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Catabolism of L-Ascorbate by *Lactobacillus rhamnosus* GG

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ABSTRACT: Catabolism of L-ascorbate by enteric bacteria is well documented, but no study has formally proved that bacteria of the *Lactobacillus* genus ferment this compound. However, some genes analogous to those of *yiaK-S* operon and *ula* regulon, which encoded proteins leading to L-ascorbate degradation by *Escherichia coli* and *Klebsiella pneumoniae*, have been identified in the recently sequenced *Lactobacillus rhamnosus* GG genome. Investigations by HPLC and in vivo ^{13}C NMR using L-[1,6-^{13}C]-ascorbate showed that *L. rhamnosus* GG, a common probiotic strain, has the ability to catabolize L-ascorbate under anaerobiosis. The main products of the ascorbate degradation have been identified as CO_2, acetate, and lactate. These results are in accordance with the metabolic pathway proposed for the fermentation of L-ascorbate by *E. coli*.

KEYWORDS: L-ascorbate, lactic bacteria, *Lactobacillus rhamnosus* GG, probiotic

INTRODUCTION

Interest in ascorbate assimilation by intestinal bacteria dates from the 1930s when Marin[1] described a clinical case of hemorrhagic scurvy that did not respond to a diet rich in vitamins but that recovered upon intramuscular injection of ascorbate. These observations, suggesting a bacterial destruction of vitamin C in the gastrointestinal tract before absorption, were confirmed by additional investigations showing that some enteric bacteria were able to assimilate L-ascorbate.[2,3] In the next decades, no important progress was made to elucidate bacterial catabolism of L-ascorbate except that resulting from molecular biology or genomic sequencing projects, which prompted workers to assign functions to unknown genes. Proteins involved in this metabolic pathway under anaerobic conditions have been consequently first identified in *Escherichia coli* by Yew et al.[4] The *ula* regulon encoding for these proteins is formed by two operons, *ulaG* and *ulaA-F*. *ulaG* encodes for a L-ascorbate-6-P lactonase,[4] whereas *ulaA-F* determines the three components of the L-ascorbate phosphotransferase transport system (PTS) UlaABC[5] as well as three catabolic enzymes (UlaDEF).[4] The three gene products UlaABC are involved in the uptake and phosphorylation of L-ascorbate.[5] The intracellular L-ascorbate 6-P generated is then transformed by UlaG to 3-keto-L-gulonate-6-P. Yew et al.[4] have proposed that this compound is decarboxylated by UlaD to form L-xylulose-5-P, which is afterward converted to D-xylulose-5-P by the sequential action of UlaE (3-epimerase activity) and UlaF (4-epimerase activity). *ula* gene products are then implicated in uptake and integration in the pentose phosphate pathway (Figure 1). The *ula* regulon is under the control of the UlaR repressor, which is expressed constitutively and binds cooperatively to four operators organized in two pairs located in both *ulaG* and *ulaA* promoters. Under aerobic conditions, an additional operon, *YiaK-S*, is required to catabolize L-ascorbate.[6] As previously described, the PTS UlaABC is used for internalization and phosphorylation of L-ascorbate.[7] The *yiaK-S* operon, under the control of the YiaJ repressor, encodes three proteins paralogous to UlaDEF, YiaQRS,[8] a dehydrogenase, and a kinase (YiaK and YiaP, respectively). These two last enzymes are theoretically able to convert 2,3-diketogulonate (autoxidation product of L-ascorbate in the presence of oxygen) to 3-keto-L-gulonate-6-phosphate.[4] It must be noted that Campos et al.[6] have demonstrated that both UlaR and YiaJ repressive activities were abolished by the presence of L-ascorbate-6-phosphate.

The *Lactobacillus* genus is well represented in the human gut microflora, and its presence was thought to beneficially affect the human host.[9] Several *Lactobacillus* strains have been identified as probiotics, living microbial food supplements that have been shown to exert beneficial effects on human health. The probiotic products represent an overall world market of around U.S.$ 16 billion in 2008 with continuous strong annual growth, including more than 500 new introduced products in the past decade (mostly in dairy foods and beverages). *Lactobacillus rhamnosus* GG is a strain found in numerous commercial products and represents one of the best clinically studied probiotic organisms.[10,11] Database analysis using the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg) showed that some genes analogous to the *yiaK-S* operon and *ula* regulon are present in the recently sequenced *L. rhamnosus* GG genome,[12,13] leading to the possibility that this strain could assimilate L-ascorbate. We present in this work clear experimental evidence that L-ascorbate is effectively catabolized by *L. rhamnosus* GG as the sole source of energy.

