The ISME Journal (2012) 6, 927–938

www.nature.com/ismej

ORIGINAL ARTICLE

Resource partitioning in relation to cohabitation of *Lactobacillus* species in the mouse forestomach

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Phylogenetic analysis of gut communities of vertebrates is advanced, but the relationships, especially at the trophic level, between commensals that share gut habitats of monogastric animals have not been investigated to any extent. *Lactobacillus reuteri* strain 100–23 and *Lactobacillus johnsonii* strain 100–33 cohabit in the forestomach of mice. According to the niche exclusion principle, this should not be possible because both strains can utilise the two main fermentable carbohydrates present in the stomach digesta: glucose and maltose. We show, based on gene transcription analysis, *in vitro* physiological assays, and *in vivo* experiments that the two strains can co-exist in the forestomach habitat because 100–23 grows more rapidly using maltose, whereas 100–33 preferentially utilises glucose. Mutation of the maltose phosphorylase gene (*malA*) of strain 100–23 prevented its growth on maltose-containing culture medium, and resulted in the numerical dominance of 100–33 in the forestomach. The fundamental niche of *L. reuteri* 100–23 in the mouse forestomach can be defined in terms of 'glucose and maltose trophism'. However, its realised niche when *L. johnsonii* 100–33 is present is 'maltose trophism'. Hence, nutritional adaptations provide niche differentiation that assists cohabitation by the two strains through resource partitioning in the mouse forestomach. This real life, trophic phenomenon conforms to a mathematical model based on *in vitro* bacterial doubling times, *in vitro* transport rates, and concentrations of maltose and glucose in mouse stomach digesta.

The ISME Journal (2012) **6,** 927–938; doi:10.1038/ismej.2011.161; published online 17 November 2011
Subject Category: microbe–microbe and microbe–host interactions
Keywords: gut; niche; adaptation; lactobacilli; resource partitioning; mathematical model

Introduction

Collections of commensal species form communities of characteristic composition in association with various body sites of vertebrates (Ley *et al.*, 2008). Gut-associated communities are temporally stable in composition suggesting that they are self-regulating assemblages (Costello *et al.*, 2009). Although gut-associated communities can be thoroughly described in terms of phylogenetic composition (Ley *et al.*, 2008), little is known about the nutritional facets of ecological niches occupied by each species within a gut community, or the mechanisms that determine how different species can share a habitat. In general, micro-ecological studies of the gut have emphasised niche (competitive) exclusion. Theoretically, two species cannot co-exist in a habitat if their ecological niches are identical (Hardin, 1960). For example, niche exclusion probably explains the control by commensal communities of opportunist pathogens such as *Clostridium difficile* (Louie *et al.*, 2009). Genomic data from gut commensals often show that bacteria have several pathways by which a variety of substrates can be degraded (Gill *et al.*, 2006). It is likely that these pathways are tightly regulated and can be switched according to the availability of particular substrates delivered by way of the host's diet. Carried a step further, evolution by natural selection could result in one of the species using a different source of resources or using one resource more efficiently than its potential competitor. Resource partitioning mediated by nutritional adaptations could thus allow potential competitors to share a habitat.

Lactobacilli are common inhabitants of proximal regions of the gut of rodents, fowls and pigs where they associate with non-secretory epithelial surfaces. *Lactobacillus reuteri* strain 100–23 colonises the forestomach epithelium of mice previously

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Received 11 July 2011; revised 14 September 2011; accepted 30 September 2011; published online 17 November 2011

naïve to lactobacilli (*Lactobacillus*-free), where it forms a biofilm (Tannock *et al*., 2005). *Lactobacillus* cells shed from this site are present in the digesta of the remainder of the gut. The largest populations of strain 100–23 are found in the forestomach and mutant strains that have impaired colonisation ability in the forestomach are present in proportionately smaller numbers in the small and large bowels (Walter *et al*., 2005). This indicates that the forestomach is the habitat of strain 100–23. The *L. reuteri* 100–23/*Lactobacillus*-free mouse paradigm has provided information about the attributes required of a gram-positive commensal to inhabit the gut of a vertebrate host (Tannock, 2004). In all of these studies, however, strain 100–23 lacked competition from other kinds of lactobacilli. We have begun to investigate features of co-colonisation of the gut by *Lactobacillus* species. In this paper, we reason that *L. reuteri* 100–23 and *Lactobacillus johnsonii* strain 100–33, although having similar capacities for gut colonisation and similar requirements for fermentable substrates, co-exist in the forestomach habitat by sharing, rather than competing for, resources.

