone to constitute the absolute safety of a species and it is important to 1723 consider, particularly when dealing with individual strains, the safety of a culture with respect 1724 to the results of in-vitro tests and an analysis of the genome. In addition, it is noteworthy that 1725 the term safe or safety, in this context, means that that there is a reasonable certainty in the minds of competent scientists that a substance or microorganism is not harmful under the 1726 intended conditions of use. However, as observed by Lauland et al. (2017), it is impossible in 1727 1728 the present state of scientific knowledge to establish with complete certainty the absolute 1729 harmlessness of the use of any microorganism (Laulund et al., 2017). It is also important to

consider recent scientific findings that may have emerged since the last peer review or scientificevaluation, to ensure that the safety of a species is still valid.

Exposure levels: The level of exposure of consumers to any new microbial FC could be 1732 1733 dependent on a number of factors, such as the proposed applications of the strain, the number of different food products in which the strain will be applied, the amount of the strain (inoculum 1734 1735 and final cell counts) within the product and the (post-fermentation) product processing conditions. A key consideration is whether groups (e.g. age groups, groups with particular 1736 medical conditions) which have not traditionally consumed or been exposed to the strain, will 1737 1738 be thus through its use as a starter culture or probiotic (Laulund et al., 2017). With respect to AAB, the fact that certain populations may not have significantly consumed products produced 1739 by this group of microorganisms (i.e. acetous fermented food and beverages) to a large extent 1740 in the past should be considered e.g. Western European counties where kombucha has not 1741 traditionally been consumed. Nevertheless, if the consumption of such products has been 1742 considered, historically, to be safe in one population (e.g. in Asia), it is unlikely to be harmful 1743 to another, albeit the gastrointestinal microbiota might be diet dependent. 1744

Any processing performed on the final food or beverage product may also effect the level of 1745 exposure to a microbial FC. For example, pasteurisation processes, depending on the conditions 1746 chosen, may significantly reduce the number of viable cells remaining. In addition, there is the 1747 1748 option to completely inactivate the strain through an intense heat treatment. This may be advisable if there is a contraindication to having live cells in the final product. An example of 1749 1750 this is the approval by EFSA in 2015 of the use of Bacteroides xylanisolvens DSM 23964 as a novel food for use in fermented milk products, under the Novel Food Regulation No 258/97 1751 (EFSA Panel on Dietetic Products, 2015; European Commission, 1997). The usage of the strain 1752 was restricted to the fermentation of pasteurized milk products and only heat-treated (75°C for 1753 1 hour) and therefore inactivated cells of *B. xylanisolvens* were allowed in the final product. At 1754

1755 the same time, the EFSA Panel on Biological Hazards carried out a QPS assessment of the 1756 species; however, while no safety concerns could be identified the panel found that the published studies about *B. xylanisolvens* were too few and not sufficient to definitively exclude 1757 1758 safety issues. Therefore, the microorganism was not included on the QPS list. The fact that the strain under investigation had no history of use in the food industry, and no strain in the genus 1759 Bacteroides has a proven history of use in food production was also considered. In addition, a 1760 1761 gene encoding β -lactam antibiotic resistance was found in the genome. However, no mobile 1762 genetic elements were found, and the Panel considered the transfer of genes therefore unlikely 1763 due to this fact, in addition to the heat inactivation of the cells (Brodmann et al., 2017; EFSA Panel on Biological Hazards, 2015). Even though B. xylanisolvens was not placed on the QPS 1764 1765 list, the positive outcome in terms of designation as novel food opens the possibility of using other species or strains of bacteria as microbial FC, even if the body of knowledge is 1766 insufficient and/or safety concerns cannot be fully excluded; however, with the qualification 1767 that no viable cells remain in the product. This is noteworthy if the strain has been applied for 1768 reasons beyond its fermentative capacity - for example, as viability is an essential condition 1769 1770 for probiotic activity, inactivated cells should not be promoted for their potentially probiotic properties, and statements such as "contains live and active cultures" should not be made if the 1771 microbial FC has been inactivated. Nevertheless, non-viable cells may still elicit immunogenic 1772 effects and may have health benefits, hence, the term "paraprobiotic" has been coined to 1773 describe such cultures (Taverniti & Guglielmetti, 2011). 1774

As an aside, one commercial beverage product which is produced via a *Gluconobacter oxydans*fermentation of malt-base (wort, or similar sugar source) is Bionade® [patent: DE4012000A1].
Following the fermentation, the *G. oxydans* cells are stated to be removed via filtration
(www.bionade.de/en/production-process).

1779 In conclusion, in light of their long history of safe consumption, it appears unlikely that the application of certain species of AAB (e.g. Acetobacter, Gluconobacter) would pose a safety 1780 risk. However, in the interest of consumer safety it would be prudent that any strains of AAB, 1781 1782 applied as microbial FC in the future, would be thoroughly evaluated for their safety using a QPS approach or following a decision tree similar to that shown in Figure 8. However, 1783 1784 relatively few studies have been conducted with regard to the safety of this microbial group, which means that the body of scientific knowledge in the area is lacking, especially when 1785 compared to the LAB (for example, prevalence and breadth of resistance to antimicrobials, 1786 1787 production of toxins etc.). In addition, distinct guidelines from the responsible regulatory authorities (e.g. EFSA) are required, that deal with this important microbial group; however, it 1788 1789 is clear and understood that such guidelines can only be built on a pre-existing body of scientific 1790 knowledge.

1791 Conclusion

AAB are most commonly known for their role in vinegar production, yet, this diverse group of 1792 bacteria play an important part in the production of other fermented products such as kombucha 1793 1794 and water kefir. Their highly efficient oxidative metabolism is unique and can be harnessed for the production of compounds that find application not only in the food and beverage area e.g. 1795 1796 GA, ascorbic acid, cellulose. The diversity of uses for this group of bacteria is particularly highlighted by *Gluconobacter oxydans*. However, an understanding of their characteristics and 1797 metabolism is critical to maximising the potential of these bacteria, especially with respect to 1798 1799 their strict oxidative requirements. Therefore, such an understanding of their metabolism can 1800 be applied to enable process optimisations for decreased process times or increased product yields, for example. In addition, further research may likely uncover other additional metabolic 1801 1802 transformations performed by these bacteria which may have as yet unknown important applications. 1803

1804 With rising consumer interest in fermented foods and beverages, linked to purported health benefits (both scientifically proven and anecdotal) and an increasing strive by consumers 1805 towards a more health-conscious lifestyle, the AAB, like the LAB, are well poised for future 1806 1807 exploitation – both in the re-imagining of traditional foods or beverages, such as kombucha, and in the development of new types of products. However, further and more extensive studies 1808 and trials are needed with regard to the health benefits related to the consumption of AAB-1809 fermented products. In addition, guidance needs to be put forward by the relevant regulatory 1810 1811 authorities, regarding the safety aspects of the application of this important group of microorganisms. However, it is clear that more fundamental studies on the safety aspects of 1812 1813 this microbial group are required, upon which guidance from regulatory authorities can be based. 1814

1815 Acknowledgements

This work has been sponsored by and performed in collaboration with AB-InBev's GITeC the core Global Research & Development Centre, where a diverse team of scientists and specialists work diligently to achieve GITeC's Dream: bringing people together to create and deliver winning innovation and technologies. To find out more, navigate to: www.youtube.com/watch?v=cbJf0MuWbJw

1821

1822

1823 Author Contributions

1824 Kieran M. Lynch and Emanuele Zannini wrote the manuscript with critical input and 1825 corrections by Elke K. Arendt. Kieran M. Lynch did the final editing. Stuart Wilkinson and 1826 Luk Daenen critically evaluated the manuscript and provided valuable input and suggestions.

1827 Literature cited

- Adachi, O., Moonmangmee, D., Toyama, H., Yamada, M., Shinagawa, E., & Matsushita, K. (2003).
 New developments in oxidative fermentation. *Applied Microbiology and Biotechnology*, 60, 643-653. doi:10.1007/s00253-002-1155-9
- Adams, M. R. (1998). Vinegar. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods* (Second ed.,
 Vol. One, pp. 1-44). London: Blackie Academic & Professional.
- Alauzet, C., Teyssier, C., Jumas-Bilak, E., Gouby, A., Chiron, R., Rabaud, C., . . . Marchandin, H. (2010).
 Gluconobacter as well as *Asaia* species, newly emerging opportunistic human pathogens
 among acetic acid bacteria. *Journal of Clinical Microbiology, 48*, 3935-3942.
- Ameyama, M., Shinagawa, E., Matsushita, K., & Adachi, O. (1981a). D-fructose dehydrogenase of
 Gluconobacter industrius: purification, characterization, and application to enzymatic
 microdetermination of D-fructose. *Journal of Bacteriology*, *145*, 814-823.
- Ameyama, M., Shinagawa, E., Matsushita, K., & Adachi, O. (1981b). D-Glucose dehydrogenase of
 Gluconobacter suboxydans: solubilization, purification and characterization. *Agricultural and Biological Chemistry*, 45, 851-861.
- Aminifarshidmehr, N. (1996). The management of chronic suppurative otitis media with acid media
 solution. *The American Journal of Otology*, *17*, 24-25.
- Andrés-Barrao, C., & Barja, F. (2017). Acetic acid bacteria strategies contributing to acetic acid
 resistance during oxidative fermentation. In I. Y. Sengun (Ed.), *Acetic Acid Bacteria: Fundamentals and Food Applications* (pp. 92-119). Japan: CRC Press.
- Andrés-Barrao, C., Barja, F., Ortega Pérez, R., Chappuis, M.-L., Braito, S., & Hospital Bravo, A. (2017).
 Identification techniques of Acetic acid bacteria: Comparison between MALDI-TOF MS and
 molecular biology techniques. In I. Y. Sengun (Ed.), *Acetic Acid Bacteria: Fundamentals and Food Applications* (pp. 162-192). Florida, USA: CRC Press.
- Andrés-Barrao, C., Benagli, C., Chappuis, M., Ortega Perez, R., Tonolla, M., & Barja, F. (2013). Rapid
 identification of acetic acid bacteria using MALDI-TOF mass spectrometry fingerprinting.
 Systematic and Applied Microbiology, 36, 75-81. doi:10.1016/j.syapm.2012.09.002
- Andrés-Barrao, C., Saad, M. M., Chappuis, M. L., Boffa, M., Perret, X., Ortega Perez, R., & Barja, F.
 (2012). Proteome analysis of *Acetobacter pasteurianus* during acetic acid fermentation.
 Journal of Proteomics, 75, 1701-1717. doi:10.1016/j.jprot.2011.11.027
- Asakura, A., & Hoshino, T. (1999). Isolation and characterization of a new quinoprotein
 dehydrogenase, L-sorbose/L-sorbosone dehydrogenase. *Bioscience, Biotechnology, and Biochemistry, 63*, 46-53.
- Baba, N., Higashi, Y., & Kanekura, T. (2013). Japanese black vinegar "Izumi" inhibits the proliferation
 of human squamous cell carcinoma cells via necroptosis. *Nutrition and Cancer, 65*, 10931097.
- 1863Barry, T., Colleran, G., Glennon, M., Dunican, L. K., & Gannon, F. (1991). The 16s/23s ribosomal1864spacer region as a target for DNA probes to identify eubacteria. *Genome Research*, 1, 51-56.
- Bartowsky, E. J., & Henschke, P. A. (2008). Acetic acid bacteria spoilage of bottled red wine -- a
 review. International Journal of Food Microbiology, 125, 60-70.
 doi:10.1016/j.ijfoodmicro.2007.10.016
- Bassetti, M., Pecori, D., Sartor, A., Londero, A., Villa, G., Cadeo, B., . . . Stefani, S. (2013). First report
 of endocarditis by *Gluconobacter spp*. in a patient with a history of intravenous-drug abuse.
 Journal of Infection, 66, 285-287. doi:10.1016/j.jinf.2012.05.006
- 1871 Beheshti, Z., Chan, Y. H., Nia, H. S., Hajihosseini, F., Nazari, R., & Shaabani, M. (2012). Influence of 1872 apple cider vinegar on blood lipids. *Life Science Journal, 9*, 2431-2440.
- Bellassoued, K., Ghrab, F., Makni-Ayadi, F., Pelt, J. V., Elfeki, A., & Ammar, E. (2015). Protective effect
 of kombucha on rats fed a hypercholesterolemic diet is mediated by its antioxidant activity.
 Pharmaceutical Biology, *53*, 1699-1709.