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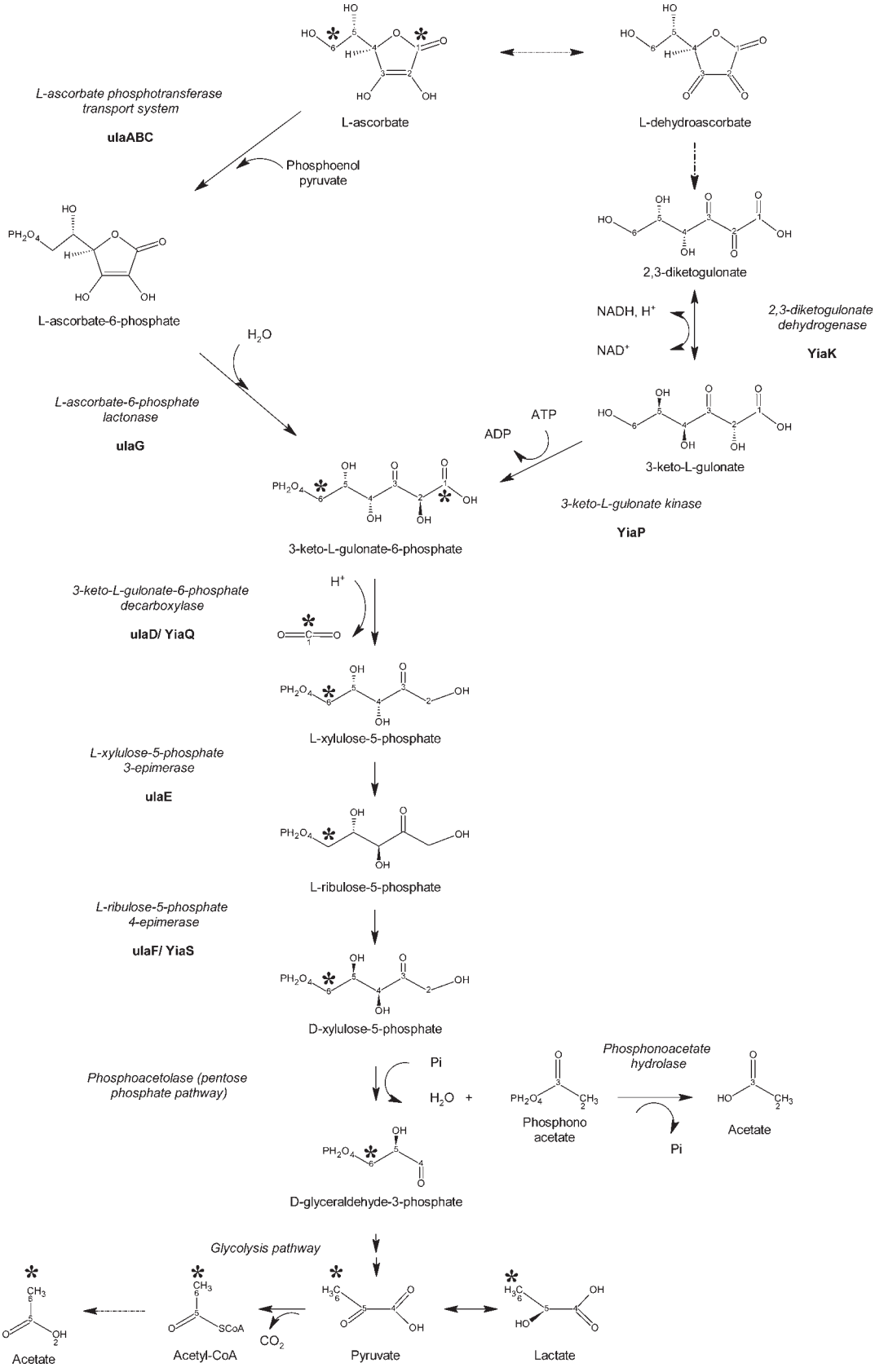

Figure 1. Metabolic map of L-ascorbate utilization by *Escherichia coli*, showing enzymes solicited for metabolic steps (italic) and corresponding proteins encoded by *ula* regulon or *yia* operon (bold). Dashed arrows indicate enzymatic or chemical reactions. Asterisks correspond to the labeled carbons used in this study.

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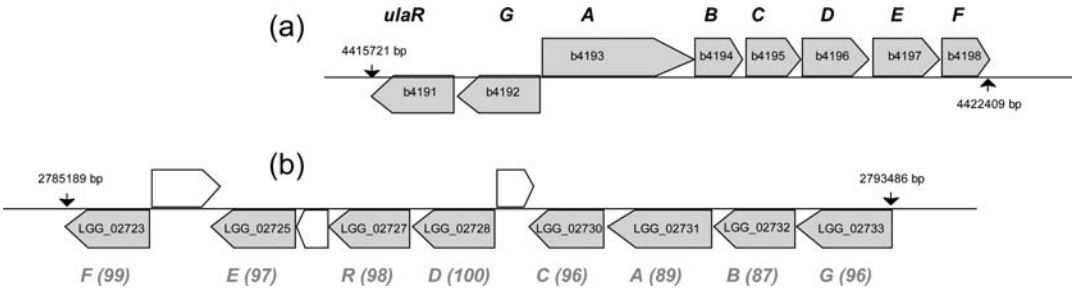

Figure 2. Schematic representation of *ula* operon of *Escherichia coli* K-12 MG1655 (a) and 2785189−2793486 bp region of *Lactobacillus rhamnosus* GG genome (b). Putative *ula* representative genes in *L. rhamnosus* are indicated in gray. In brackets: Query coverage (in %) of gene products is given in parentheses.