Materials and methods

Lactobacillus *strains*

L. reuteri 100–23 (DSM 17509) and *L. johnsonii* 100–33 were isolated from conventional rodents at the University of Otago and have been maintained in the laboratory culture collection. It is not known whether the two strains originated from the same animal host. They were cultured in Lactobacilli MRS medium (Difco, Becton Dickinson Co., Sparks, MD, USA) incubated anaerobically at 37 °C. Lactobacilli MRS medium was made from scratch (per litre: proteose peptone 10.0 g, beef extract 10.0 g, yeast extract 5.0 g, tween 80 1.0 ml, ammonium citrate 2.0 g, sodium acetate 5.0 g, magnesium sulphate 0.1 g, manganese sulphate 0.05 g, di-potassium phosphate 2.0 g) and contained either glucose or maltose at 2% (w/v) according to experimental requirements. Liquid media were filter-sterilised; agar media were autoclaved. Genetic manipulations of strain 100–23 described in this report utilised a plasmid-free derivative, 100–23C. The plasmids present in strain 100–23 do not encode metabolic activities (GenBank: GU108604.1; U21859.1). Both 100–23 and 100–33 fermented glucose and maltose when tested in medium containing fermentable carbohydrate at 0.5% (w/v) concentration. The pH of the cultures decreased from 6.8 to <4.5 after 48-h incubation.

Lactobacillus*-free mice*

Lactobacillus-free mice were used in animal experiments. The colony of BALB/c mice was maintained in gnotobiotic isolators as described previously (Tannock *et al*., 1988). Lactobacilli are absent from the gut of the animals but a complex microbiota consisting of about 600 bacterial phylotypes is present in the cecum (Tannock, 2009). The mice, therefore, provide animal hosts that are naïve with respect to lactobacilli but whose tissues have been conditioned by exposure to a complex microbiota. The constituents of the sterile diet fed to the animals are given in Table 1.

Analysis of soluble mono- and disaccharides in mouse stomach contents

Concentrations of mono- and disaccharides in stomach contents were measured as follows. Four batches of pooled stomach contents from 12 to 13 mice (*Lactobacillus*-free, or ex-*Lactobacillus*-free mice colonised by strain 100–23 or 100–33, or colonised by both strains) were homogenised in sterile water, centrifuged at low speed ($106 \times g$) to remove particulate material, then lyophilised. Soluble sugars were extracted from the lyophilised material by extraction in methanol (62.5% v/v) at 55 °C for 1 h with vortexing. Extracts were centrifuged to remove particulates, and the supernatants were analysed by high-performance liquid chromatography (Dionex Ultimate 3000, Bannockburn, IL, USA). Aliquots (20 μl) were separated by high-performance liquid chromatography on a Prevail carbohydrate ES 5 μ column (250×4.6 mm; Grade Davidson Discovery Science, Bannockburn, IL, USA), in a solvent mix of 25% water and 75% acetonitrile at a flow rate of 1 ml min^{-1}. The column temperature was kept at 30 °C. Peaks were detected using an ELSD (Polymer Laboratories, Alphatech Systems Ltd, Vancouver, WA, USA; gas flow 1.0, nebulising temperature 40 °C, evaporating temperature 90 °C), and identified by their retention times against standards (fructose (BDH, Cedar Rapids, IA, USA) 7.8 min, glucose (BDH) 10.4 min, sucrose (Merck, Whitehouse Station, NJ, USA) 14.7 min, maltose (BDH) 18.0 min). The area under the peaks was integrated using Millennium (software version 3.2, Waters Corporation, Milford, MA, USA) and quantified against standard curves produced during the analysis.

Table 1 Composition of mouse diet (diet 86)

Ingredient	*Concentration (% w/w)*
Wheat	40.00
Broll	21.50
Barley	20.00
Meat and bone meal	6.00
Fish meal	5.00
Mollases	2.50
Prelac	2.00
Dried blood meal	1.70
Sodium chloride	0.50
Tallow	0.45
Limestone	0.2
Premix	0.15

Colonisation experiments with Lactobacillus*-free mice*
Colonisation of the gut of *Lactobacillus*-free mice was accomplished by inoculating the mice by gavage on a single occasion with about 10^6 lactobacilli per dose (Walter *et al*., 2005). To determine population sizes of 100–23, *malA* mutant or 100–33 as sole *Lactobacillus* inhabitants of the gut, mice were inoculated with a pure culture of the appropriate strain. The animals were killed 2 weeks after inoculation and samples were taken for culture. Mixed populations of 100–23 and 100–33 were produced by inoculating mice with a 1:1 mixture of cultures of the two strains. Groups of mice were examined 2, 3, 4 and 5 weeks after inoculation. *In vivo* competition was measured by inoculating mice with a 1:1 mixture of the *malA* mutant and appropriate wild-type (100–23C or 100–33) cultures. The animals were killed and samples were taken for culture 7 days after inoculation. The *Lactobacillus* populations present in the forestomach were quantified by plating dilutions of forestomach, ileal or caecal homogenates on Rogosa SL agar plates (Difco) that were then incubated anaerobically at 37 °C for 48 h. Differential counts on Rogosa SL agar with or without erythromycin (5 μg per ml) were used to determine the proportion formed by the *malA* mutant of the total *Lactobacillus* population in the competition experiment, and to measure *in vivo* stability of the mutation when mice were colonised by the mutant alone. The population sizes of 100–23 and 100–33 when co-colonising the forestomach were determined by culturing forestomach homogenates on Rogosa SL agar (total lactobacilli) and modified Rogosa agar in which glucose was replaced with fructose. Strain 100–33 produced colonies of about 2 mm diameter on modified Rogosa agar plates whereas strain 100–23 did not. Statistical comparisons were made using the Mann–Whitney test.