1876 Berry, A., Lee, C., Mayer, A., & Shinjoh, M. (2003). Microbial production of L-ascorbic acid. (Patent EP 1877 2348113). 1878 Bertelli, D., Maietti, A., Papotti, G., Tedeschi, P., Bonetti, G., Graziosi, R., . . . Plessi, M. (2015). 1879 Antioxidant activity, phenolic compounds, and NMR characterization of Balsamic and 1880 Traditional Balsamic Vinegar of Modena. Food Analytical Methods, 8, 371-379. 1881 doi:10.1007/s12161-014-9902-y Beschkov, V., Velizarov, S., & Peeva, L. (1995). Some kinetic aspects and modelling of 1882 1883 biotransformation of D-glucose to keto-D-gluconates. *Bioprocess Engineering*, 13, 301-305. 1884 Blanc, P. J. (1996). Characterization of the tea fungus metabolites. Biotechnology Letters, 18, 139-1885 142. 1886 Bokulich, N. A., Bamforth, C. W., & Mills, D. A. (2012). Brewhouse-resident microbiota are 1887 responsible for multi-stage fermentation of American coolship ale. PLoS One, 7, e35507. 1888 Bourassa, L., & Butler-Wu, S. M. (2015). MALDI-TOF mass spectrometry for microorganism 1889 identification. In Methods in Microbiology (Vol. 42, pp. 37-85): Elsevier. 1890 Bourdichon, F., Berger, B., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., . . . Laulund, S. 1891 (2012c). Building an inventory of microbial food cultures with a technological role in 1892 fermented food products. Bulletin of the International Dairy Federation, 455, 13-21. 1893 Bourdichon, F., Boyaval, P., Casaregola, S., Dupont, J., Farrokh, C., Frisvad, J. C., . . . Laulund, S. 1894 (2012b). The 2012 Inventory of Microbial Species with technological beneficial role in 1895 fermented food products. Bulletin of the International Dairy Federation, 455, 22-61. 1896 Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., ... Hansen, E. 1897 B. (2012a). Food fermentations: microorganisms with technological beneficial use. 1898 International Journal of Food Microbiology, 154, 87-97. 1899 doi:10.1016/j.ijfoodmicro.2011.12.030 1900 Bourgeois, J. F., & Barja, F. (2009). The history of vinegar and of its acetification systems. Archieves 1901 des Sciences, 62, 147-160. 1902 Bremus, C., Herrmann, U., Bringer-Meyer, S., & Sahm, H. (2006). The use of microorganisms in L-1903 ascorbic acid production. Journal of Biotechnology, 124, 196-205. 1904 doi:10.1016/j.jbiotec.2006.01.010 1905 Brighenti, F., Castellani, G., Benini, L., Casiraghi, M. C., Leopardi, E., Crovetti, R., & Testolin, G. (1995). 1906 Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed 1907 meal in healthy subjects. European journal of Clinical Nutrition, 49, 242-247. 1908 Bringer, S., & Bott, M. (2016). Central carbon metabolism and respiration in *Gluconobacter oxydans*. 1909 In K. Matsushita, H. Toyama, N. Tonouchi, & A. Okamoto-Kainuma (Eds.), Acetic Acid 1910 Bacteria: Ecology and Physiology (pp. 235-253). Japan: Springer Nature. 1911 Brodmann, T., Endo, A., Gueimonde, M., Vinderola, G., Kneifel, W., de Vos, W. M., . . . Gómez-1912 Gallego, C. (2017). Safety of novel microbes for human consumption: practical examples of 1913 assessment in the European Union. Frontiers in Microbiology, 8, 1725. 1914 Budak, N. H., Aykin, E., Seydim, A. C., Greene, A. K., & Guzel-Seydim, Z. B. (2014). Functional 1915 properties of vinegar. Journal of Food Science, 79, R757-R764. doi:10.1111/1750-3841.12434 1916 Buse, R., Qazi, G. N., & Onken, U. (1992). Influence of constant and oscillating dissolved oxygen 1917 concentrations on keto acid production by Gluconobacter oxydans subsps. melanogenum. 1918 Journal of Biotechnology, 26, 231-244. 1919 Camu, N., Gonzalez, A., De Winter, T., Van Schoor, A., De Bruyne, K., Vandamme, P., . . . De Vuyst, L. 1920 (2008). Influence of turning and environmental contamination on the dynamics of 1921 populations of lactic acid and acetic acid bacteria involved in spontaneous cocoa bean heap 1922 fermentation in Ghana. Applied and Environmental Microbiology, 74, 86-98. 1923 doi:10.1128/AEM.01512-07 1924 Cañete-Rodríguez, A. M., Santos-Dueñas, I. M., Jiménez-Hornero, J. E., Ehrenreich, A., Liebl, W., & 1925 García-García, I. (2016). Gluconic acid: Properties, production methods and applications—An 1926 excellent opportunity for agro-industrial by-products and waste bio-valorization. Process Biochemistry, 51, 1891-1903. doi:10.1016/j.procbio.2016.08.028 1927 1928 Castro, C., Zuluaga, R., Putaux, J.-L., Caro, G., Mondragon, I., & Gañán, P. (2011). Structural 1929 characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from 1930 Colombian agroindustrial wastes. Carbohydrate Polymers, 84, 96-102. 1931 doi:10.1016/j.carbpol.2010.10.072 1932 Cetojevic-Simin, D. D., Bogdanovic, G. M., Cvetkovic, D. D., & Velicanski, A. S. (2008). 1933 Antiproliferative and antimicrobial activity of traditional Kombucha and Satureja montana L. 1934 Kombucha. Journal of BUON, 13, 395-401. 1935 Chang, J. M., & Fang, T. J. (2007). Survival of Escherichia coli O157:H7 and Salmonella enterica 1936 serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against 1937 E. coli O157:H7. Food Microbiology, 24, 745-751. doi:10.1016/j.fm.2007.03.005 1938 Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during 1939 prolonged fermentation. Journal of Applied Microbiology, 89, 834-839. 1940 Chen, F., & Gullo, M. (2015). 4th international conference on acetic acid bacteria-vinegar and other 1941 products (AAB 2015). Acetic Acid Bacteria, 4. 1942 Chen, H., Chen, T., Giudici, P., & Chen, F. (2016). Vinegar functions on health: Constituents, sources, 1943 and formation mechanisms. Comprehensive Reviews in Food Science and Food Safety, 15, 1944 1124-1138. doi:10.1111/1541-4337.12228 1945 Chinese National Standard. (2004). Edible vinager. No. 14834, N5239. 1946 Cleenwerck, I., & De Vos, P. (2008). Polyphasic taxonomy of acetic acid bacteria: an overview of the 1947 currently applied methodology. International Journal of Food Microbiology, 125, 2-14. 1948 doi:10.1016/j.ijfoodmicro.2007.04.017 1949 Cleenwerck, I., De Vos, P., & De Vuyst, L. (2010). Phylogeny and differentiation of species of the 1950 genus Gluconacetobacter and related taxa based on multilocus sequence analyses of 1951 housekeeping genes and reclassification of Acetobacter xylinus subsp. sucrofermentans as 1952 Gluconacetobacter sucrofermentans (Toyosaki et al. 1996) sp. nov., comb. nov. International 1953 Journal of Systematic and Evolutionary Microbiology, 60, 2277-2283. 1954 doi:10.1099/ijs.0.018465-0 1955 Cleenwerck, I., De Wachter, M., González, Á., De Vuyst, L., & De Vos, P. (2009). Differentiation of 1956 species of the family Acetobacteraceae by AFLP DNA fingerprinting: Gluconacetobacter 1957 kombuchae is a later heterotypic synonym of Gluconacetobacter hansenii. International 1958 Journal of Systematic and Evolutionary Microbiology, 59, 1771-1786. 1959 Codex Alimentarius Commission. (1987). Draft european regional standard for vinegar. 1960 Croxatto, A., Prod'hom, G., & Greub, G. (2012). Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Reviews, 36, 380-407. doi:10.1111/j.1574-1961 1962 6976.2011.00298.x 1963 Dabija, A., & Hatnean, C. A. (2014). Study concerning the quality of apple vinegar obtained through 1964 classical method. Journal of Agroalimentary Processes and Technologies, 20, 304-310. 1965 Dağbağlı, S., & Göksungur, Y. (2017). Exopolysaccharide production of acetic acid bacteria. In I. Y. 1966 Sengun (Ed.), Acetic Acid Bacteria: Fundamentals and Food Applications (pp. 120-141). 1967 Florida, USA: CRC Press. 1968 De Dios Lozano, J., Juárez-Flores, B. I., Pinos-Rodríguez, J. M., Aguirre-Rivera, J. R., & Álvarez-Fuentes, 1969 G. (2012). Supplementary effects of vinegar on body weight and blood metabolites in 1970 healthy rats fed conventional diets and obese rats fed high-caloric diets. Journal of Medicinal 1971 Plants Research, 6, 4135-4141. 1972 De Filippis, F., Troise, A. D., Vitaglione, P., & Ercolini, D. (2018). Different temperatures select 1973 distinctive acetic acid bacteria species and promotes organic acids production during 1974 Kombucha tea fermentation. Food Microbiology, 73, 11-16. doi:10.1016/j.fm.2018.01.008 1975 De Keersmaecker, J. (1996). The mystery of lambic beer. Scientific American, 275, 74-80.

1976 De Muynck, C., Pereira, C. S., Naessens, M., Parmentier, S., Soetaert, W., & Vandamme, E. J. (2007).
1977 The genus *Gluconobacter oxydans*: comprehensive overview of biochemistry and
1978 biotechnological applications. *Critical Reviews in Biotechnology, 27*, 147-171.
1979 doi:10.1080/07388550701503584

1980 De Roos, J., & De Vuyst, L. (2018a). Acetic acid bacteria in fermented foods and beverages. *Current* 1981 *Opinion in Biotechnology, 49*, 115-119. doi:10.1016/j.copbio.2017.08.007

- De Roos, J., & De Vuyst, L. (2018b). Microbial acidification, alcoholization, and aroma production
 during spontaneous lambic beer production. *Journal of the Science of Food and Agriculture*,
 In Press. doi:10.1002/jsfa.9291
- 1985De Roos, J., Van der Veken, D., & De Vuyst, L. (2018b). The interior surfaces of wooden barrels are an1986additional microbial inoculation source for lambic beer production. Applied and1987Environmental Microbiology, In Press. doi:10.1128/AEM.02226-18
- De Roos, J., Verce, M., Aerts, M., Vandamme, P., & De Vuyst, L. (2018a). Temporal and spatial
 distribution of the acetic acid bacterium communities throughout the wooden casks used for
 the fermentation and maturation of lambic beer underlines their functional role. *Applied and Environmental Microbiology, 84*, AEM. 02846-02817.
- 1992De Vero, L., Gala, E., Gullo, M., Solieri, L., Landi, S., & Giudici, P. (2006). Application of denaturing1993gradient gel electrophoresis (DGGE) analysis to evaluate acetic acid bacteria in traditional1994balsamic vinegar. Food Microbiology, 23, 809-813. doi:10.1016/j.fm.2006.01.006
- 1995 De Vero, L., Gullo, M., & Giudici, P. (2017). Preservation of Acetic acid bacteria. In I. Y. Sengun (Ed.), 1996 Acetic Acid Bacteria: Fundamentals and Food Applications (pp. 193-209). Japan: CRC Press.
- 1997De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. Food1998Technology and Biotechnology, 38, 105-112.
- De Vuyst, L., Camu, N., De Winter, T., Vandemeulebroecke, K., Van de Perre, V., Vancanneyt, M., . . .
 Cleenwerck, I. (2008). Validation of the (GTG)(5)-rep-PCR fingerprinting technique for rapid
 classification and identification of acetic acid bacteria, with a focus on isolates from
 Ghanaian fermented cocoa beans. *International Journal of Food Microbiology*, *125*, 79-90.
 doi:10.1016/j.ijfoodmicro.2007.02.030
- Deppenmeier, U., & Ehrenreich, A. (2009). Physiology of acetic acid bacteria in light of the genome
 sequence of *Gluconobacter oxydans*. *Journal of Molecular Microbiology and Biotechnology*,
 16, 69-80. doi:10.1159/000142895
- 2007Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: a review. Food Research2008International, 33, 409-421.
- 2009 Dutta, D., & Gachhui, R. (2007). Nitrogen-fixing and cellulose-producing *Gluconacetobacter* 2010 *kombuchae* sp. nov., isolated from Kombucha tea. *International Journal of Systematic and* 2011 *Evolutionary Microbiology, 57*, 353-357.
- 2012 Ebihara, K., & Nakajima, A. (1988). Effect of acetic acid and vinegar on blood glucose and insulin
 2013 responses to orally administered sucrose and starch. *Agricultural and Biological Chemistry*,
 2014 52, 1311-1312.
- 2015 Ebner, H., Sellmer, Sylvia, Follmann, Heinrich. (2008). Acetic Acid. In H. J. Rehm, Reed, G. (Ed.),
 2016 Ullmann's Encyclopedia of Industrial Chemistry.
- 2017 EFSA FEEDAP. (2012). Panel on additives products or substances used in animal feed (FEEDAP),
 2018 guidance on the assessment of bacterial susceptibility to antimicrobials of human and
 2019 veterinary importance. *EFSA Journal*, 10, 2740.
- 2020 EFSA Panel on Biological Hazards. (2013). Scientific Opinion on the maintenance of the list of QPS
 2021 biological agents intentionally added to food and feed (2013 update). *EFSA Journal, 11*,
 2022 3449.
- 2023EFSA Panel on Biological Hazards. (2015). Statement on the update of the list of QPS-recommended2024biological agents intentionally added to food or feed as notified to EFSA 1: Suitability of2025taxonomic units notified to EFSA until October 2014. EFSA Journal, 13, 4138.

- EFSA Panel on Biological Hazards. (2018). Update of the list of QPS-recommended biological agents
 intentionally added to food or feed as notified to EFSA 8: suitability of taxonomic units
 notified to EFSA until March 2018. *EFSA Journal, 16*, e05315.
- EFSA Panel on Dietetic Products, N. A. (2015). Scientific Opinion on the safety of 'heat-treated milk
 products fermented with Bacteroides xylanisolvens DSM 23964'as a novel food. *EFSA Journal, 13*, 3956.
- 2032Emde, F. (2006). State-of-the-art technologies in submersible vinegar production. Paper presented at2033the Second Symposium on R+D+I for Vinegar Production: Córdoba, Abril 2006.
- 2034 Emde, F. (2014). Vinegar. *Ullmann's Encyclopedia of Industrial Chemistry*, 1-24.
- Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y., & Ohta, M. (1998). Antibacterial action of vinegar
 against food-borne pathogenic bacteria including *Escherichia coli* O157: H7. *Journal of Food Protection, 61*, 953-959.
- 2038European Commission. (1997). Regulation (EC) No. 258/97 of the European Parliament and of the2039Council of 27 January 1997 concerning novel foods and novel food ingredients.
- European Commission. (2008). Regulation (EC) No 1331/2008 of the European Parliament and of the
 Council of 16 December 2008 establishing a common authorisation procedure for food
 additives, food enzymes and food flavourings.
- 2043European Commission. (2015). Regulation (EU) 2015/2283 of the European Parliament and of the2044Council of 25 November 2015 on novel foods, amending regulation (EU) No 1169/2011 of2045the European Parliament and of the Council and Repealing Regulation (EC) No 258/97 of the2046European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.
- 2047European Commission. (2016). Regulation (EU) 2016/263 of 25 February 2016 amending Annex II to2048Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the2049title of the food category 12.3 Vinegars.
- 2050European Food and Feed Cultures Association. (2018). Definition of Food Cultures (FC). Available2051online: https://effca.org/microbial-cultures/about-food-cultures/ (accessed on 20 August20522018).
- 2053European Food Safety Authority. (2005). Opinion of the Scientific Committee on a request from EFSA2054related to a generic approach to the safety assessment by EFSA of microorganisms used in2055food/feed and the production of food/feed additives. EFSA Journal (226), 1-12.
- European Food Safety Authority. (2007). Introduction of a Qualified Presumption of Safety (QPS)
 approach for assessment of selected microorganisms referred to EFSA-Opinion of the
 Scientific Committee. *EFSA Journal*, *5*, 587.
- 2059European Food Safety Authority. (2008). Foodborne antimicrobial resistance as a biological hazard-2060Scientific Opinion of the Panel on Biological Hazards. *EFSA Journal, 6*, 765.
- Fan, J., Zhang, Y., Chang, X., Zhang, B., Jiang, D., Saito, M., & Li, Z. (2009). Antithrombotic and
 fibrinolytic activities of methanolic extract of aged sorghum vinegar. *Journal of Agricultural and Food Chemistry*, *57*, 8683-8687. doi:10.1021/jf901680y
- Feng, S., Zhang, Z., & Zhang, C. (2000). Effect of *Bacillus megaterium* on *Gluconobacter oxydans* in
 mixed culture. *Ying Yong Sheng Tai Xue Bao, 11*, 119-122.
- Fiorda, F. A., de Melo Pereira, G. V., Thomaz-Soccol, V., Rakshit, S. K., Pagnoncelli, M. G. B.,
 Vandenberghe, L. P. S., & Soccol, C. R. (2017). Microbiological, biochemical, and functional
 aspects of sugary kefir fermentation A review. *Food Microbiology, 66*, 86-95.
 doi:10.1016/j.fm.2017.04.004
- Food and Drug Administration. (2018). GRAS Notices;
 https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices (accessed 21 November
- 2072 2018).
- 2073 Food Safety and Standards Authority of India. (2012). Manual of methods of analysis of foods.
- 2074 Food Standards Australia New Zealand Act. (1991). Vinegar and Related Products.
- 2075 *Food: A Culinary History*. (2000). New York: Columbia University Press.