MATERIALS AND METHODS

Bacterial Strains. *Lactobacillus* strains were obtained from ATCC and DSMZ culture collections (American Type Culture Collection, Rockville, MD, and Deutsche Sammlung von Mikroorganismen and Zellkulturen, Braunschweig, Germany, respectively). The five strains used in this study were *Lactobacillus gasseri* DSMZ 20243, *Lactobacillus paracasei* subsp. *paracasei* DSMZ 20312, *Lactobacillus casei* DSMZ 20011, *Lactobacillus acidophilus* DSMZ 20079, and *L. rhamnosus* GG ATCC 53103.

Culture on Multiwell Plates. Bacterial growth was measured with an automatic turbidometer and the Bioscreen C MBR analyzing system (Labsystems, Helsinki, Finland), which records kinetic changes in the absorbance of liquid samples in a multiwell plate. Each well of the plate was filled with 150 μL of carbohydrate substrates (D-glucose or L-ascorbate) solubilized in distilled water (final concentration was 5 or 10 g/L according to experiments) and with 150 μL of double-concentrated semisynthetic medium.[14] The double-concentrated semisynthetic medium was composed (for 1 L) of casamino acids (Difco) (30 g), yeast nitrogen base (Difco) (13.4 g), sodium acetate·3H_2O (20 g), $(NH_4)_2SO_4$ (2 g), urea (4 g), $MgSO_4 \cdot 7H_2O$ (0.4 g), $FeSO_4 \cdot 7H_2O$ (0.02 g), $MnSO_4 \cdot H_2O$ (0.014 g), cystein-HCl·H_2O (1 g), and Tween 80 (2 mL). The pH was adjusted to 6.4, and the medium was sterilized by filtration at 0.2 μm. Medium was inoculated with cell suspension, after sterilization and before well filling, to have 10^7 CFU/mL. All of the strains were incubated at 37 °C for 48 h, and the absorbance was measured at 600 nm every 2 h after low agitation for 5 s. Experiments were performed in triplicate.

Culture in Flask. Culture was performed in a 250 mL Schott bottle filled with 100 mL of modified Morishita et al.[15] minimal medium (composition for 1 L: $MgSO_4 \cdot 7H_2O$ (0.2 g), $MnSO_4 \cdot H_2O$ (0.05 g), KH_2PO_4 (3 g), Na_2HPO_4 (3 g), L-tyrosine (0.05 g), L-aspartate (0.2 g), L-cysteine (0.2 g), L-glycine (0.2 g), L-glutamate (0.5 g), L-alanine (0.1 g), L-arginine (0.1 g), L-histidine (0.1 g), L-isoleucine (0.1 g), L-leucine (0.1 g), L-lysine (0.1 g), L-methionine (0.1 g), L-phenylalanine (0.1 g), L-proline (0.1 g), L-serine (0.1 g); L-threonine (0.1 g); L-tryptophan (0.1 g), L-valine (0.1 g), riboflavin (0.0005 g), 4-aminobenzoate (0.0002 g), folate (0.0001 g), nicotinate (0.001 g), D-penthotenate, calcium salt (0.001 g), and pyridoxine (0.001 g); the pH was adjusted to 6.4, and medium was sterilized by filtration at 0.2 μm. L-Ascorbate solution (sterilized by filtration at 0.2 μm) and cell suspension of *L. rhamnosus* GG were added to the medium to have final concentrations of 10 g/L and 10^7 CFU/mL, respectively. To attain anaerobiosis, the bottle was sparged for 15 min with argon. Sampling (10 mL) was done at 0, 43, 68, 88, 112, and 135 h, and the samples were immediately centrifuged (10000*g*, 10 min at 4 °C). Supernatants were filtered through preweighed 0.2 μm nitrocellulose filters (Sartorius). Filters were used for the determination of biomass dry weight (after drying for 24 h at 110 °C), and filtrates were stored at −20 °C for amino acid and HPLC assays. Experiments were done in triplicate.

Amino Acid Analysis. Two methods were used in this study. Total nitrogen concentration was determined using the Hach Total Nitrogen HR Test'N Tube (10−150 mg/L N) TNT persulfate method (no. 10072). Samples (0.5 mL of filtered supernatant diluted by 5 or distilled water for zero reference) were first digested with persulfate in alkaline conditions (105 °C, 30 min in a DCO reactor). Sodium metabisulfite was added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex. Absorbance was read at 420 nm using a Hach DR 820 colorimeter. Amino acid concentrations were estimated by dividing total nitrogen concentrations by 7.35 (nitrogen/amino acid ratio of the medium used). Soluble amino nitrogen was also determined with ninhydrin following the method of Lee et al.[16] with L-glutamic acid as a standard. Samples (0.2 mL filtered supernatant diluted by 5 or distilled water for zero reference) were added with 3.8 mL of ninhydrin reagent (0.5 g of ninhydrin with 128 mL of glycerol in sqf 200 mL of 0.5 M sodium citrate buffer), and sealed tubes were incubated for 5 min at 100 °C before reaction was stopped with ice. Absorbance was read at 570 nm with a UV-1700 spectrophotometer (Shimadzu).