- Fushimi, T., Suruga, K., Oshima, Y., Fukiharu, M., Tsukamoto, Y., & Goda, T. (2006). Dietary acetic
 acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *British Journal of Nutrition, 95*, 916-924.
- Gao, L., Zhou, J., Liu, J., Du, G., & Chen, J. (2012). Draft genome sequence of *Gluconobacter oxydans* WSH-003, a strain that is extremely tolerant of saccharides and alditols. *Journal of Bacteriology, 194*, 4455-4456. doi:10.1128/JB.00837-12
- 2082García-García, I., Cañete-Rodríguez, A. M., Santos-Dueñas, I. M., Jiménez-Hornero, J. E., Ehrenreich,2083A., Liebl, W., . . . Mauricio, J. C. (2017). Biotechnologically relevant features of gluconic acid2084production by acetic acid bacteria. Acetic Acid Bacteria, 6. doi:10.4081/aab.2017.6458
- 2085 Garcia-Parrilla, M. C., Torija, M. J., Mas, A., Cerezo, A. B., & Troncoso, A. M. (2017). Vinegars and
 2086 other fermented condiments. In J. Frias, C. Martinez-Villaluenga, & E. Peñas (Eds.),
 2087 *Fermented Foods in Health and Disease Prevention* (First ed., pp. 577-591). London, U.K.:
 2088 Academic Press.
- 2089 Ge, X., Zhao, Y., Hou, W., Zhang, W., Chen, W., Wang, J., . . . Xiong, X. (2013). Complete genome
 2090 sequence of the industrial strain *Gluconobacter oxydans* H24. *Genome Announcements, 1*.
 2091 doi:10.1128/genomeA.00003-13
- 2092 Giudici, P., De Vero, L., & Gullo, M. (2017). Vinegars. In I. Y. Sengun (Ed.), Acetic Acid Bacteria:
 2093 Fundamentals and Food Applications (pp. 261-287). Japan: CRC Press.
- Gómez-Manzo, S., Chavez-Pacheco, J. L., Contreras-Zentella, M., Sosa-Torres, M. E., Arreguín Espinosa, R., De La Mora, M. P., . . . Escamilla, J. E. (2010). Molecular and catalytic properties
 of the aldehyde dehydrogenase of *Gluconacetobacter diazotrophicus*, a quinoheme protein
 containing pyrroloquinoline quinone, cytochrome b, and cytochrome c. *Journal of Bacteriology*, *192*, 5718-5724.
- Gomez-Manzo, S., Escamilla, J. E., Gonzalez-Valdez, A., Lopez-Velazquez, G., Vanoye-Carlo, A.,
 Marcial-Quino, J., . . . Sosa-Torres, M. E. (2015). The oxidative fermentation of ethanol in
 Gluconacetobacter diazotrophicus is a two-step pathway catalyzed by a single enzyme:
 alcohol-aldehyde Dehydrogenase (ADHa). *International Journal of Molecular Sciences, 16*,
 1293-1311. doi:10.3390/ijms16011293
- Gomez-Manzo, S., Gonzalez-Valdez, A. A., Oria-Hernandez, J., Reyes-Vivas, H., Arreguin-Espinosa, R.,
 Kroneck, P. M., . . . Escamilla, J. E. (2012). The active (ADHa) and inactive (ADHi) forms of the
 PQQ-alcohol dehydrogenase from *Gluconacetobacter diazotrophicus* differ in their
 respective oligomeric structures and redox state of their corresponding prosthetic groups.
 FEMS Microbiology Letters, 328, 106-113. doi:10.1111/j.1574-6968.2011.02487.x
- Gonzalez, A., & De Vuyst, L. (2009). Vinegars from tropical Africa. In *Vinegars of the World* (pp. 209-221): Springer.
- 2111 Gonzalez, A., Hierro, N., Poblet, M., Mas, A., & Guillamon, J. M. (2005). Application of molecular
 2112 methods to demonstrate species and strain evolution of acetic acid bacteria population
 2113 during wine production. *International Journal of Food Microbiology, 102*, 295-304.
 2114 doi:10.1016/j.ijfoodmicro.2004.11.020
- Gonzalez, A., & Mas, A. (2011). Differentiation of acetic acid bacteria based on sequence analysis of
 16S-23S rRNA gene internal transcribed spacer sequences. *International Journal of Food Microbiology, 147*, 217-222. doi:10.1016/j.ijfoodmicro.2011.04.005
- Greenberg, D. E., Porcella, S. F., Stock, F., Wong, A., Conville, P. S., Murray, P. R., . . . Zelazny, A. M.
 (2006). *Granulibacter bethesdensis* gen. nov., sp. nov., a distinctive pathogenic acetic acid
 bacterium in the family *Acetobacteraceae*. *International Journal of Systematic and Evolutionary Microbiology*, *56*(11), 2609-2616.
- Gu, X., Zhao, H.-L., Sui, Y., Guan, J., Chan, J. C. N., & Tong, P. C. Y. (2012). White rice vinegar improves
 pancreatic beta-cell function and fatty liver in streptozotocin-induced diabetic rats. *Acta Diabetologica, 49*, 185-191.

- 2125Guerreiro, T. M., de Oliveira, D. N., Ferreira, M. S., & Catharino, R. R. (2014). High-throughput2126analysis by SP-LDI-MS for fast identification of adulterations in commercial balsamic2127vinegars. Analytica Chimica Acta, 838, 86-92. doi:10.1016/j.aca.2014.06.009
- Gulitz, A., Stadie, J., Ehrmann, M. A., Ludwig, W., & Vogel, R. F. (2013). Comparative phylobiomic
 analysis of the bacterial community of water kefir by 16S rRNA gene amplicon sequencing
 and ARDRA analysis. *Journal of Applied Microbiology*, *114*, 1082-1091.
- Gullo, M., Caggia, C., De Vero, L., & Giudici, P. (2006). Characterization of acetic acid bacteria in
 "traditional balsamic vinegar". *International Journal of Food Microbiology*, *106*, 209-212.
 doi:10.1016/j.ijfoodmicro.2005.06.024
- Gullo, M., & Giudici, P. (2008). Acetic acid bacteria in traditional balsamic vinegar: phenotypic traits
 relevant for starter cultures selection. *International Journal of Food Microbiology*, *125*, 46 doi:10.1016/j.ijfoodmicro.2007.11.076
- Gullo, M., Verzelloni, E., & Canonico, M. (2014). Aerobic submerged fermentation by acetic acid
 bacteria for vinegar production: Process and biotechnological aspects. *Process Biochemistry*,
 49, 1571-1579. doi:10.1016/j.procbio.2014.07.003
- Halstead, F. D., Rauf, M., Moiemen, N. S., Bamford, A., Wearn, C. M., Fraise, A. P., . . . Webber, M. A.
 (2015). The antibacterial activity of acetic acid against biofilm-producing pathogens of
 relevance to burns patients. *PLoS One, 10*, e0136190. doi:10.1371/journal.pone.0136190
- Hancock, R. D., & Viola, R. (2002). Biotechnological approaches for L-ascorbic acid production.
 Trends in Biotechnology, 20, 299-305.
- Hanke, T., Noh, K., Noack, S., Polen, T., Bringer, S., Sahm, H., . . . Bott, M. (2013). Combined fluxomics
 and transcriptomics analysis of glucose catabolism via a partially cyclic pentose phosphate
 pathway in *Gluconobacter oxydans* 621H. *Applied and Environmental Microbiology, 79*,
 2336-2348. doi:10.1128/AEM.03414-12
- Hanke, T., Richhardt, J., Polen, T., Sahm, H., Bringer, S., & Bott, M. (2012). Influence of oxygen
 limitation, absence of the cytochrome bc1 complex and low pH on global gene expression in *Gluconobacter oxydans* 621H using DNA microarray technology. *Journal of Biotechnology*,
 157, 359-372.
- Hermann, M., Petermeier, H., & Vogel, R. F. (2015). Development of novel sourdoughs with in situ
 formed exopolysaccharides from acetic acid bacteria. *European Food Research and Technology, 241*, 185-197. doi:10.1007/s00217-015-2444-8
- Higashide, T., Okumura, H., Kawamura, Y., Teranishi, K., Hisamatsu, M., & Yamada, T. (1996).
 Membrane components and cell form of *Acetobacter polyoxogenes* (vinegar producing strain) under high acidic conditions. *Journal of the Japanese Society for Food Science and Technology, 43*, 117-123.
- 2160 Hirshfield, I. N., Terzulli, S., & O'Byrne, C. (2003). Weak organic acids: a panoply of effects on
 2161 bacteria. *Science Progress, 86*, 245-269.
- Hlebowicz, J., Darwiche, G., Bjorgell, O., & Almer, L. O. (2007). Effect of apple cider vinegar on
 delayed gastric emptying in patients with type 1 diabetes mellitus: a pilot study. *BMC Gastroenterology, 7*, 46. doi:10.1186/1471-230X-7-46
- Ho, C. W., Lazim, A. M., Fazry, S., Zaki, U., & Lim, S. J. (2017). Varieties, production, composition and
 health benefits of vinegars: A review. *Food Chemistry*, 221, 1621-1630.
 doi:10.1016/j.foodchem.2016.10.128
- Hoelscher, T., Weinert-Sepalage, D., & Goerisch, H. (2007). Identification of membrane-bound
 quinoprotein inositol dehydrogenase in *Gluconobacter oxydans* ATCC 621H. *Microbiology*,
 153, 499-506.
- Hoshino, T., Sugisawa, T., & Fujiwara, A. (1991). Isolation and characterization of NAD (P)-dependent
 L-sorbosone dehydrogenase from *Gluconobacter melanogenus* UV10. *Agricultural and Biological Chemistry, 55*, 665-670.
- Hutkins, R. W. (2006). Vinegar Fermentation. In *Microbiology and Technology of Fermented Foods* (pp. 397-417). Iowa, USA: Blackwell Publishing.

- 2176 lizuka, M., Tani, M., Kishimoto, Y., Saita, E., Toyozaki, M., & Kondo, K. (2010). Inhibitory effects of
 2177 balsamic vinegar on LDL oxidation and lipid accumulation in THP-1 macrophages. *Journal of* 2178 *Nutritional Science and Vitaminology, 56*, 421-427.
- 2179 Illeghems, K., De Vuyst, L., & Weckx, S. (2013). Complete genome sequence and comparative
 2180 analysis of *Acetobacter pasteurianus* 386B, a strain well-adapted to the cocoa bean
 2181 fermentation ecosystem. *BMC Genomics*, *14*, 526.
- Inagaki, S., Morimura, S., Gondo, K., Tang, Y., Akutagawa, H., & Kida, K. (2007). Isolation of
 tryptophol as an apoptosis-inducing component of vinegar produced from boiled extract of
 black soybean in human monoblastic leukemia U937 cells. *Bioscience, Biotechnology, and Biochemistry, 71*, 371-379. doi:10.1271/bbb.60336
- International Organization for Standardization. (2010). ISO 10932:2010—Milk and Milk Products—
 Determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to
 bifidobacteria and non-enterococcal lactic acid bacteria (LAB). International Organization for
 Standardization: Geneva, Switzerland.
- Ishida, T., Sugano, Y., & Shoda, M. (2002). Novel glycosyltransferase genes involved in the acetan
 biosynthesis of Acetobacter xylinum. Biochemical and Biophysical Research Communications,
 295, 230-235.
- Jakob, F., Pfaff, A., Novoa-Carballal, R., Rubsam, H., Becker, T., & Vogel, R. F. (2013). Structural
 analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid
 function. *Carbohydrate Polymers*, *92*, 1234-1242. doi:10.1016/j.carbpol.2012.10.054
- Jakob, F., Steger, S., & Vogel, R. F. (2012). Influence of novel fructans produced by selected acetic
 acid bacteria on the volume and texture of wheat breads. *European Food Research and Technology, 234*, 493-499. doi:10.1007/s00217-011-1658-7
- Jarrell, J., Cal, T., & Bennett, J. W. (2000). The Kombucha consortia of yeasts and bacteria.
 Mycologist, 14, 166-170.
- Jayabalan, R., Baskaran, S., Marimuthu, S., Swaminathan, K., & Yun, S. E. (2010). Effect of Kombucha
 tea on Aflatoxin B1 induced acute hepatotoxicity in Albino rats-prophylactic and curative
 studies. Journal of the Korean Society for Applied Biological Chemistry, 53, 407-416.
- Jayabalan, R., Chen, P.-N., Hsieh, Y.-S., Prabhakaran, K., Pitchai, P., Marimuthu, S., . . . Yun, S. E.
 (2011). Effect of solvent fractions of kombucha tea on viability and invasiveness of cancer
 cells—characterization of dimethyl 2-(2-hydroxy-2-methoxypropylidine) malonate and
 vitexin. *NISCAIR-CSIR*.
- Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on
 Kombucha tea-Microbiology, composition, fermentation, beneficial effects, toxicity, and tea
 fungus. *Comprehensive Reviews in Food Science and Food Safety, 13*, 538-550.
 doi:10.1111/1541-4337.12073
- Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008).
 Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, *109*, 227-234. doi:10.1016/j.foodchem.2007.12.037
- Ji, A., & Gao, P. (2001). Substrate selectivity of *Gluconobacter oxydans* for production of 2, 5-diketo d-gluconic acid and synthesis of 2-keto-l-gulonic acid in a multienzyme system. *Applied Biochemistry and Biotechnology, 94*, 213-223.
- 2218Jia, S., Ou, H., Chen, G., Choi, D., Cho, K., Okabe, M., & Cha, W. S. (2004). Cellulose production from2219Gluconobacter oxydans TQ-B2. Biotechnology and Bioprocess Engineering, 9, 166.
- Jiao, Y., Zhang, W., Xie, L., Yuan, H., & Chen, M. (2002). Effects of Bacillus cereus on *Gluconobacter oxydans* in vitamin C fermentation process. *Wei sheng wu xue tong bao, 29*, 35-38.
- Johnston, C. S., & Gaas, C. A. (2006). Vinegar: medicinal uses and antiglycemic effect. *Medscape General Medicine*, *8*, 61.
- Johnston, C. S., Kim, C. M., & Buller, A. J. (2004). Vinegar improves insulin sensitivity to a high carbohydrate meal in subjects with insulin resistance or type 2 diabetes. *Diabetes Care, 27*,
 281-282.

- Johnston, C. S., Quagliano, S., & White, S. (2013). Vinegar ingestion at mealtime reduced fasting
 blood glucose concentrations in healthy adults at risk for type 2 diabetes. *Journal of Functional Foods, 5*, 2007-2011. doi:10.1016/j.jff.2013.08.003
- Johnston, C. S., Steplewska, I., Long, C. A., Harris, L. N., & Ryals, R. H. (2010). Examination of the
 antiglycemic properties of vinegar in healthy adults. *Annals of Nutrition and Metabolism, 56*,
 74-79. doi:10.1159/000272133
- Johnston, C. S., White, A. M., & Kent, S. M. (2009). Preliminary evidence that regular vinegar
 ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes
 mellitus. *Diabetes Research and Clinical Practice, 84*, e15-17.
 doi:10.1016/j.diabres.2009.02.005
- Jozala, A. F., de Lencastre-Novaes, L. C., Lopes, A. M., de Carvalho Santos-Ebinuma, V., Mazzola, P.
 G., Pessoa, A., Jr., . . . Chaud, M. V. (2016). Bacterial nanocellulose production and
 application: a 10-year overview. *Applied Microbiology and Biotechnology, 100*, 2063-2072.
 doi:10.1007/s00253-015-7243-4
- Jung, H. H., Cho, S. D., Yoo, C. K., Lim, H. H., & Chae, S. W. (2002). Vinegar treatment in the
 management of granular myringitis. *The Journal of Laryngology & Otology, 116*, 176-180.
- Kadas, Z., Akdemir Evrendilek, G., & Heper, G. (2014). The metabolic effects of hawthorn vinegar in
 patients with high cardiovascular risk group. *Journal of Food and Nutrition Research*, *2*, 539545. doi:10.12691/jfnr-2-9-2
- Kanchanarach, W., Theeragool, G., Inoue, T., Yakushi, T., Adachi, O., & Matsushita, K. (2010a). Acetic
 acid fermentation of acetobacter pasteurianus: relationship between acetic acid resistance
 and pellicle polysaccharide formation. *Bioscience, Biotechnology, and Biochemistry, 74*,
 1591-1597. doi:10.1271/bbb.100183
- Kanchanarach, W., Theeragool, G., Yakushi, T., Toyama, H., Adachi, O., & Matsushita, K. (2010b).
 Characterization of thermotolerant *Acetobacter pasteurianus* strains and their quinoprotein alcohol dehydrogenases. *Applied Microbiology and Biotechnology*, *85*, 741-751.
 doi:10.1007/s00253-009-2203-5
- Karabiyikli, S., & Sengun, I. Y. (2017). Beneficial effects of Acetic acid bacteria and their food
 products. In I. Y. Sengun (Ed.), *Acetic Acid Bacteria: Fundamentals and Food Applications* (pp. 321-342). Florida, USA: CRC Press.
- Keshk, S., & Sameshima, K. (2006). Influence of lignosulfonate on crystal structure and productivity
 of bacterial cellulose in a static culture. *Enzyme and Microbial Technology*, 40, 4-8.
 doi:10.1016/j.enzmictec.2006.07.037
- Keshk, S. M. (2014). Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*.
 Carbohydrate Polymers, 99, 98-100. doi:10.1016/j.carbpol.2013.08.060
- Khajavi, R., Esfahani, E. J., & Sattari, M. (2011). Crystalline structure of microbial cellulose compared
 with native and regenerated cellulose. *International Journal of Polymeric Materials, 60*,
 1178-1192.
- Kimura, S., Chen, H. P., Saxena, I. M., Brown, R. M., Jr., & Itoh, T. (2001). Localization of c-di-GMP binding protein with the linear terminal complexes of Acetobacter xylinum. *Journal of Bacteriology, 183*, 5668-5674. doi:10.1128/JB.183.19.5668-5674.2001
- Kishi, M., Fukaya, M., Tsukamoto, Y., Nagasawa, T., Takehana, K., & Nishizawa, N. (1999). Enhancing
 effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats.
 Bioscience, Biotechnology, and Biochemistry, 63, 905-910.
- Klemm, D., Kramer, F., Moritz, S., Lindstrom, T., Ankerfors, M., Gray, D., & Dorris, A. (2011).
 Nanocelluloses: a new family of nature-based materials. *Angewandte Chemie International Edition in English, 50*, 5438-5466. doi:10.1002/anie.201001273
- Komagata, K., Iino, T., & Yamada, Y. (2014). The family Acetobacteraceae. In E. Rosenberg, E. F. De
 Long, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria* (pp. 3-78). Berlin Heidelberg: Springer-Verlag.