HPLC Analysis. Acetate and lactate were assessed by HPLC analysis of centrifuged (10 min, 10000*g* at 4 °C) and deproteinized samples. Protein removal was carried out using the $Ba(OH)_2$−$ZnSO_4 \cdot 7H_2O$ method of Slein.[17] The HPLC system (Agilent 1100, Agilent Technology) was fitted with two columns (Resex ROA 300 × 7.8 mm, Phenomenex) mounted in serial assembly in an oven thermostated at 50 °C. Columns were eluted at 0.7 mL/min by a solution of 2 mM H_2SO_4 in ultrapure water. A refractometer (RID G1362A) was used as detector. Lactate and acetate were respectively eluted at 24.30 and 28.48 min.

^{1}H and ^{13}C NMR Spectroscopy. The metabolic behavior of L-ascorbate after consumption by *L. rhamnosus* GG was studied in vivo by ^{13}C NMR spectroscopy with a Bruker Avance 500 spectrometer equipped with a 10 mm broadband probe. For these experiments, *L. rhamnosus* GG cells were first cultivated for 48 h in semisynthetic medium with L-ascorbate (10 g/L) and washed twice by centrifugation (10000*g*, 10 min at 20 °C). Cells were then held in suspension in a phosphate buffer (20 mM, pH 6.4) at 10^8 CFU/mL under anaerobic conditions as described by Grivet et al.[18] Benzene contained in an in situ capillary was used as internal reference to calibrate chemical shifts (128.39 ppm relative to TMS (0 ppm)). Incubation medium was composed of 4 mL of cell suspension in phosphate buffer (15 mM, pH 6.4) containing 10% D_2O and 10 mM L-[1,6-^{13}C]-ascorbate from Campro Scientific. Spectra were acquired with power-gated ^{1}H decoupling (WALTZ16 sequence). Acquisition parameters were as follows: number of scans, 700 (corresponding to 5 min acquisition for each spectrum); 90° pulse time, 20 μs; relaxation delay, 1 s; acquisition time, 0.14 s; spectral window, 30303 Hz; data points, 8K, zero filled to 16K. Experiments were done anaerobically (tube was flushed for 10 min with argon and hermetically sealed) at 22 °C.

Table 1. Maximal Absorbance ± Standard Deviations (λ = 600 nm) Reached after 48 h of *Lactobacillus* Incubations in Semisynthetic Medium Supplemented or Not with Carbohydrates

	A_{600}			
strain	glucose (10 g/L)	no substrate	L-ascorbate (5 g/L)	L-ascorbate (10 g/L)
none (abiotic control)	0.00 ± 0.01	0.00 ± 0.01	0.21 ± 0.01	0.26 ± 0.01
L. gasseri	1.22 ± 0.06	0.00 ± 0.01	0.21 ± 0.02	0.24 ± 0.04
L. paracasei	1.24 ± 0.07	0.00 ± 0.01	**0.32 ± 0.09**	**0.52 ± 0.10**
L. rhamnosus GG	1.30 ± 0.13	0.00 ± 0.01	**0.43 ± 0.12**	**0.63 ± 0.10**
L. casei	0.64 ± 0.14	0.01 ± 0.01	0.21 ± 0.04	0.25 ± 0.02
L. acidophilus	0.58 ± 0.03	0.00 ± 0.01	0.24 ± 0.06	0.24 ± 0.04

At the end of the in vivo experiment (see above), biomass was harvested by centrifugation (10000*g*, 10 min, 4 °C), and the resulting supernatant was frozen in liquid nitrogen and lyophilized before analysis by ^{1}H NMR. This freeze-dried material containing nonvolatile compounds was dissolved in 2.5 mL of D_2O, buffered with 50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and finally lyophilized. Divalent cations (particularly Mn^{2+} and Mg^{2+}) were chelated by the addition of sufficient amounts of CDTA ranging from 50 to 100 μmol/L depending on the samples. Chelating paramagnetic cations is a prerequisite for obtaining sharp resonance signals. In vitro ^{1}H NMR experiments were performed with a Bruker Avance 500 equipped with a 5 mm triple-tuned ^{1}H−^{13}C−^{15}N probe fitted with a *z*-gradient coil. Water resonance was suppressed by low presaturation method. $TSPd_4$ (5 mM) dissolved in D_2O was used as internal reference. Acquisition parameters were as follows: number of scans, 128; impulsion time, 8.4 μs; relaxation delay, 5 s; acquisition time, 4.67 s; spectral window, 7002 Hz; data points, 65536. Spectra were treated with Topspin version 2.0. For the preparation of samples, 60 μL of $TSPd_4$ solution in D_2O (5 mM) and 540 μL of D_2O were added to the dried material.