- Kondo, T., Kishi, M., Fushimi, T., & Kaga, T. (2009b). Acetic acid upregulates the expression of genes
 for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *Journal of Agricultural and Food Chemistry, 57*, 5982-5986. doi:10.1021/jf900470c
- Kondo, T., Kishi, M., Fushimi, T., Ugajin, S., & Kaga, T. (2009a). Vinegar intake reduces body weight,
 body fat mass, and serum triglyceride levels in obese Japanese subjects. *Bioscience*,
 Biotechnology, and Biochemistry, *73*, 1837-1843. doi:10.1271/bbb.90231
- 2283 Kraft, F. F. (1959). Le champignon du thé [The Tea Fungus]. *Nova Hedwigia*, 297-304.
- Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Goncalves-Miskiewicz, M., Turkiewicz, M., &
 Bielecki, S. (2002). Factors affecting the yield and properties of bacterial cellulose. *Journal of Industrial Microbiology & Biotechnology, 29*, 189-195. doi:10.1038/sj.jim.7000303
- Landete, J. M., Ferrer, S., & Pardo, I. (2007). Biogenic amine production by lactic acid bacteria, acetic
 bacteria and yeast isolated from wine. *Food Control, 18*, 1569-1574.
- Laulund, S., Wind, A., Derkx, P. M. F., & Zuliani, V. (2017). Regulatory and safety requirements for food cultures. *Microorganisms*, *5*. doi:10.3390/microorganisms5020028
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2018). Oxygen and diverse nutrients influence
 the water kefir fermentation process. *Food Microbiology*, *73*, 351-361.
 doi:10.1016/j.fm.2018.02.007
- Laureys, D., & De Vuyst, L. (2014). Microbial species diversity, community dynamics, and metabolite
 kinetics of water kefir fermentation. *Applied and Environmental Microbiology, 80*, 2564 2572. doi:10.1128/AEM.03978-13
- Laureys, D., & De Vuyst, L. (2017). The water kefir grain inoculum determines the characteristics of
 the resulting water kefir fermentation process. *Journal of Applied Microbiology*, *122*, 719 732. doi:10.1111/jam.13370
- Laureys, D., Van Jean, A., Dumont, J., & De Vuyst, L. (2017). Investigation of the instability and low
 water kefir grain growth during an industrial water kefir fermentation process. *Applied Microbiology and Biotechnology, 101*, 2811-2819.
- Leuschner, R. G. K., Robinson, T. P., Hugas, M., Cocconcelli, P. S., Richard-Forget, F., Klein, G., . . .
 Richardson, M. (2010). Qualified presumption of safety (QPS): a generic risk assessment
 approach for biological agents notified to the European Food Safety Authority (EFSA). *Trends in Food Science & Technology, 21*, 425-435. doi:10.1016/j.tifs.2010.07.003
- Li, B., Li, Z., Wei, Y., Zhang, X. L., Wu, R. Q., Fan, Y. L., & Bu, L. J. (2009). Study on the effects of brans
 and Aspergillus niger about corn vinegar on reducing obesity and blood lipids in rat. Journal
 of Northwest A & F University-Natural Science Edition, 37, 194-198.
- Liatis, S., Grammatikou, S., Poulia, K. A., Perrea, D., Makrilakis, K., Diakoumopoulou, E., &
 Katsilambros, N. (2010). Vinegar reduces postprandial hyperglycaemia in patients with type
 Il diabetes when added to a high, but not to a low, glycaemic index meal. *European Journal of Clinical Nutrition, 64*, 727-732. doi:10.1038/ejcn.2010.89
- Lin, S.-P., Loira Calvar, I., Catchmark, J. M., Liu, J.-R., Demirci, A., & Cheng, K.-C. (2013). Biosynthesis,
 production and applications of bacterial cellulose. *Cellulose, 20*, 2191-2219.
 doi:10.1007/s10570-013-9994-3
- Liu, C. H., Hsu, W. H., Lee, F. L., & Liao, C. C. (1996). The isolation and identification of microbes from
 a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology*, *13*, 407-415.
- Liu, L., Han, Y. W., Wang, N., Zhao, L., Kou, X., & Li, Z. X. (2015). Effect of purple sweet potato vinegar
 on hepatoprotective of acute liver injury and mass-reducing, hypolipidemic in mice. *Acta Agriculturae Boreali-occidentalis Sinica, 24*, 28-33.
- Liu, L., Li, Y., Zhang, J., Zhou, Z., Liu, J., Li, X., . . . Chen, J. (2011). Complete genome sequence of the
 industrial strain *Ketogulonicigenium vulgare* WSH-001. *Journal of Bacteriology*, *193*, 61086109. doi:10.1128/JB.06007-11
- Liu, L., & Yang, X. (2015). Hypolipidemic and antioxidant effects of freeze-dried powder of Shanxi
 mature vinegar in hyperlipidaemic mice. *Food Science, 36*, 141-151.

- Lončar, E. S., Petrovič, S. E., Malbača, R. V., & Verac, R. M. (2000). Biosynthesis of glucuronic acid by means of tea fungus. *Molecular Nutrition & Food Research, 44*, 138-139.
- Lu, P. J., & Zhou, Y. Z. (2002). Anti-fatigue function of Hengshun vinegar capsules. *China Condiment*, 10, 8-13.
- Lu, Z. M., Wang, Z. M., Zhang, X. J., Mao, J., Shi, J. S., & Xu, Z. H. (2017). Microbial ecology of cereal
 vinegar fermentation: insights for driving the ecosystem function. *Current Opinion in Biotechnology, 49*, 88-93. doi:10.1016/j.copbio.2017.07.006
- Luttik, M., Van Spanning, R., Schipper, D., Van Dijken, J. P., & Pronk, J. T. (1997). The low biomass
 yields of the acetic acid bacterium *Acetobacter pasteurianus* are due to a low stoichiometry
 of respiration-coupled proton translocation. *Applied and Environmental Microbiology, 63*,
 3345-3351.
- Lynch, K. M., Coffey, A., & Arendt, E. K. (2018). Exopolysaccharide producing lactic acid bacteria:
 Their techno-functional role and potential application in gluten-free bread products. *Food Research International, 110*, 52-61.
- 2342Lynch, K. M., Zannini, E., Coffey, A., & Arendt, E. K. (2018). Lactic acid bacteria exopolysaccharides in2343foods and beverages: Isolation, properties, characterization, and health benefits. Annual2344Review of Food Science and Technology, 9, 155-176. doi:10.1146/annurev-food-030117-2345012537
- 2346 Ma, T. J., Xia, F., & Jia, C. X. (2010). The influence of bitter buckwheat vinegar in blood glucose of 2347 diabetic model mice. *Journal of the Chinese Cereals and Oils Association, 25*, 42-48.
- Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. (2015). *Brock Biology of Microorganisms 14th edition*: Pearson Education, Inc.
- Magalhaes, K. T., Pereira, G. V. d. M., Dias, D. R., & Schwan, R. F. (2010). Microbial communities and
 chemical changes during fermentation of sugary Brazilian kefir. *World Journal of Microbiology and Biotechnology, 26*, 1241-1250.
- Malaysian Food Regulations. (1985). Standards and Particular Labelling Requirements for Food:
 Vinegar Sauce, Chutney and Pickle. Malaysia: Food Act 1983.
- Malbaša, R., Lončar, E., Djurić, M., Klašnja, M., Kolarov, L. J., & Markov, S. (2006). Scale-up of black
 tea batch fermentation by kombucha. *Food and Bioproducts Processing, 84*, 193-199.
- Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Čanadanović-Brunet, J. M. (2011). Influence of starter
 cultures on the antioxidant activity of kombucha beverage. *Food Chemistry*, *127*, 1727-1731.
 doi:10.1016/j.foodchem.2011.02.048
- Malimas, T., Thi Lan Vu, H., Muramatsu, Y., Yukphan, P., Tanasupawat, S., & Yamada, Y. (2017).
 Systematics of Acetic Acid Bacteria. In I. Y. Sengun (Ed.), *Acetic Acid Bacteria: Fundamentals* and Food Applications (pp. 3-43). Japan: CRC Press.
- Mamlouk, D., & Gullo, M. (2013). Acetic Acid bacteria: physiology and carbon sources oxidation.
 Indian Journal of Microbiology, 53, 377-384. doi:10.1007/s12088-013-0414-z
- Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2013). Sequence-based analysis of the
 microbial composition of water kefir from multiple sources. *FEMS Microbiology Letters, 348*,
 79-85.
- Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the
 bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology, 38*, 171-178. doi:10.1016/j.fm.2013.09.003
- 2371Mas, A., Torija, M. J., García-Parrilla, M. d. C., & Troncoso, A. M. (2014). Acetic acid bacteria and the2372production and quality of wine vinegar. *The Scientific World Journal, 2014*.
- Mas, A., Troncoso, A. M., Garcı´a-Parrilla, M. C., & Torija, M. J. (2016). Vinegar. In B. Caballero, P. M.
 Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (Vol. Volume 5, pp. 418-423).
 Oxford, UK: Elsevier Ltd.
- Matsushita, K., Fujii, Y., Ano, Y., Toyama, H., Shinjoh, M., Tomiyama, N., . . . Adachi, O. (2003). 5 Keto-D-gluconate production is catalyzed by a quinoprotein glycerol dehydrogenase, major

- polyol dehydrogenase, in *Gluconobacter* species. *Applied and Environmental Microbiology*,
 69, 1959-1966. doi:10.1128/aem.69.4.1959-1966.2003
- Matsushita, K., Inoue, T., Adachi, O., & Toyama, H. (2005). *Acetobacter aceti* possesses a protonmotive force-dependent efflux system for acetic acid. *Journal of Bacteriology*, *187*, 43464352.
- Matsushita, K., & Matsutani, M. (2016). Distribution, evolution, and physiology of oxidative
 fermentation. In K. Matsushita, H. Toyama, N. Tonouchi, & A. Okamoto-Kainuma (Eds.),
 Acetic Acid Bacteria: Ecology and Physiology (pp. 159-187). Japan: Springer Nature.
- Matsushita, K., Nagatani, Y., Shinagawa, E., Adachi, O., & Ameyama, M. (1989). Effect of extracellular
 pH on the respiratory chain and energetics of *Gluconobacter suboxydans*. *Agricultural and Biological Chemistry*, *53*, 2895-2902.
- Matsushita, K., Toyama, H., & Adachi, O. (1994). Respiratory chains and bioenergetics of acetic acid
 bacteria. In *Advances in Microbial Physiology* (Vol. 36, pp. 247-301): Elsevier.
- Matsushita, K., Yakushi, T., Takaki, Y., Toyama, H., & Adachi, O. (1995). Generation mechanism and
 purification of an inactive form convertible in vivo to the active form of quinoprotein alcohol
 dehydrogenase in *Gluconobacter suboxydans*. *Journal of Bacteriology*, *177*, 6552-6559.
- Matsushita, K., Yakushi, T., Toyama, H., Shinagawa, E., & Adachi, O. (1996). Function of multiple
 heme c moieties in intramolecular electron transport and ubiquinone reduction in the
 quinohemoprotein alcohol dehydrogenase-cytochrome c complex of *Gluconobacter suboxydans. Journal of Biological Chemistry, 271*, 4850-4857.
- Matsutani, M., Fukushima, K., Kayama, C., Arimitsu, M., Hirakawa, H., Toyama, H., . . . Matsushita, K.
 (2014). Replacement of a terminal cytochrome c oxidase by ubiquinol oxidase during the
 evolution of acetic acid bacteria. *Biochimica et Biophysica Acta, 1837*, 1810-1820.
 doi:10.1016/j.bbabio.2014.05.355
- Matsutani, M., Hirakawa, H., Yakushi, T., & Matsushita, K. (2011). Genome-wide phylogenetic
 analysis of *Gluconobacter*, *Acetobacter*, and *Gluconacetobacter*. *FEMS Microbiology Letters*,
 315, 122-128. doi:10.1111/j.1574-6968.2010.02180.x
- 2405 Mayser, P., Fromme, S., Leitzmann, G., & Gründer, K. (1995). The yeast spectrum of the 'tea fungus
 2406 Kombucha'. *Mycoses, 38*, 289-295.
- Mazza, S., & Murooka, Y. (2009). Vinegars through the ages. In L. Solieri & P. Giudici (Eds.), *Vinegars of the World* (pp. 17-39). Milano: Springer Milan.
- McArthur, A. G., Waglechner, N., Nizam, F., Yan, A., Azad, M. A., Baylay, A. J., . . . Ejim, L. (2013). The
 comprehensive antibiotic resistance database. *Antimicrobial Agents and Chemotherapy*, *57*,
 3348-3357.
- Miguel, M. G. d. C. P., Cardoso, P. G., Magalhães, K. T., & Schwan, R. F. (2011). Profile of microbial
 communities present in tibico (sugary kefir) grains from different Brazilian States. *World Journal of Microbiology and Biotechnology, 27*, 1875-1884. doi:10.1007/s11274-010-0646-6
- Mimura, A., Suzuki, Y., Toshima, Y., Yazaki, S. I., Ohtsuki, T., Ui, S., & Hyodoh, F. (2004). Induction of
 apoptosis in human leukemia cells by naturally fermented sugar cane vinegar (kibizu) of
 Amami Ohshima Island. *Biofactors, 22*, 93-97.
- 2418 Ministry of Food and Drugs Safety. (2014). New South Korea organic regulation.
- Mir, S. A., Shah, M. A., Naik, H. R., & Zargar, I. A. (2016). Influence of hydrocolloids on dough
 handling and technological properties of gluten-free breads. *Trends in Food Science & Technology, 51*, 49-57. doi:10.1016/j.tifs.2016.03.005
- Mitrou, P., Petsiou, E., Papakonstantinou, E., Maratou, E., Lambadiari, V., Dimitriadis, P., . . .
 Dimitriadis, G. (2015). The role of acetic acid on glucose uptake and blood flow rates in the
 skeletal muscle in humans with impaired glucose tolerance. *European Journal of Clinical Nutrition, 69*, 734-739. doi:10.1038/ejcn.2014.289
- Mitrou, P., Raptis, A. E., Lambadiari, V., Boutati, E., Petsiou, E., Spanoudi, F., . . . Raptis, S. A. (2010).
 Vinegar decreases postprandial hyperglycemia in patients with type 1 diabetes. *Diabetes Care, 33*, e27. doi:10.2337/dc09-1354

- Miura, H., Mogi, T., Ano, Y., Migita, C. T., Matsutani, M., Yakushi, T., . . . Matsushita, K. (2013).
 Cyanide-insensitive quinol oxidase (CIO) from *Gluconobacter oxydans* is a unique terminal
 oxidase subfamily of cytochrome bd. *Journal of Biochemistry*, *153*, 535-545.
 doi:10.1093/jb/mvt019
- 2433 Miyazaki, T., Sugisawa, T., & Hoshino, T. (2006). Pyrroloquinoline quinone-dependent
 2434 dehydrogenases from *Ketogulonicigenium vulgare* catalyze the direct conversion of L2435 sorbosone to L-ascorbic acid. *Applied and Environmental Microbiology, 72*, 1487-1495.
 2436 doi:10.1128/AEM.72.2.1487-1495.2006
- 2437 Mohite, B. V., & Patil, S. V. (2014a). A novel biomaterial: bacterial cellulose and its new era 2438 applications. *Biotechnology and Applied Biochemistry, 61*, 101-110. doi:10.1002/bab.1148
- Mohite, B. V., & Patil, S. V. (2014b). Physical, structural, mechanical and thermal characterization of
 bacterial cellulose by *G. hansenii* NCIM 2529. *Carbohydrate Polymers, 106*, 132-141.
 doi:10.1016/j.carbpol.2014.02.012
- Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R. M., & Remaud-Siméon, M. (2001).
 Homopolysaccharides from lactic acid bacteria. *International Dairy Journal*, *11*, 675-685.
- 2444 Moon, Y.-J., & Cha, Y.-S. (2008). Effects of persimmon-vinegar on lipid metabolism and alcohol 2445 clearance in chronic alcohol-fed rats. *Journal of Medicinal Food, 11*, 38-45.
- 2446 Motizuki, K., Kanzaki, T., Okazaki, H., Yoshino, H., Nara, K., Isono, M., . . . Sasajima, K. (1962).
- 2447 Murray, J. C. F. (2009). Cellulosics. In *Handbook of Hydrocolloids* (Second ed., pp. 710-723): Elsevier.
- Naessens, M., Cerdobbel, A., Soetaert, W., & Vandamme, E. J. (2005). Dextran dextrinase and
 dextran of *Gluconobacter oxydans*. *Journal of Industrial Microbiology & Biotechnology*, *32*,
 323-334. doi:10.1007/s10295-005-0259-5
- Nakano, S., & Ebisuya, H. (2016). Physiology of Acetobacter and Komagataeibacter spp.: Acetic acid
 resistance mechanism in acetic acid fermentation. In K. Matsushita, H. Toyama, N. Tonouchi,
 & A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp. 222-234).
 Japan: Springer Nature.
- Nakano, S., & Fukaya, M. (2008). Analysis of proteins responsive to acetic acid in *Acetobacter*:
 molecular mechanisms conferring acetic acid resistance in acetic acid bacteria. *International Journal of Food Microbiology*, *125*, 54-59. doi:10.1016/j.ijfoodmicro.2007.05.015
- Nakano, S., Fukaya, M., & Horinouchi, S. (2004). Enhanced expression of aconitase raises acetic acid
 resistance in *Acetobacter aceti. FEMS Microbiology Letters, 235*, 315-322.
 doi:10.1016/j.femsle.2004.05.007
- Nakano, S., Fukaya, M., & Horinouchi, S. (2006). Putative ABC transporter responsible for acetic acid
 resistance in *Acetobacter aceti*. *Applied and Environmental Microbiology*, *72*, 497-505.
 doi:10.1128/AEM.72.1.497-505.2006
- Nanda, K., Miyoshi, N., Nakamura, Y., Shimoji, Y., Tamura, Y., Nishikawa, Y., . . . Tanaka, T. (2004).
 Extract of vinegar "Kurosu" from unpolished rice inhibits the proliferation of human cancer
 cells. Journal of Experimental and Clinical Cancer Research, 23, 69-76.
- 2467 NCBI. (2018). <u>www.ncbi.nlm.nih.gov/genome</u> (accessed 22 August 2018).
- Nishidai, S., Nakamura, Y., Torikai, K., Yamamoto, M., Ishihara, N., Mori, H., & Ohigashi, H. (2000).
 Kurosu, a traditional vinegar produced from unpolished rice, suppresses lipid peroxidation in vitro and in mouse skin. *Bioscience, Biotechnology, and Biochemistry, 64*, 1909-1914.
- Ogawa, N., Satsu, H., Watanabe, H., Fukaya, M., Tsukamoto, Y., Miyamoto, Y., & Shimizu, M. (2000).
 Acetic acid suppresses the increase in disaccharidase activity that occurs during culture of
 caco-2 cells. *The Journal of Nutrition, 130*, 507-513.
- 2474Okamoto-Kainuma, A., Ishikawa, M., Nakamura, H., Fukazawa, S., Tanaka, N., Yamagami, K., &2475Koizumi, Y. (2011). Characterization of rpoH in Acetobacter pasteurianus NBRC3283. Journal2476of Bioscience and Bioengineering, 111, 429-432. doi:10.1016/j.jbiosc.2010.12.016
- Olijve, W., & Kok, J. J. (1979). Analysis of growth of *Gluconobacter oxydans* in glucose containing
 media. *Archives of microbiology*, *121*, 283-290.

- Östman, E., Granfeldt, Y., Persson, L., & Björck, I. (2005). Vinegar supplementation lowers glucose
 and insulin responses and increases satiety after a bread meal in healthy subjects. *European* Journal of Clinical Nutrition, 59, 983.
- Östman, E. M., Liljeberg Elmståhl, H. G. M., & Björck, I. M. E. (2001). Inconsistency between glycemic
 and insulinemic responses to regular and fermented milk products. *The American Journal of Clinical Nutrition, 74*(1), 96-100.
- Papalexandratou, Z., & De Vuyst, L. (2011). Assessment of the yeast species composition of cocoa
 bean fermentations in different cocoa-producing regions using denaturing gradient gel
 electrophoresis. *FEMS Yeast Research, 11*, 564-574. doi:10.1111/j.1567-1364.2011.00747.x
- Papalexandratou, Z., Lefeber, T., Bahrim, B., Lee, O. S., Daniel, H. M., & De Vuyst, L. (2013).
 Hanseniaspora opuntiae, Saccharomyces cerevisiae, Lactobacillus fermentum, and
 Acetobacter pasteurianus predominate during well-performed Malaysian cocoa bean box
 fermentations, underlining the importance of these microbial species for a successful cocoa
 bean fermentation process. Food Microbiology, 35, 73-85. doi:10.1016/j.fm.2013.02.015
- Pappenberger, G., & Hohmann, H.-P. (2014). Industrial production of L-ascorbic acid (Vitamin C) and
 D-isoascorbic acid. In *Biotechnology of Food and Feed Additives* (pp. 143-188): Springer.
- Pappenberger, G., & Hohmann, H.-P. (2016). Direct microbial routes to Vitamin C production. In E. J.
 Vandamme & J. L. Revuelta (Eds.), *Industrial Biotechnology of Vitamins, Biopigments, and* Antioxidants (pp. 193-225). Germany: Wiley-VCH Verlag GmbH & Co.
- Pariza, M. W. (2007). A scientific perspective on labeling genetically modified food. In P. Weirich
 (Ed.), Labeling Genetically Modified Food: The Philosophical and Legal Debate (pp. 3-9). New
 York: Oxford University Press.
- Pariza, M. W., Gillies, K. O., Kraak-Ripple, S. F., Leyer, G., & Smith, A. B. (2015). Determining the
 safety of microbial cultures for consumption by humans and animals. *Regulatory Toxicology and Pharmacology*, *73*, 164-171. doi:10.1016/j.yrtph.2015.07.003
- Park, J. K., & Khan, T. (2009). Bacterial cellulose. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids* (Second ed., pp. 724-739). United Kingdom: Woodhead Publishing Limited.
- Parrondo, J., Garcia, L. A., & Diaz, M. (2009). Whey Vinegar. In L. Solieri & P. Giudici (Eds.), *Vinegars of the World* (pp. 273-288). Milan, Italy: Springer-Verlag.
- Pasteur, L. (1864). *Mémoire sur la fermentation acétique*. Paper presented at the Annales
 Scientifiques de l'École Normale Supérieure.
- Pauline, T., Dipti, P., Anju, B., Kavimani, S., Sharma, S. K., Kain, A. K., . . . Devendra, K. (2001). Studies
 on toxicity, anti-stress and hepato-protective properties of Kombucha tea. *Biomedical and Environmental Sciences: BES, 14*, 207-213.
- Pothakos, V., Illeghems, K., Laureys, D., Spitaels, F., Vandamme, P., & De Vuyst, L. (2016). Acetic acid
 bacteria in fermented food and beverage ecosystems. In K. Matsushita, H. Toyama, N.
 Tonouchi, & A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp.
 73-99). Japan: Springer Nature.
- Prust, C., Hoffmeister, M., Liesegang, H., Wiezer, A., Fricke, W. F., Ehrenreich, A., . . . Deppenmeier,
 U. (2005). Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*.
 Nature Biotechnology, 23, 195-200. doi:10.1038/nbt1062
- Rainieri, S., & Zambonelli, C. (2009). Organisms associated with acetic acid bacteria in vinegar
 production. In L. Solieri & P. Giudici (Eds.), *Vinegars of the World* (pp. 73-95). Milano:
 Springer Milan.
- 2523Raspor, P., & Goranovic, D. (2008). Biotechnological applications of acetic acid bacteria. Critical2524Reviews in Biotechnology, 28, 101-124. doi:10.1080/07388550802046749
- Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R., . . . Nørrung, B. (2017a).
 Scientific Opinion on the update of the list of QPS-recommended biological agents
 intentionally added to food or feed as notified to EFSA. *EFSA Journal*, 15.
- Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R., . . . Robertson, L. (2017b).
 Update of the list of QPS-recommended biological agents intentionally added to food or

2530 feed as notified to EFSA 6: suitability of taxonomic units notified to EFSA until March 2017. 2531 EFSA Journal, 15. 2532 Richhardt, J., Luchterhand, B., Bringer, S., Buchs, J., & Bott, M. (2013). Evidence for a key role of 2533 cytochrome bo3 oxidase in respiratory energy metabolism of *Gluconobacter oxydans*. 2534 Journal of Bacteriology, 195, 4210-4220. doi:10.1128/JB.00470-13 2535 Roussin, M. R. (1996). Analyses of kombucha ferments: Report on growers. Information Resources, 2536 LC, Salt Lake City, Utah, USA. 2537 Rubio-Fernandez, H., Desamparados Salvador, M., & Fregapane, G. (2004). Influence of fermentation 2538 oxygen partial pressure on semicontinuous acetification for wine vinegar production. 2539 European Food Research and Technology, 219. doi:10.1007/s00217-004-0947-9 Ruiz, A., Poblet, M., Mas, A., & Guillamon, J. M. (2000). Identification of acetic acid bacteria by RFLP 2540 2541 of PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer. International Journal of 2542 Systematic and Evolutionary Microbiology, 50, 1981-1987. 2543 Russell, J. B., & Diez-Gonzalez, F. (1997). The effects of fermentation acids on bacterial growth. In 2544 Advances in Microbial Physiology (Vol. 39, pp. 205-234): Academic Press. 2545 Russo, P., Spano, G., & Capozzi, V. (2017). Safety evaluation of starter cultures. In B. Speranza, A. Bevilacqua, M. R. Corbo, & M. Sinigaglia (Eds.), Starter Cultures in Food Production (pp. 101-2546 2547 128). West Sussex, UK: Wiley Blackwell. Sadahiro, J., Mori, H., Saburi, W., Okuyama, M., & Kimura, A. (2015). Extracellular and cell-associated 2548 2549 forms of Gluconobacter oxydans dextran dextrinase change their localization depending on 2550 the cell growth. Biochemical and Biophysical Research Communications, 456, 500-505. doi:10.1016/j.bbrc.2014.11.115 2551 2552 Saichana, N., Matsushita, K., Adachi, O., Frebort, I., & Frebortova, J. (2015). Acetic acid bacteria: A group of bacteria with versatile biotechnological applications. Biotechnology Advances, 33(6 2553 2554 Pt 2), 1260-1271. doi:10.1016/j.biotechadv.2014.12.001 Saito, Y., Ishii, Y., Hayashi, H., Imao, Y., Akashi, T., Yoshikawa, K., . . . Niwa, M. (1997). Cloning of 2555 2556 genes coding for L-sorbose and L-sorbosone dehydrogenases from Gluconobacter oxydans 2557 and microbial production of 2-keto-L-gulonate, a precursor of L-ascorbic acid, in a 2558 recombinant G. oxydans strain. Applied and Environmental Microbiology, 63, 454-460. 2559 Sakakibara, S., Murakami, R., Takahashi, M., Fushimi, T., Murohara, T., Kishi, M., . . . Kaga, T. (2010). 2560 Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric 2561 oxide synthase activity. Bioscience, Biotechnology, and Biochemistry, 74, 1055-1061. 2562 doi:10.1271/bbb.90953 2563 Sakakibara, S., Yamauchi, T., Oshima, Y., Tsukamoto, Y., & Kadowaki, T. (2006). Acetic acid activates 2564 hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. Biochemical and 2565 Biophysical Research Communications, 344, 597-604. doi:10.1016/j.bbrc.2006.03.176 2566 Sakurai, K., Arai, H., Ishii, M., & Igarashi, Y. (2012). Changes in the gene expression profile of 2567 Acetobacter aceti during growth on ethanol. Journal of Bioscience and Bioengineering, 113, 2568 343-348. doi:10.1016/j.jbiosc.2011.11.005 2569 Samad, A., Azlan, A., & Ismail, A. (2016). Therapeutic effects of vinegar: a review. Current Opinion in 2570 *Food Science, 8*, 56-61. doi:10.1016/j.cofs.2016.03.001 2571 Schedel, M. (2000). Regioselective oxidation of aminosorbitol with *Gluconobacter oxydans*, key 2572 reaction in the industrial 1-deoxynojirimycin synthesis. *Biotechnology*, 8, 295-308. 2573 Schüller, G., Hertel, C., & Hammes, W. P. (2000). Gluconacetobacter entanii sp. nov., isolated from submerged high-acid industrial vinegar fermentations. International Journal of Systematic 2574 2575 and Evolutionary Microbiology, 50, 2013-2020. Seki, T., Morimura, S., Shigematsu, T., Maeda, H., & Kida, K. (2004). Antitumor activity of rice-shochu 2576 2577 post-distillation slurry and vinegar produced from the post-distillation slurry via oral 2578 administration in a mouse model. *Biofactors*, 22, 103-105. 2579 Sengun, I. Y. (2015). Acetic Acid Bacteria: Prospective applications in food biotechnology. In R. Ray & 2580 M. Didier (Eds.), Fermented Foods, Part I (pp. 106-120). Boca Raton: CRC Press.