Bioinformatic Analysis. Putative *ula* representative genes in the *L. rhamnosus* genome were found by querying the BLAST program of the National Center of Biotechnology Information (NCBI) with amino acid sequences of *E. coli* K-12 MG1655 (taxid: 83333) *ulaRGABCDEF* gene products (http://blast.ncbi.nlm.nih.gov/Blast.cgi): database, nonredundant protein sequences; organism, *L. rhamnosus* GG (taxid: 568703); algorithm, BlastP 2.2.24+.[19]

RESULTS

Screening of Lactobacilli for L-Ascorbate Fermentation. The genome sequence (3 010 111 bp) of *L. rhamnosus* GG, the original strain of *L. rhamnosus* ATCC 53103, was recently deposited in a public database (Genbank accession no. FM17-9322).[12] Strong similarities were found between *ula* regulon gene products from *E. coli* and some gene products found in the *L. rhamnosus* proteome obtained with this genome (Figure 2). As a result, the ability of *Lactobacillus* species to assimilate L-ascorbate was suspected, and in vitro experiments were performed to evaluate the putative catabolitic activity.

In vitro fermentations of different carbohydrate substrates by five *Lactobacillus* strains were screened by a multiwell plate test developed in the laboratory (see Materials and Methods). Results showed a global increase of maximal absorbance reached in the presence of D-glucose or L-ascorbate (Table 1). This was correlated with the presence of carbohydrate as no absorbance elevation was seen in the negative control (without carbohydrate). However, abiotic control tests (done without cell suspension) revealed that this phenomenon was partially due to yellow coloration of the medium observed in the presence of

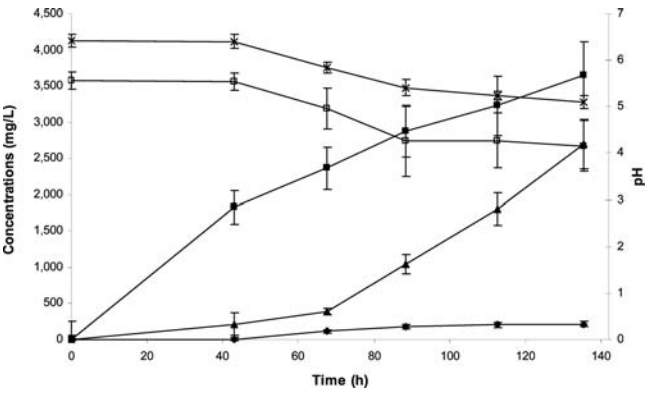

Figure 3. Production of biomass (◆), acetate (■), and lactate (▲), evolution of pH (×), and concentration of amino acids obtained with Hach total nitrogen measurement (□) during culture of *Lactobacillus rhamnosus* GG in a flask maintained under anaerobic conditions. Error bars = standard deviation.

L-ascorbate. Maximal absorbance was reached in the presence of *L. paracasei* and *L. rhamnosus* GG and was dose-dependent. A putative catabolitic activity of these strains on L-ascorbate was then hypothesized. At this stage, it was not clear if the increase of absorbance was correlated to biotic or abiotic phenomenon. The occurrence of growth could be linked to the consumption of L-ascorbate or one of its degradation products. It was also possible that L-ascorbate could serve as a reducing agent or cofactor favoring the use of other carbon sources present in the medium (amino acids, for example)

Furthermore, cultures in flask of *L. rhamnosus* GG were done under anaerobic condition to avoid autoxidation of L-ascorbate and in minimal medium to control the catabolism of amino acid. A correlation between absorbance (600 nm) and dry weight was carried out to link the following two phenomena: "increase of absorbance" with "production of biomass". The consumption of added amino acids was measured by Hach total nitrogen measurement and correlated with the method of Lee et al.[16] Productions of organic acids (lactate and acetate) resulting from putative fermentation of L-ascorbate were analyzed by ion exclusion HPLC method. Acquired results confirmed clearly the production of biomass combined with a pH decrease due to the formation of lactate and acetate (Figure 3). These data led to the conclusion that a fermentation process occurred. The concentration of amino acids decreased, but the consumed mass stayed inferior to the produced one (biomass and organic acids). Thus, the growth of *L. rhamnosus* GG was also supported by L-ascorbate as it was the sole compound with amino acids that could be used as carbon source.