- Sengun, I. Y., & Karapinar, M. (2004). Effectiveness of lemon juice, vinegar and their mixture in the
 elimination of *Salmonella typhimurium* on carrots (Daucus carota L.). *International Journal of Food Microbiology*, *96*, 301-305. doi:10.1016/j.ijfoodmicro.2004.04.010
- Serrato, R. V., Meneses, C. H., Vidal, M. S., Santana-Filho, A. P., Iacomini, M., Sassaki, G. L., & Baldani,
 J. I. (2013). Structural studies of an exopolysaccharide produced by *Gluconacetobacter diazotrophicus* Pal5. *Carbohydrate Polymers, 98*, 1153-1159.
 doi:10.1016/j.carbpol.2013.07.025
- Setorki, M., Asgary, S., Eidi, A., & Khazaei, M. (2010). Acute effects of vinegar intake on some
 biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. *Lipids in Health and Disease, 9*, 10.
- Settembre, E. C., Chittuluru, J. R., Mill, C. P., Kappock, T. J., & Ealick, S. E. (2004). Acidophilic
 adaptations in the structure of *Acetobacter aceti* N5-carboxyaminoimidazole ribonucleotide
 mutase (PurE). *Acta Crystallographica Section D: Biological Crystallography, 60*, 1753-1760.
- Shi, Z., Zhang, Y., Phillips, G. O., & Yang, G. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids*, *35*, 539-545. doi:10.1016/j.foodhyd.2013.07.012
- Shimoji, Y., Kohno, H., Nanda, K., Nishikawa, Y., Ohigashi, H., Uenakai, K., & Tanaka, T. (2004). Extract
 of Kurosu, a vinegar from unpolished rice, inhibits azoxymethane-induced colon
 carcinogenesis in male F344 rats. *Nutrition and Cancer, 49*, 170-173.
- Shinagawa, E., Ano, Y., Yakushi, T., Adachi, O., & Matsushita, K. (2009). Solubilization, purification,
 and properties of membrane-bound D-glucono-delta-lactone hydrolase from *Gluconobacter oxydans. Bioscience, Biotechnology, and Biochemistry, 73*, 241-244. doi:10.1271/bbb.80554
- Shinagawa, E., Matsushita, K., Adachi, O., & Ameyama, M. (1982). Purification and characterization
 of D-sorbitol dehydrogenase from membrane of *Gluconobacter suboxydans* var. α.
 Agricultural and Biological Chemistry, 46, 135-141.
- Shinagawa, E., Matsushita, K., Adachi, O., & Ameyama, M. (1984). D-Gluconate dehydrogenase, 2 keto-D-gluconate yielding, from *Gluconobacter dioxyacetonicus*: purification and
 characterization. *Agricultural and Biological Chemistry, 48*, 1517-1522.
- Shinjoh, M., & Toyama, H. (2016). Industrial application of Acetic acid bacteria (Vitamin C and
 others). In K. Matsushita, H. Toyama, N. Tonouchi, & A. Okamoto-Kainuma (Eds.), Acetic Acid
 Bacteria: Ecology and Physiology (pp. 321-338). Japan: Springer Nature.
- Sievers, M., & Swings, J. (2005). Family II. Acetobacteraceae. In D. J. Brenner, N. R. Krieg, J. T. Staley,
 & G. M. Garrity (Eds.), *Bergey's Manual of Systematic Bacteriology, 2nd Ed, Vol. 2, Part C.*(pp. 41-95). New York: Springer-Verlag.
- Solieri, L., & Giudici, P. (2009). Vinegars of the World. In L. Solieri & P. Giudici (Eds.), *Vinegars of the World* (pp. 1-16): Springer.
- Soltan, S. S. A., & Shehata, M. M. E. M. (2012). Antidiabetic and hypocholesrolemic effect of different
 types of vinegar in rats. *Life Science Journal, 9*, 2141-2151.
- Spitaels, F., Li, L., Wieme, A. D., Balzarini, T., Cleenwerck, I., Van Landschoot, A., . . . Vandamme, P.
 (2014b). Acetobacter lambici sp. nov., isolated from fermenting lambic beer. International Journal of Systematic and Evolutionary Microbiology, 64, 1083-1089.
- Spitaels, F., Wieme, A. D., Balzarini, T., Cleenwerck, I., Van Landschoot, A., De Vuyst, L., &
 Vandamme, P. (2014c). *Gluconobacter cerevisiae* sp. nov., isolated from the brewery
 environment. *International Journal of Systematic and Evolutionary Microbiology*, 64, 11341141.
- Spitaels, F., Wieme, A. D., Janssens, M., Aerts, M., Daniel, H. M., Van Landschoot, A., . . . Vandamme,
 P. (2014a). The microbial diversity of traditional spontaneously fermented lambic beer. *PLoS One*, *9*, e95384. doi:10.1371/journal.pone.0095384
- Spitaels, F., Wieme, A. D., Janssens, M., Aerts, M., Van Landschoot, A., De Vuyst, L., & Vandamme, P.
 (2015). The microbial diversity of an industrially produced lambic beer shares members of a
 traditionally produced one and reveals a core microbiota for lambic beer fermentation. *Food Microbiology*, 49, 23-32. doi:10.1016/j.fm.2015.01.008

- Spitaels, F., Wieme, A. D., & Vandamme, P. (2016). MALDI-TOF MS as a Novel Tool for Dereplication
 and Characterization of Microbiota in Bacterial Diversity Studies. In P. Demirev & Todd R.
 Sandrin (Eds.), *Applications of Mass Spectrometry in Microbiology* (pp. 235-256).
 Switzerland: Springer.
- Srdic-Rajic, T., & Konic Ristic, A. (2016). Antioxidants: Role on Health and Prevention. In B. Caballero,
 P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (Vol. Volume 1, pp. 227-233). Oxford, UK: Elsevier Ltd.
- Srihari, T., Arunkumar, R., Arunakaran, J., & Satyanarayana, U. (2013a). Downregulation of signalling
 molecules involved in angiogenesis of prostate cancer cell line (PC-3) by kombucha
 (lyophilized). *Biomedicine & Preventive Nutrition, 3*, 53-58.
- Srihari, T., Karthikesan, K., Ashokkumar, N., & Satyanarayana, U. (2013b). Antihyperglycaemic
 efficacy of kombucha in streptozotocin-induced rats. *Journal of Functional Foods, 5*, 17941802. doi:10.1016/j.jff.2013.08.008
- Srikanth, R., Reddy, C. H., Siddartha, G., Ramaiah, M. J., & Uppuluri, K. B. (2015). Review on
 production, characterization and applications of microbial levan. *Carbohydrate Polymers*,
 120, 102-114. doi:10.1016/j.carbpol.2014.12.003
- Stadie, J., Gulitz, A., Ehrmann, M. A., & Vogel, R. F. (2013). Metabolic activity and symbiotic
 interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiology*,
 35, 92-98. doi:10.1016/j.fm.2013.03.009
- Sugisawa, T., & Hoshino, T. (2002). Purification and properties of membrane-bound D-sorbitol
 dehydrogenase from *Gluconobacter suboxydans* IFO 3255. *Bioscience, Biotechnology, and Biochemistry, 66*, 57-64. doi:10.1271/bbb.66.57
- Sugisawa, T., Hoshino, T., Masuda, S., Nomura, S., Setoguchi, Y., Tazoe, M., . . . Fujiwara, A. (1990).
 Microbial production of 2-keto-L-gulonic acid from L-sorbose and D-sorbitol by
 Gluconobacter melanogenus. Agricultural and Biological Chemistry, 54, 1201-1209.
- Sugisawa, T., Hoshino, T., Nomura, S., & Fujiwara, A. (1991). Isolation and characterization of
 membrane-bound L-sorbose dehydrogenase from *Gluconobacter melanogenus* UV10.
 Agricultural and Biological Chemistry, 55, 363-370.
- Sugisawa, T., Miyazaki, T., & Hoshino, T. (2005). Microbial production of L-ascorbic acid from D sorbitol, L-sorbose, L-gulose, and L-sorbosone by *Ketogulonicigenium vulgare* DSM 4025.
 Bioscience, Biotechnology, and Biochemistry, 69, 659-662. doi:10.1271/bbb.69.659
- Taban, B. M., & Saichana, N. (2017). Physiology and biochemistry of Acetic acid bacteria. In I. Y.
 Sengun (Ed.), *Acetic Acid Bacteria: Fundamentals and Food Applications* (pp. 71-91). Florida,
 USA: CRC Press.
- Tagliazucchi, D., Verzelloni, E., & Conte, A. (2008). Antioxidant properties of traditional balsamic
 vinegar and boiled must model systems. *European Food Research and Technology, 227*, 835 843. doi:10.1007/s00217-007-0794-6
- Tagliazucchi, D., Verzelloni, E., & Conte, A. (2010). Contribution of melanoidins to the antioxidant
 activity of Traditional Balsamic Vinegar during aging. *Journal of Food Biochemistry, 34*, 1061 1078. doi:10.1111/j.1745-4514.2010.00349.x
- Tan, L. L., Ren, L., Cao, Y. Y., Chen, X. L., & Tang, X. Y. (2012). Bacterial cellulose synthesis in
 Kombucha by Gluconacetobacter sp and Saccharomyces sp. Advanced Materials Research,
 Vols. 554-556, pp. 1000-1003.
- Taverniti, V., & Guglielmetti, S. (2011). The immunomodulatory properties of probiotic
 microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept).
 Genes & Nutrition, 6, 261.
- Tesfaye, W., Morales, M. L., Garcıa-Parrilla, M. C., & Troncoso, A. M. (2002). Wine vinegar:
 technology, authenticity and quality evaluation. *Trends in Food Science & Technology, 13*,
 12-21.

- Thurner, C., Vela, C., Thöny-Meyer, L., Meile, L., & Teuber, M. (1997). Biochemical and genetic
 characterization of the acetaldehyde dehydrogenase complex from *Acetobacter europaeus*.
 Archives of Microbiology, *168*, 81-91.
- Tonouchi, N. (2016). Cellulose and other capsular polysaccharides of Acetic acid bacteria. In K.
 Matsushita, H. Toyama, N. Tonouchi, & A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp. 299-320). Japan: Springer Nature.
- Toyama, H., Furuya, N., Saichana, I., Ano, Y., Adachi, O., & Matsushita, K. (2007). Membrane-bound,
 2-keto-D-gluconate-yielding D-gluconate dehydrogenase from "*Gluconobacter dioxyacetonicus*" IFO 3271: molecular properties and gene disruption. *Applied and Environmental Microbiology, 73*, 6551-6556. doi:10.1128/AEM.00493-07
- Trcek, J. (2005). Quick identification of acetic acid bacteria based on nucleotide sequences of the
 16S–23S rDNA internal transcribed spacer region and of the PQQ-dependent alcohol
 dehydrogenase gene. *Systematic and Applied Microbiology, 28*, 735-745.
- 2694Trovatti, E., Serafim, L. S., Freire, C. S. R., Silvestre, A. J. D., & Neto, C. P. (2011). Gluconacetobacter2695sacchari: an efficient bacterial cellulose cell-factory. Carbohydrate Polymers, 86, 1417-1420.
- Ua-Arak, T., Jakob, F., & Vogel, R. F. (2016). Characterization of growth and exopolysaccharide
 production of selected acetic acid bacteria in buckwheat sourdoughs. *International Journal of Food Microbiology, 239*, 103-112. doi:10.1016/j.ijfoodmicro.2016.04.009
- Ua-Arak, T., Jakob, F., & Vogel, R. F. (2017). Influence of levan-producing acetic acid bacteria on
 buckwheat-sourdough breads. *Food Microbiology*, *65*, 95-104. doi:10.1016/j.fm.2017.02.002
- United States of America Food and Drug Administration. (1977). Vinegar, Definitions Adulteration
 with Vinegar Eels. *CPG Sec. 525.825*.
- Urbance, J. W., Bratina, B. J., Stoddard, S. F., & Schmidt, T. M. (2001). Taxonomic characterization of *Ketogulonigenium vulgare* gen. nov., sp. nov. and *Ketogulonigenium robustum* sp. nov.,
 which oxidize L-sorbose to 2-keto-L-gulonic acid. *International Journal of Systematic and Evolutionary Microbiology*, *51*, 1059-1070.
- Valera, M. J., Torija, M. J., Mas, A., & Mateo, E. (2015). Cellulose production and cellulose synthase
 gene detection in acetic acid bacteria. *Applied Microbiology and Biotechnology*, *99*, 13491361. doi:10.1007/s00253-014-6198-1
- Vangnai, A. S., Toyama, H., De-eknamkul, W., Yoshihara, N., Adachi, O., & Matsushita, K. (2004).
 Quinate oxidation in *Gluconobacter oxydans* IFO3244: purification and characterization of
 quinoprotein quinate dehydrogenase. *FEMS Microbiology Letters, 241*, 157-162.
- Vegas, C., Mateo, E., Gonzalez, A., Jara, C., Guillamon, J. M., Poblet, M., . . . Mas, A. (2010).
 Population dynamics of acetic acid bacteria during traditional wine vinegar production. *International Journal of Food Microbiology, 138*, 130-136.
 doi:10.1016/j.ijfoodmicro.2010.01.006
- Velizarov, S., & Beschkov, V. (1994). Production of free gluconic acid by cells of *Gluconobacter oxydans*. *Biotechnology Letters*, *16*, 715-720.
- Velizarov, S., & Beschkov, V. (1998). Biotransformation of glucose to free gluconic acid by
 Gluconobacter oxydans: substrate and product inhibition situtations. *Process Biochemistry*,
 33, 527-534.
- Verzelloni, E., Tagliazucchi, D., & Conte, A. (2007). Relationship between the antioxidant properties
 and the phenolic and flavonoid content in traditional balsamic vinegar. *Food Chemistry*, *105*,
 564-571. doi:10.1016/j.foodchem.2007.04.014
- Verzelloni, E., Tagliazucchi, D., & Conte, A. (2010). From balsamic to healthy: traditional balsamic
 vinegar melanoidins inhibit lipid peroxidation during simulated gastric digestion of meat.
 Food and Chemical Toxicology, 48, 2097-2102. doi:10.1016/j.fct.2010.05.010
- Wang, P., Zeng, W., Xu, S., Du, G., Zhou, J., & Chen, J. (2018). Current challenges facing one-step
 production of I-ascorbic acid. *Biotechnology Advances, 36*, 1882-1899.
 doi:10.1016/j.biotechadv.2018.07.006

- Wang, Y., Ji, B., Wu, W., Wang, R., Yang, Z., Zhang, D., & Tian, W. (2014). Hepatoprotective effects of
 kombucha tea: identification of functional strains and quantification of functional
 components. *Journal of the Science of Food and Agriculture, 94*, 265-272.
 doi:10.1002/jsfa.6245
- Watanabe, K., Tabuchi, M., Morinaga, Y., & Yoshinaga, F. (1998). Structural features and properties
 of bacterial cellulose produced in agitated culture. *Cellulose*, 5, 187-200.
- Weenk, G., Olijve, W., & Harder, W. (1984). Ketogluconate formation by *Gluconobacter* species.
 Applied Microbiology and Biotechnology, 20, 400-405.
- Wei, Z. P., Li, Z. X., Yu, X. Z., Liu, Z. M., Cui, X. Y., & Jin, J. (2005). Effect of mulberry vinegar on
 reducing obesity of animals. *China Brewing*, *12*, 5-7.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification
 for phylogenetic study. *Journal of Bacteriology*, *173*, 697-703.
- Wells, J. S., Hunter, J. C., Astle, G. L., Sherwood, J. C., Ricca, C. M., Trejo, W. H., ... Sykes, R. B.
 (1982). Distribution of beta-lactam and beta-lactone producing bacteria in nature. *Journal of*Antibiotics, 35, 814-821.
- White, A. M., & Johnston, C. S. (2007). Vinegar ingestion at bedtime moderates waking glucose
 concentrations in adults with well-controlled type 2 diabetes. *Diabetes Care, 30*, 2814-2185.
 doi:10.2337/dc07-1062
- Wieme, A. D., Spitaels, F., Aerts, M., De Bruyne, K., Van Landschoot, A., & Vandamme, P. (2014).
 Effects of growth medium on matrix-assisted laser desorption-ionization time of flight mass
 spectra: a case study of acetic acid bacteria. *Applied and Environmental Microbiology, 80*,
 1528-1538. doi:10.1128/AEM.03708-13
- Wu, D., Kimura, F., Takashima, A., Shimizu, Y., Takebayashi, A., Kita, N., . . . Murakami, T. (2013).
 Intake of vinegar beverage is associated with restoration of ovulatory function in women
 with polycystic ovary syndrome. *The Tohoku Journal of Experimental Medicine, 230*, 17-23.
- 2756Xu, Q. P., Tao, W. Y., & Ao, Z. H. (2005). Antioxidation effects of vinegar on ageing accelerating model2757mice. Food Science, 12, 049.
- Yakushi, T., & Matsushita, K. (2010). Alcohol dehydrogenase of acetic acid bacteria: structure, mode
 of action, and applications in biotechnology. *Applied Microbiology and Biotechnology, 86*,
 1257-1265.
- Yamada, Y. (2016). Systematics of acetic acid bacteria. In K. Matsushita, H. Toyama, N. Tonouchi, &
 A. Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp. 1-49). Japan:
 Springer Nature.
- Yamada, Y., Hoshino, K. I., & Ishikawa, T. (1997). The phylogeny of acetic acid bacteria based on the
 partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconoacetobacter*to the generic level. *Bioscience, Biotechnology, and Biochemistry, 61*, 1244-1251.
- Yamada, Y., Yukphan, P., Vu, H. T. L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., & Nakagawa, Y.
 (2012). Description of *Komagataeibacter* gen. nov., with proposals of new combinations
 (Acetobacteraceae). The Journal of General and Applied Microbiology, 58, 397-404.
- Yang, W., & Xu, H. (2016). Industrial fermentation of Vitamin C. In E. J. Vandamme & J. L. Revuelta
 (Eds.), *Industrial Biotechnology of Vitamins, Biopigments, and Antioxidants* (pp. 161-192).
 Germany: Wiley-VCH Verlag GmbH & Co.
- Zahid, N. (2017). Osmotic stress response in the industrially important bacterium Gluconobacter
 oxydans. (Doctoral thesis), Rheinische Friedrich-Wilhelms University of Bonn, Germany.
- Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., . . . Larsen, M. V.
 (2012). Identification of acquired antimicrobial resistance genes. *Journal of antimicrobial chemotherapy*, *67*, 2640-2644.
- Zhang, L., Li, Z., & Du, S. (2007). Study on the effects of mulberry vinegar on weight losing and
 antifatigue in rat. *Journal of Northwest Sci-Tech University of Agriculture and Forestry*.