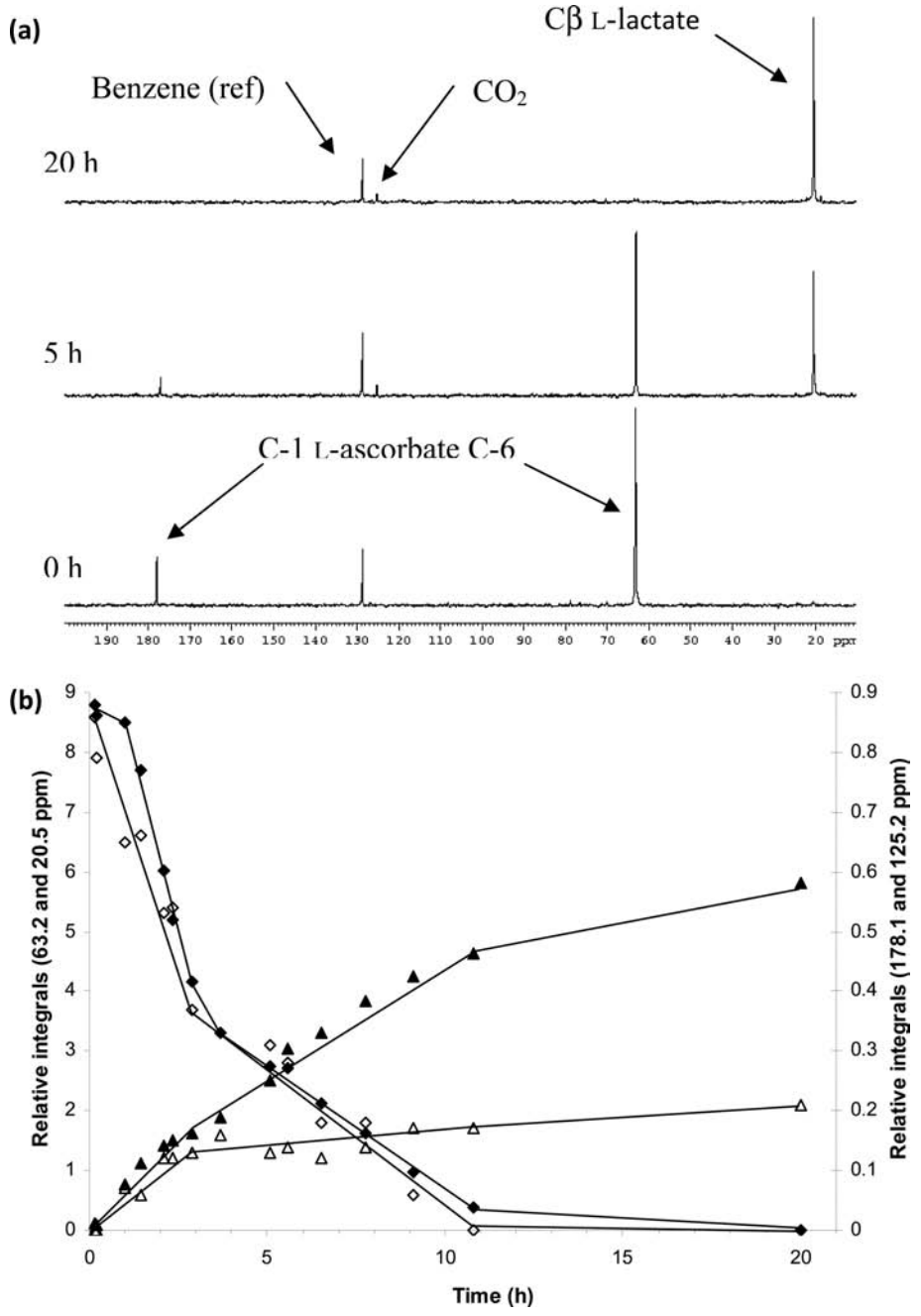

Figure 4. Catabolism of [1,6-^{13}C]-L-ascorbate by *Lactobacillus rhamnosus* GG: (a) in vivo ^{13}C NMR spectra; (b) time courses of the relative integrals of C1 (◇) and C6 (◆) of L-ascorbate, solubilized carbon dioxide (△), and Cβ of lactate (▲).

Fermentation of L-Ascorbate by *Lactobacillus rhamnosus* GG. Figure 4a presents the in vivo ^{13}C NMR spectra collected after 0, 5, and 20 h of incubation with 10 mM L-[1,6-^{13}C]-ascorbate under anaerobiosis at 22 °C. This labeled L-ascorbate was used to confirm its degradation and to identify the products of its catabolic pathway (CO_2, acetate, and lactate) from the observation of two coupled signals at the expected chemical shifts. Figure 1 removes any ambiguity regarding the first step under anaerobiosis so as L-ascorbate could not be oxidized into L-dehydroascorbate. The spectrum obtained at 0 h of incubation (Figure 4a) showed two signals at 178.1 and 63.2 ppm, which were assigned respectively to L-ascorbate C-1 and C-6.[20] These signals decreased during incubation while two others appeared concomitantly. These two new signals were assigned to the carbon of solubilized carbon dioxide (125.2 ppm) and Cβ of lactate (20.5 ppm) according to the literature.[21,22] L-Ascorbate was entirely consumed after 12 h without a lag phase (data not shown), contrary to previous results observed in Figure 3. This phenomenon was presumably due to the precultivation of *L. rhamnosus* GG on L-ascorbate and indicated that the consumption of this compound was inducible. Kinetics showed that the apparition of solubilized carbon dioxide was connected to the disappearance of C-1 of L-ascorbate and to the appearance of Cβ of lactate followed by the decrease of C-6 of L-ascorbate (Figure 4b). This confirmed that the anaerobic catabolism of L-ascorbate by *L. rhamnosus* GG corresponded to the metabolic

pathway described in Figure 1. The presence of lactate and acetate at equimolar concentration (5 mM) was established by HPLC analysis. The lack of signals for acetate on ^{13}C NMR was explained by the very long relaxation time (T1) of its labeled carbon. This T1 was not compatible with the in vivo ^{13}C NMR experiment, by which spectra were recorded with a relaxation delay of 1 s. To confirm this hypothesis and to validate the presence of lactate, a ^{1}H NMR spectrum was performed on the supernatant recovered after the in vivo ^{13}C NMR experiment. Signals of ^{13}C- and ^{12}C-linked protons from methyl groups of lactate and acetate were specifically detected (Figure 5). In each case, the central peak corresponds to protons bound to ^{12}C atoms, and the satellite peaks correspond to those bound to ^{13}C atoms.[23] The synthesis of ^{13}C- and ^{12}C-acetates could result from xylulose-5-phosphate degradation pathways via acetyl-CoA and phosphonoacetate, respectively.[24] The presence of ^{13}C- and ^{12}C-lactates was more ambiguous as only ^{13}C-lactate should be identified in regard to the metabolic map proposed for L-ascorbate (Figure 1). The main hypothesis to explain this presence of ^{12}C-lactate is the catabolism of glucogenic amino acids. Indeed, the biotransformation of ^{12}C-amino acids could generate ^{12}C-pyruvate and consequently ^{12}C-lactate by the activity of lactate dehydrogenase (Figure 6). The pathway could lead also to the production of ^{12}C-acetate. This hypothesis has been confirmed by the quantification of various isotopomers (^{12}C and ^{13}C) of lactate and acetate generated by the degradation of ^{13}C-ascorbate and ^{12}C-amino acids (Figure 6 and Table 2). ^{13}C-Acetate and ^{13}C-lactate were produced only from ^{13}C-ascorbate. The integration of proton signals from their $^{13}CH_3$ groups (8.00 + 1.97 = 9.97) is equal to that of the $^{12}CH_3$ group from ^{12}C-acetate generated by ^{13}C-ascorbate catabolism (cleavage of ^{13}C-D-xylulose-5-P). According to the ^{13}C-ascorbate metabolic pathway (Figure 1), ^{12}C-lactate can only be issued from the catabolism of amino acids. Because the ratio of ^{13}C-lactate/^{13}C-acetate is known (4.06), the integration of proton from $^{12}CH_3$ groups of ^{12}C-acetate from the degradation of amino acid can be calculated using the measured value of proton integrals from $^{12}CH_3$ of ^{12}C-lactate (integral = 3.51). This value is 0.86. The total amount of ^{12}C-acetate can be thus evaluated as the sum of proton integrals from $^{12}CH_3$ groups of ^{12}C-acetate from ^{13}C-ascorbate and amino acids. The calculated integral with 10.83 can be compared to the corresponding measured integral with 12.56. It can be estimated that these two values are in good agreement with each other considering the experimental errors of integral measurements is around 10%. This demonstrates that amino acids can really be at the origin of the ^{12}C-lactate and part of the ^{12}C-acetate. In addition, these integrals can give a quantitative evaluation of the fluxes in the various metabolic routes. This result was also partially reinforced by those obtained when bacteria were grown in a minimal medium (Figure 3). After 67 h of culture, the outcome of the experiments showed clearly that when amino acids were assimilated by bacteria, the kinetics of lactate production demonstrated a significantly higher increase compared to the acetate kinetics, which kept rising more or less consistently. This is in full agreement with our hypothesis due to the fact that the degradation of amino acids generates more lactate than acetate (approximately 3 times more), whereas it is the reverse when only ascorbate is degraded.

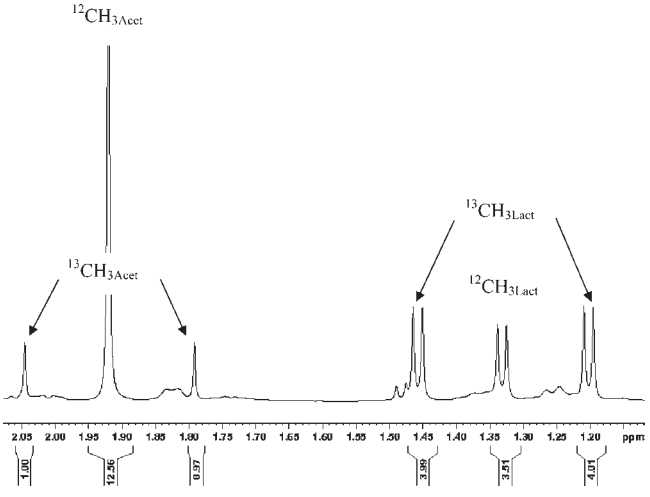

Figure 5. ^{1}H NMR spectrum of supernatant from in vivo ^{13}C NMR experiment of catabolism of [1,6-^{13}C]-L-ascorbate by *Lactobacillus rhamnosus* GG.