- Zhou, C. E., Smith, J., Lam, M., Zemla, A., Dyer, M. D., & Slezak, T. (2006). MvirDB—a microbial
 database of protein toxins, virulence factors and antibiotic resistance genes for bio-defence
 applications. *Nucleic Acids Research*, *35*, D391-D394.
- Zhu, C., Li, F., Zhou, X. X., Lin, L., & Zhang, T. (2014). Kombucha-synthesized bacterial cellulose:
 Preparation, characterization, and biocompatibility evaluation. *Journal of Biomedical Materials Research*, *102*, 1548-1557.

Food product	AAB species found associated with the fermentation	References
Vinegar (Traditional	Acetobacter pasteurianus	(De Vero et al. 2006)
Balsamic Vinegar)	A. aceti. Komagataeibacter xylinus.	(Gullo, Caggia, De Vero, & Giudici,
	K. europaeus, K. hansenii	2006),
Kombucha	K. xylinus, A. pasteurianus, K.	(Jayabalan et al., 2014), (Tan et al.,
	hansenii, A. aceti, Ga.	2012), (Liu et al., 1996), (Wang et
	saccharivorans, other Acetobacter,	al., 2014), (Marsh et al., 2014)
	Gluconobacter, and	
	Gluconacetobacter spp.	
Water kefir	A. lovaniensis, A. fabarum, A.	(Magalhaes et al., 2010), (Miguel,
	cerevisiae, A. aceti, A. ghanensis,	Cardoso, Magalhães, & Schwan,
	A. lovaniensis/fabarum. A. sicerae	2011), (Gulitz et al., 2013) (Stadie,
		Gulitz, Ehrmann, & Vogel, 2013).
		(Laureys & De Vuyst, 2014)
Lambic beer	A. lambici, G. cerevisiae	(De Roos & De Vuyst, 2018_9
		2018b Spitaels et al $2014b$
		Spitaels et al. $2014c$)
		······································

2794 <u>Table 1: Acetic acid bacteria commonly isolated from vinegar and fermented beverages</u>

Characteristic	Acetobacter	Gluconobacter	Gluconacetobacter	Komagataeibacter
Type strain	A. aceti NBRC 14818 ^T	<i>G. oxydans</i> NBRC 14819 ^T	<i>Ga. liquefaciens</i> NBRC 12388 ^T	K. xylinus JCM 7644 ^T
Production of acetic acid from ethanol	+	+	+	+
Flagellation	Per	Pol	Per	N
Oxidation of (to CO ₂ and				
H ₂ O)				
Acetate	+	-	+	+
Lactate	+	-	+	+
Growth on				
30% glucose (w/v)	-	_ ^b	-	nd
1% glucose (w/v)	+	+	+	+
Glutamate agar	-	-	+	+
Mannitol agar	VW	+	+	+
Raffinose	-	-	-	nd
Utilisation of methanol	-	-	-	nd
Growth in the presence				
of				
0.35% acetic acid (w/v)	+	+	+	+
1% KNO ₃ (w/v)	-	-	-	nd
Water soluble brown	-	_ ^b	+	-
pigment production				
Production of dihydroxygostong from	+	+	+	+
glycerol				
Production of levan-like	_	_b	_	_b
polysaccharide				
Assimilation of				
ammoniac nitrogen on				
Glucose	-	+	+	nd
Mannitol	-	+	+	+
Ethanol	W	-	-	nd
Production of				
2-ketogluconate	+	+	+	+
5-ketogluconate	+	+	+	+
2,5-diketogluconate	-	_b	+	-
Acid production from				
Mannitol	-	+	-	-
Sorbitol	-	+	-	-
Dulcitol	-	W	-	nd
Glycerol	-	+	-	nd
Raffinose	-	-	-	nd
Ethanol	+	+	+	+

2808 Table 2: Key characteristics of the four genera of AAB commonly associated with food

2809 2810 2811 2812	Per: peritrichous; Pol: polar; N: none; +, positive; -, negative; nd, not determined; b: some strains in the genus are positive; w, weakly positive; vw, very weakly positive. Modified from Yamada, 2016.
2813	
2814	
2815	
2816	
2817	
2818	
2819	
2820	
2821	
2822	
2823	
2824	
2825	
2826	
2827	
2828	
2829	
2830	
2831	

2832	Table 3: Media comm	only used for the	e growth of AAB	and their composition
			0	

Ingredient (%)	GYC	AE	YPM	MYA	DMS	mDMS
Glucose	10	0.5	-	-	0.1	0.1
Yeast extract	1	0.3	0.5	0.5	0.3	0.3
Peptone	-	0.4	0.3	-	1.0	1.0
Calcium carbonate	2	-	-	-	-	
Ethanol	-	3	-	6	-	0.5
Glacial acetic acid	-	3	-	-	-	0.3
Lactic acid	-	-	-	-	-	0.6
Mannitol	-	-	2.5	-	0.1	0.1
Sorbitol	-	-	-	-	0.1	0.1
Malt extract	-	-	-	1.5	-	-
Calcium lactate	-	-	-	-	1.5	-
Potassium phosphate	-	-	-	-	0.1	0.1
Sodium deoxycholate	-	-	-	-	0.01	0.01
Magnesium sulfate	-	-	-	-	0.002	0.002
Bromocresol	-	-	-	-	0.003	0.003
Bacteriological agar	1.5	0.9	1.2	1.5	1.0	1.8
2836 2837 2838 2839 2840 2841 2842 2843 2844						
2844 2845						

2848	Table	4:	Primary	membrane-bound	dehydrogenases	of	AAB	performing	oxidative
2849	ferment	tatic	ons.						

Enzyme	Abbreviation	Prosthetic group	Substrate	Product	Importance	References
Alcohol dehydrogenase	ADH	PQQ	Ethanol	Acetaldehyde	Vinegar production	(Yakushi & Matsushita, 2010)
Aldehyde dehydrogenase	ALDH	MCD	Acetaldehyde	Acetic acid	Vinegar production	(Thurner et al., 1997)
Glucose dehydrogenase	GDH	PQQ	D-glucose	δ-glucono-lactone	Gluconate and keto-gluconate(s) production	(Ameyama, Shinagawa, Matsushita, & Adachi, 1981b)
Gluconate dehydrogenase	GADH	FAD	D-gluconate	2-keto-D- gluconate	Gluconate and keto-gluconate(s) production	(Shinagawa, Matsushita, Adachi, & Ameyama, 1984)
2-keto-D- gluconate dehydrogenase	GADH	FAD	2-Keto-D- gluconate	2,5-diketo-D- gluconate	Gluconate and keto-gluconate(s) production	(Toyama et al., 2007)
Glycerol dehydrogenase	GLDH	PQQ	(Polyols)	(ketones)	Polyol oxidation	(Matsushita et al., 2003)
			D-gluconate Glycerol D-mannitol D-sorbitol D-arabitol Ribitol Meso- erythritol	5-keto-D- gluconate Dihydroxyacetone D-fructose L-sorbose D-xylulose L-ribulose Erythrulose		
Sorbitol dehydrogenase	SLDH	FAD	D-sorbitol	L-sorbose	Vitamin C production	(Shinagawa, Matsushita, Adachi, & Ameyama, 1982)
L-sorbose dehydrogenase	SDH	FAD	L-sorbose	L-sorbosone	Potential Vitamin C production	(Sugisawa, Hoshino, Nomura, & Fujiwara, 1991)
L-sorbosone dehydrogenase*	SNDHai	PQQ	L-sorbosone	L-ascorbic acid	Potential Vitamin C production	(Berry et al., 2003; Pappenberger & Hohmann, 2014)
Fructose dehydrogenase	FDH	FAD	D-fructose	5-keto-D-fructose	Potential low- calorie sweetener	(Ameyama, Shinagawa, Matsushita, & Adachi, 1981a)
Quinate dehydrogenase	QDH	PQQ	Quinate	3-dehydroquinate	Precursor for protocatechuic acid production	(Vangnai et al., 2004)

						(antioxidant and anti- inflammatory compound)	
	Myo-Inositol dehydrogenase	IDH	PQQ	Myo-inositol	2-keto-myo- inositol	No clear industrial importance	(Hoelscher, Weinert- Sepalage, & Goerisch, 2007)
	Glycerol dehydrogenase	GLDH	PQQ	(N- hydroxyethyl)- 1-amino-1- deoxy-d- sorbitol	(N-hydroxyethyl)- 6-amino-6- deoxy-l-sorbose	Production of the antidiabetic drugs, 1- deoxynojirimycin and miglitol	(Schedel, 2000)
2850 2851	*Note: Anothe converts L-sort	r enzyme o bosone to 2-	of the same r keto-L-gulon	name, SNDH-F	AD, is located in	the cytoplasm	and
2852							
2853							

2856Table 5. Definition of vinegar around the world.

Regulation	Definition	Reference
Malaysian Food Act 1983 and Food Regulation 1985,	Liquid product prepared from the alcoholic fermentation and subsequent acetous fermentation of any suitable food. The final product shall not contain less than 4 percent weight per volume (w/v) of acetic acid and shall not contain any mineral acid. The vinegar may also contain permitted preservatives, caramel as a colouring substance and spices as permitted flavouring substances	(Malaysian Food Regulations, 1985)
<i>Codex Alimentarius</i> Commission	Liquid suitable for human consumption and produced exclusively from appropriate products containing starch or sugars or starch and sugars by double fermentation processes, alcoholic and acetous. Vinegar shall not contain more than 0.5% alcohol, and stabiliser is not permitted for use in fermented vinegars according to European law. The vinegar itself shall not contain less than 50 g per litre (w/v) of acetic acid	(Codex Alimentarius Commission, 1987)
U.S. Food and Drug Administration (FDA)	There are no standards of identity for vinegar established under the Federal Food, Drug and Cosmetic Act. Nevertheless, the FDA considers that an acceptable guideline for vinegars that they must contain in excess of 4 g of acetic acid per 100 mL. Vinegar is made by the alcoholic and subsequent acetous fermentation of fruit juice	(United States of America Food and Drug Administration, 1977)
Commission Regulation (EU) 2016/263	 Liquid produced by double fermentation, i.e., alcoholic and acetic from agricultural origin such as fruit, cereal, grains, wine, cider or malt. Plants or part of plants, including fruit, spices, salt or sugar may be added for flavouring. Diluted acetic acid (diluted with water to 4-30 % by volume). 	(European Commission, 2016)
Food Standards Australia New Zealand 2.10.1	Sour liquid prepared by the acetous fermentation with or without alcoholic fermentation of any suitable foodstuff and includes blends and mixtures of vinegar. This vinegar must contain not less than 40 g/kg of acetic acid.	(Food Standards Australia New Zealand Act, 1991)
Food Safety and Standards Authority of India	Products obtained by the alcoholic and acetic acid fermentation of any suitable medium such as fruit, malt, or molasses, with or without the addition of caramel and spices. They shall not be fortified with acetic acid. The acidity, calculated as the acetic acid content, shall not be less than 3.75% (m/v), the total solids (m/v) shall not less than 1.5% , and the total ash content shall not be less than 0.18%	(Food Safety and Standards Authority of India, 2012)

	The Ministry of Food and Drug Safety - Korea (MFDS)	 Brewed vinegar that is produced by fermenting grains, fruits or alcoholic drinks or by mixing and ripening them with a grain-saccharified solution or fruit juice Synthetic vinegar that is manufactured by diluting glacial acetic acid or acetic acid with drinking water. The total acid content is quantified as the acetic acid content, which is in the range of 4.0 to 29.0% (w/v), and tar colour should not be detected 	(Ministry of Food and Drugs Safety, 2014)
	Chinese National Standard code of condiments (2004) Edible vinegar. No.14834, N5239	Products obtained from both fermentation or artificial process (acetic acid blended with other ingredients, such as flavours)	(Chinese National Standard, 2004)
2858			
2859			
2860			
2861			
2862			
2863			
2864			
2865			
2866			

Category	Raw material	Edible part	Intermediate	Main carbon sources	Vinegar name	Geographical distribution ^d
Vegetable ^a	Bamboo sap	Bamboo sap	Fermented bamboo sap	Sucrose	Bamboo vinegar ^b	Japan, Korea
	Palm sap	Sap (xylem fluid)	Palm wine (toddy, tari, tuack, tuba)	Sucrose	Palm vinegar, toddy vinegar	Southeast Asia, Africa
	Tea and Sugar		Kombucha	Sucrose	Kombucha vinegar	Russia, Asia (China, Japan, Indonesia)
	Onion	Bulbs	Onion alcohol	Fructose, glucose, sucrose	Onion vinegar	East and Southeast Asia
	Tomato		-		Tomato vinegar	Japan, East Asia
	Sugarcane	Stalks	Fermented sugar cane juice	Sucrose	Cane vinegar	France, USA
			Basi		Sukang iloko	Philippines
					Kibizu	Japan
Grains	Malt	Seeds (caryopsis)	Beer	Maltose	Malt vinegar	Northern Europa, USA
	Rice	Seeds (caryopsis)	Koji/moromi	Starch	Komesu, kurosu (Japanese) Heicu (Chinese)	East and Southeast Asia
	Barley	Seeds (caryopsis)	Beer	Starch	Vinegar	Germany, Austria, Netherlands
	Millet	Seeds	Koji/moromi	Starch	Black vinegar	China, East Asia
	Wheat	Seeds (caryopsis)	Koji/moromi	Starch	Black vinegar	China, East Asia
	Sorghum	Seeds (caryopsis	Koji/moromi	Starch	Black vinegar	China, East Asia
Fruit	Apple	Fruit (pome)	Cider	Fructose, glucose, sucrose	Cider vinegar	USA, Canada
	Grape	Fruits (berry)	Raisin	Fructose, glucose	Raisin (grape) vinegar	Turkey and Middle East
			Red or white wine		Wine vinegar	Widespread
			Sherry wine		Sherry (jerez) vinegar	Spain
			Cooked must		Balsamic vinegar	Italy
	Coconut	Coconut water	Fermented coconut water	Glucose, fructose	Coconut water vinegar	Philippines, Sri Lanka
	Date	Fruits (drupe)	Fermented date juice	Sucrose	Date vinegar	Middle East
	Mango	Fruits	Fermented mango juice		Mango vinegar	East and Southeast Asia
	Red date	Fruits (drupe)	Fermented jujube juice	Sucrose	Jujibe vinegar	China
	Raspberry	Fruits (berry)	Fermented raspberry juice	Fructose, glucose	Raspberry vinegar	East and Southeast Asia
	Black currant	Fruits (berry)	Fermented black currant juice	Fructose, glucose	Blackcurrant vinegar	East and Southeast Asia
	Blackberry	Fruits (berry)	Fermented blackberry juice	Fructose, glucose	Blackberry vinegar	East and Southeast Asia
	Mulberry	Fruits (berry)	Fermented mulberry juice	Fructose, glucose	Mulberry vinegar	East and Southeast Asia
	Plum	Fruits (drupe)	Umeboshi ^c fermented plum juice	Sucrose, fructose, glucose	Ume-su	Japan
	Cranberry	Fruits (drupe)	Fermented cranberry juice	Fructose, glucose	Cranberry vinegar	East and Southeast Asia
	Kaki	Fruit (pome)	Fermented persimmon juice	Fructose, glucose, sucrose	Persimmon vinegar	South Korea
		-		-	Kakisu	Japan
Animal	Whey	Whey	Fermented whey	Lactose	Whey vinegar	Europe
	Honey	Honey	Diluted honey win, tej	Fructose, glucose	Honey vinegar	Europe, America, Africa

2867 Table 6. Overview of vinegars from around the world: raw materials, intermediate product, vinegar name and geographical distribution.