DISCUSSION

In contrast to the *Lactobacillus* genus bacteria, L-ascorbate fermentation has been described thoroughly for several enteric bacteria such as *Enterobacter aerogenes*,[25] *E. coli*,[4] and *K. pneumoniae*,[8] which are normal inhabitants of the mammalian intestine. Under anaerobiosis, degradation of L-ascorbate by *E. coli* and *K. pneumoniae* is carried out by *ula* regulon-encoded proteins. The *ula* regulon is induced by growth on L-ascorbate and is under the control of the UlaR repressor constitutively expressed by *ulaR*,[7] which belongs to the DeoR repressor family.[4] These

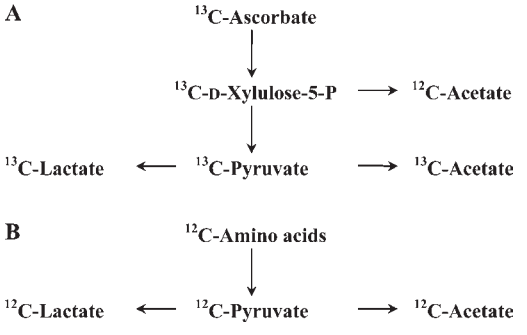

Figure 6. Generation of ^{12}C and ^{13}C isotopomers of acetate and lactate via ascorbate pathways (A) and glucogenic amino acid (B) degradations.

Table 2. Integrals of $^{12}C/^{13}C$-Lactate and -Acetate Measured by ^{1}H NMR Spectroscopy or Calculated

	isotopomers			
	^{13}C-lactate	^{12}C-lactate	^{13}C-acetate	^{12}C-acetate
labeling origin	^{13}C-ascorbate	^{12}C-amino acids	^{13}C-ascorbate	^{13}C-ascorbate and ^{12}C-amino acids
measured integrals	8.00	3.51	1.97	12.56
calculated integrals				10.83

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types of repressors recognize generally a phosphorylated sugar as an effector molecule. Other data also obtained with *E. coli* showed that another operon, *yiaK-S*, participates in the aerobic dissimilation of L-ascorbate. Transcriptional fusions and proteomic analysis indicated that the *ula* regulon and *yiaK-S* operons are required for the aerobic use of L-ascorbate by *E. coli* and *K. pneumoniae*.[7,8] Investigations on L-ascorbate catabolism by *K. pneumoniae* elucidated the role of L-ascorbate-6-phosphate (the product of UlaABC, the phosphotransferase transport system) as an effector of UlaR, releasing the UlaR−DNA complex.[6,8]

Interestingly, the anaerobic part of this pathway supported by *ula* genes has been clearly identified on the *L. rhamnosus* GG genome[12,13] containing grouped genes whose products possess strong similarities with Ula proteins (Figure 2). The growth of *L. rhamnosus* GG on semisynthetic medium using L-ascorbate as the sole carbon source gave evidence of its capability to use this compound under anaerobiosis. The detection of acetate and lactate in the culture medium reinforced this analysis. Additional in vivo ^{13}C NMR experiments using L-[1,6-^{13}C]-ascorbate as carbon substrate showed that *L. rhamnosus* GG has the capability to catabolize this compound. CO_2, lactate, and acetate have been detected again as products of this catabolic pathway.

Our results demonstrate undoubtedly that an intestinal bacteria of the *Lactobacillus* genus has the capability to ferment L-ascorbate, and positive elements were retrieved with a *L. paracasei* species (Table 1). These two *Lactobacillus* strains as well as others are actually the subject of abundant literature as probiotics, and some of them are intensively employed in food products for their beneficial effects on the health of the host.[26] It is reasonably possible to hypothesize that the faculty to ferment ascorbate should be present among the majority of these strains. In this context, the competition of these intestinal bacteria with the intestinal mucosal cells for L-ascorbate could be significant when they are abundantly brought by feeding. Zhang et al.[5] have shown that an L-ascorbate specific transporter localized in mammalian intestine epithelial cells displayed a lower affinity than the L-ascorbate phosphotransferase transport system of *E. coli*. These authors suggested that the physiological consequences of the competitive use of ascorbate by intestinal bacteria to human and animal health have to be evaluated in vivo. It will then be necessary to measure the potential of some probiotics complemented or not with prebiotic(s)/nutraceutic(s) in food to reinforce the ability of several enteric bacteria to compete with the host for ascorbate assimilation. Experiments on animal models with controlled and specific gut microflora could evaluate the impact of probiotic supplementation on L-ascorbate assimilation by the host.

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