2868 2869 2870 2871 *Vegetable is not a botanical term and is used to refer to an edible plant part; some botanical fruits, such as tomatoes, are also generally considered to be vegetables. b Obtained by bamboo sap fermentation (González & De Vuyst, 2009).

^c Umeboshi are pickled *ume* fruits. *Ume* is a species of fruit-bearing tree of the genus *Prunus*, which is often called a plum but is actually more closely related to the apricot. ^d Listed in order, from the largest to the

smallest amount.

2872 Reprinted with permission from (Solieri & Giudici, 2009).
2873	Table 7. Studies	examining the	potential health	benefits associa	ated with vinegar	consumption.
		<i>U</i>			U	1

Vinegar type	Function	Country	Subject(s)	Results	Reference
Spirit vinegar	Antibacterial	Japan	Food-borne pathogenic bacteria	The growth of all strains evaluated was inhibited with a 0.1% concentration of acetic acid in the vinegar.	(Entani et al., 1998)
Rice vinegar	Antibacterial	China	<i>E. coli</i> O157:H7	Treatment of inoculated lettuce (10^7 CFU/g bacteria) with vinegar (5% acetic acid) for 5 min would reduce 3 logs population at 25 °C.	(Chang & Fang, 2007)
Grape vinegar	Antibacterial	Turkey	Salmonella typhimurium	Treatment of carrot samples with vinegar (4.03% acetic acid) for different exposure times (0, 15, 30 and 60 min) caused significant reductions ranging between 1.57 and 3.58 log CFU/g.	(Sengun & Karapinar, 2004)
Acetic acid solution	Anti-infection	Kuwait	96 patients with chronic suppurative otitis media	The patients received ear irrigation with 2% acetic acid solution three times per week (3 weeks, followed for up to 3 years). 55 patients had resolution of their original otorrhen, whereas 19 patients developed healed ear drum perforation. 14 patients (15%) showed recurrence and 8 of them had no response to the treatment.	(Aminifarshidmehr, 1996)
Fermented vinegar	Anti-infection	Korea	15 patients with chronic granular myringitis	The patients were treated with irrigation of the external canal with dilute vinegar solution ($pH = 2.43$) twice to four times per day. All patients had resolution of their original otorrhoea within three weeks.	(Jung, Cho, Yoo, Lim, & Chae, 2002)
Shanxi aged vinegar	Antioxidative	China	Hyperlipidemic mouse	Fed with a diet with 1% freeze-dried powder of Shanxi aged vinegar for 35 d resulted in a significant increase in antioxidant activity in mice.	(Liu & Yang, 2015)
Zhenjiang aromatic vinegar	Antioxidative	China	Ageing accelerating mice	Instilled with 1.2 g/kg/d vinegar for 35 d resulted in a significant increase in antioxidant activity in mice.	(Xu, Tao, & Ao, 2005)

Kurosu	Antioxidative	Japan	Mice	The ethyl acetate extract of Kurosu significantly suppressed the 12-O- tetradecanoylphorbol-13-acetate induced myeloperoxidase activity and H ₂ O ₂ generation in mouse.	(Nishidai et al., 2000)
Traditional balsamic vinegar	Antioxidative	Italy	In vitro	Reducing capacity: 218.85 ± 6.86 mg Vc/100 mL Antiradical activity: 298.10 ± 6.25 mg Vc/100 mL	(Tagliazucchi et al., 2008)
Traditional balsamic vinegar	Antioxidative	Italy	In vitro	Reducing capacity: $27.12 \pm 11.1 \ \mu M$ Tes/mL Antiradical activity: $33.52 \pm 19.3 \ \mu M$ Tes/mL	(Bertelli et al., 2015)
Traditional balsamic vinegar	Antioxidative	Italy	Simulated gastric	The vinegar melanoidins (4.5 mg/mL) significantly inhibited the lipid peroxidation during simulated gastric digestion of meat.	(Verzelloni et al., 2010)
Balsamic vinegar	Antioxidative	Japan	Macrophage:THP-1	Balsamic vinegar (0.01%) significantly inhibited the low density lipoprotein (LDL) oxidation and lipid accumulation in macrophages	(Iizuka et al., 2010)
Red wine vinegar	Antioxidative	Italy	In vitro	Reducing capacity: $48.18 \pm 2.00 \text{ mg Vc}/100 \text{ mL}$ Antiradical activity: $85.40 \pm 1.73 \text{ mg Vc}/100 \text{ mL}$	(Verzelloni et al., 2007)
White vinegar	Blood glucose control	Sweden	12 healthy volunteers	Supplementation of a meal with vinegar (18 g) reduced postprandial responses of blood glucose and insulin and increased the subjective rating of satiety.	(E. Östman, Granfeldt, Persson, & Björck, 2005)
Bitter buckwheat vinegar	Blood glucose control	China	Diabetic rats	Oral intake of vinegar (2 mL/kg/d) for 4 weeks reduced about 17% blood glucose in rats.	(Ma, Xia, & Jia, 2010)
Rice vinegar	Blood glucose control	China	Diabetic rats	Oral intake of vinegar (2 mL/kg/d) for 30 d improved fasting hyperglycemia and body weight loss through attenuating insulin deficiency, pancreatic beta-cell deficit, and hepatic glycogen depletion in rats.	(Gu et al., 2012)
Apple vinegar	Blood glucose control	America	11 patients with type 2 diabetes	Vinegar ingestion (30 mL) at bedtime moderates waking glucose concentrations in adults with well-controlled type 2 diabetes mellitus.	(White & Johnston, 2007)

Apple vinegar	Blood glucose control	America	27 patients with type 2 diabetes	Oral intake of vinegar (1.4g/d) for 12 weeks significantly reduced haemoglobin A1c values in individuals with type 2 diabetes mellitus.	(Johnston et al., 2009)
Apple vinegar	Blood glucose control	America	8 healthy volunteers	Supplementation of a meal with vinegar (10 g) reduced about 23% postprandial responses of blood glucose.	(Johnston et al., 2010)
Apple vinegar	Blood glucose control	Japan	7 patients with polycystic ovary syndrome	Oral intake of vinegar (15 g/d) for 90 - 110 d improved insulin sensitivity in individuals with polycystic ovary syndrome.	(Wu et al., 2013)
Vinegar	Blood glucose control	Greece	10 patients with type 1 diabetes	Supplementation of a meal with vinegar (30 mL) reduced about 20% postprandial responses of blood glucose.	(Mitrou et al., 2010)
Acetic acid solution	Regulation of lipid metabolism	Japan	Human umbilical vein endothelial cell	Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric oxide synthase activity.	(Sakakibara et al., 2010)
Shanxi aged vinegar	Regulation of lipid metabolism	China	Hyperlipidemic mice	Fed with a diet with 1% freeze-dried powder of vinegar for 35 d resulted in a significant reduction of triglyceride, total cholesterol and LDL in mouse.	(Liu & Yang, 2015)
Sorghum vinegar	Regulation of lipid metabolism	China	Rats	Fed with a diet with extract of vinegar (100 mg/kg) protected the rats against thrombotic death induced by collagen and epinephrine.	(Fan et al., 2009)
Grape vinegar	Regulation of lipid metabolism	Iran	Rabbits with high cholesterol diet	Oral intake of 10 mL vinegar significantly reduced LDL- cholesterol, oxidized-LDL malondialdehyde and total cholesterol in rabbits after 3 hours.	(Setorki, Asgary, Eidi, & Khazaei, 2010)
Grape vinegar	Regulation of lipid metabolism	Egypt	Diabetic rats	Fed with a diet with 15% vinegar for 6 weeks significantly reduced LDL-cholesterol and total cholesterol in rats.	(Soltan & Shehata, 2012)

Apple vinegar	Regulation of lipid metabolism	Iran	19 patients with hyperlipidemia	Oral intake of vinegar (30 mL/d, twice) for 8 weeks significantly reduced triglyceride, total cholesterol and LDL in individuals with hyperlipidemia.	(Beheshti et al., 2012)
Persimmon vinegar	Regulation of lipid metabolism	Korea	Mouse with high lipid diet	Oral intake of vinegar (2 mL/kg/d) for 16 weeks significantly reduced triglyceride and total cholesterol in mouse.	(Moon & Cha, 2008)
Acetic acid solution	Weight loss	Japan	Obese mice	Fed with 0.3 or 1.5% acetic acid solution for 6 weeks significantly inhibited the accumulation of body fat and hepatic lipids without changing food consumption or skeletal muscle weight.	(Kondo, Kishi, Fushimi, & Kaga, 2009b)
Corn vinegar	Weight loss	China	Obese mice	Oral intake of vinegar (0.3 mL/d) for 30 d significantly reduced body weight, fat coefficient, triglyceride and total cholesterol in mouse.	(Li et al., 2009)
Purple sweet potato vinegar	Weight loss	China	Obese mice	Oral intake of vinegar (10 mL/ kg/d) for 30 d significantly reduced body weight, fat coefficient, LDL, triglyceride and total cholesterol in mouse.	(Liu et al., 2015)
Apple vinegar	Weight loss	Japan	150 obese Japanese	Oral intake of vinegar (15 mL/d) for 12 weeks significantly reduced body weight, body fat mass and serum triglyceride levels in subjects.	(Kondo et al., 2009a)
Apple vinegar	Weight loss	Mexico	Rats with high-caloric diets	Oral intake of vinegar (0.8 mL/ kg/d) for 4 weeks significantly reduced body weight, fat coefficient, LDL, triglyceride and total cholesterol in rats.	(De Dios Lozano, Juárez- Flores, Pinos-Rodríguez, Aguirre-Rivera, & Álvarez- Fuentes 2012)
Mulberry vinegar	Weight loss	China	Obese mice	Oral intake of vinegar (0.1 mL/d) for 30 d significantly reduced body weight, fat coefficient, triglyceride and total cholesterol in mouse.	(Wei et al., 2005)
Hawthorn Vinegar	Weight loss	Turkey	37 Obese patients with cardiovascular disease	Oral intake of vinegar (40 mL/d) for 4 weeks significantly reduced body weight, body fat mass and serum triglyceride levels in subjects.	(Kadas, Akdemir Evrendilek, & Heper, 2014)

Shanxi aged vinegar	Anti-carcinogenic	China	Cancer cells (A549, Hep-G2, MDA-MDB- 231, HeLa)	Ethyl acetate extract of vinegar (0.01%) significantly inhibited the proliferation of cancer cells <i>in vitro</i> .	(Chen & Gullo, 2015)
Kurosu	Anti-carcinogenic	Japan	Rats with colon cancer	Fed with water containing 0.05% ethyl acetate extract of Kurosu for 35 weeks significantly inhibited azoxymethane-induced colon carcinogenesis in rats.	(Shimoji et al., 2004)
Kurosu	Anti-carcinogenic	Japan	Cancer cell (Caco-2, A549, MCF-7, 5637, LNCaP)	Ethyl acetate extract of vinegar (0.025%) significantly inhibited the proliferation of cancer cells <i>in vitro</i> .	(Nanda et al., 2004)
Black soybeans vinegar	Anti-carcinogenic	Japan	Leukaemia U937 cells	Ethyl acetate extract of vinegar (10 mg/mL) significantly inhibited the proliferation of cancer cells <i>in vitro</i> .	(Inagaki et al., 2007)
Post-distillation slurry vinegar	Anti-carcinogenic	Japan	Mice with Sarcoma 180 and Colon 38 tumour cells	Fed with a diet with 0.5% vinegar for 72 d significantly decreased the sizes of tumours and prolonged life spans of mouse.	(Seki, Morimura, Shigematsu, Maeda, & Kida, 2004)
Sugarcane vinegar	Anti-carcinogenic	Japan	Leukaemia cells: HL- 60, THP-1, Molt-4, U- 937, K-562	Fraction eluted by 40% methanol from vinegar significantly inhibited the proliferation of leukaemia cells <i>in vitro</i> .	(Mimura et al., 2004)
Zhenjiang Aromatic Vinegar	Anti-fatigue	China	Mouse	Fed with a diet with vinegar (300 mg/kg/d) for 28 d significantly improved anti-fatigue abilities of mouse.	(Lu & Zhou, 2002)
Mulberry vinegar	Anti-fatigue	China	Mouse	Fed with a diet with vinegar (0.2 mL/d) for 20 d significantly improved anti-fatigue abilities of mouse.	(Zhang, Li, & Du, 2007)
Grain vinegar	Prevention of osteoporosis	Japan	Ovariectomized rats	Fed with a diet with 0.4% vinegar for 32 d significantly increased intestinal absorption of calcium in rats.	(Kishi et al., 1999)

2874 Reproduced from Chen et al., 2016 with permission, and with slight modification







Figure 2. Strategies of acetic acid resistance employed by Acetic acid bacteria. a) adaption of and protection of intracellular proteins to and against acid stress, b) metabolism (overoxidation) of intracellular acetic acid, c) efflux of acetic acid from the cell and d) prevention of acetic acid from entering the cell.





Figure 4. Schematic representation of vinegar production in submerged system. ^aAt concentrations specified by legislation; ^b Blending with high acidity vinegar, to block undesired alcoholic fermentation; c nutrients containing carbon and nitrogen sources, vitamins and minerals are supplemented especially to produce high acidity vinegar (>12% of acetic acid) from alcoholic stocks containing no carbon sources except for ethanol. Reprinted with permission from Gullo et al., 2014.



2918	Figure 5. Kombucha cellulose pellicle (SCOBY).
2919	
2920	
2921	
2922	
2923	
2924	
2925	
2927	
2927	
2320	



Figure 6. Different non-dairy products from sugary kefir fermentation. Reprinted with permission fromFiorda et al., 2017.



2932

2933 Figure 7. Processes used in, or having the potential for, Vitamin C production. (a) The Reichstein Process, microbial production of L-sorbose from

D-sorbitol; (b) The Two-Step Fermentation Process, microbial production of 2-KLGA from L-sorbose via a 2-step fermentation; (c) Microbial production of 2-KLGA by *G. oxydans*; (d) direct microbial production of Vitamin C by *G. oxydans*; (e) microbial production of 2-KLGA via the 2,5-diKGA pathway. C: chemical reaction, M: microbial bioconversion.

Strain characterization and genome sequencing

	Has the strain been characterized to genus and species level ? AND Has the strain been completely genome sequenced ?	If NO do it
1	Screening for undesirable attributes and metabolites <	' If YES go on
	Is the strain free from genetic elements encoding known virulence factors and/or toxins associated with pathogenicity ? AND	
	Is the strain free of functional and transferable antibiotic resistance gene DNA ?	Any NO: additional safety studies are required If YES go on
	Does the strain produce antimicrobial substances useful in human medicine?	↓ If YES: strain is not appropriate for human consumption
(Genetic modification 🗧	If NO go on
	Has the strain been genetically modified ?	If YES: additional safety studies are required (+regulatory approval required in most countries) If NO co on
5	Strain origin <	n i o go on
	Was the strain isolated from a food that has a history of safe consumption for which the species is a substantial and characterizing component (not simply an incidental isolate)? AND Has the species undergone comprehensive peer reviewed safety evaluation (e.g. QPS) or has it been affirmed safe by authoritative group/qualified scientific experts? AND Do findings published since completion of the peer review continue to support safety?	Any NO: additional safety studies
	····//	are required
1	Exposure levels <	
	Will the intended use of the strain expand exposure to the species beyond the group(s) that typically consume the species in traditional foods (in which it is typically found)? AND Will the intended use of the strain expand overall intake of the species (e.g. increasing the number of foods beyond traditional	
	foods in which the species is found or using the strain as probiotic rather than starter culture)	If NO: Strain is considered safe for human consumption Any YES: additional safety studies
	Additional safety studies 🗧 🧲	are required
	Does the strain induce undesirable physiological effects in appropriately designed safety studies (e.g. animal models or clinical trials) ?	If NO: Strain is considered safe for human consumption If YES: strain is not appropriate for human consumption
		manurconsumption

2938 2939

Figure 8. Decision tree for the safety assessment of microbial strains to be used in food applications. Reprinted from Laulund et al., 2017.