Transcriptional analysis of 100–23 cultures
Total RNA was extracted from cultures of strain 100–23 grown in Lactobacilli MRS medium containing 2% (w/v) glucose or maltose until in exponential growth phase ($A_{600} = 0.46$). Bacterial cells were harvested by brief centrifugation at $12\,000 \times g$ and washed with 750 μl of RNAprotect reagent (Qiagen, Germantown, MD, USA). The bacterial cells were disrupted by bead beating (5000 r.p.m., 2×40 s) in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and extracted with chloroform. RNA was precipitated with iso-propanol and dried after removal of iso-propanol. The dried RNA was then dissolved in nuclease-free water. The RNA was further purified using the RNeasy Mini Kit (Qiagen) and DNase treated using a DNA-free kit (Ambion, Austin, TX, USA). RNA quality was assessed using a Bioanalyzer 2100 instrument (Agilent Technologies, Palo Alto, CA, USA) and quantified by NanoDrop ND-1000 (Thermo Fisher, Waltham, MA, USA).

RNA was also extracted from stomach contents (four *Lactobacillus*-free and four mice colonised with 100–23) by the method described above, except that samples suspended in RNAprotect were subjected to two slow speed centrifugations ($150 \times g$ and $300 \times g$) before pelleting the bacterial cells for suspension in TRIzol.

The draft genome sequence of *L. reuteri* 100–23 (http://www.ncbi.nlm.nih.gov/nuccore/AAPZ00000000) was used to design high-density 4×44K arrays from Agilent Technologies using eArray 4.5. A minimum of nine sense-strand probes per transcript were included on the array, representing the 2449 predicted ORF of *L. reuteri* 100–23. Details of the microarray are provided in GEO submission GSE24472.

To compare the transcriptional profiles of *L. reuteri* grown in the presence of glucose or maltose, 2 μg of total RNA was labelled with either Cy5- or Cy3-dCTP during first-strand synthesis with SuperScript II (Invitrogen) reverse transcriptase kit following the manufacturer's protocol. Labelled complementary DNA was purified using a MinElute PCR purification kit (Qiagen) and the dye incorporation rate was determined by NanoDrop ND-1000 UV-Vis spectrophotometer. The labelled samples were mixed and competitively hybridised using an Agilent Gene Expression Hybridisation Kit (part number: 5188–5242) in an Agilent hybridisation oven (G2545A) at 65 °C for 24 h. Microarrays were scanned using the Agilent Microarray Scanner System (G2505B) and Agilent scan control software version 7.0 at a resolution of 5 μm. Feature extraction was performed using Agilent Feature Extraction software version 9.1 and microarray data were processed as described by Garcia de al Nava *et al*. (2003) and Hijum van *et al*. (2005). Differential gene expression was determined by Cyber-T test (Long *et al*., 2001). Genes with a *P*-value < 0.001 and greater than twofold difference under the two conditions were considered differentially expressed.

Reverse-transcription quantitative PCR utilised primers (Table 2) that were designed using the Primer3 software package (Rozen and Skaletsky, 2000). Reverse transcription was performed with Superscript II (Invitrogen) according to the manufacturer's instructions with 2 μg of RNA used as starting material and 250 ng of random nonamers (Invitrogen). Reverse-transcription quantitative PCR amplifications were performed in an ABI prism detection system (Life Technologies, Mulgrave, Australia) using SYBR Green for the fluorescent detection of amplicons. Each well contained 10 μl of Fast-SYBR Green master mix ABI, 5 pmol of each primer and complementary DNA template equivalent to 20 ng. Reactions were performed in duplicate. No-template control reactions were included and temperature-dependent melting curves were checked after amplification to ensure PCR specificity. Relative changes in gene expression were calculated using the comparative $2^{-\Delta CT}$ method as described by others using 16S ribosomal RNA as

Table 2 PCR primers

Target gene	*Primer*	*Sequence (5′–3′)*	*Application*
Mfs1 (Lreu23DRAFT_4916)	LrP1_mutF	TGACTGGATCCTAAGGCGTTGTTCCAGATTCAGT	Insert primer
	LrP1_mutR	TGACTGAATTCATGAACCCAAGAGCACCAAG	
	LrP1_testF	TTGCTTTGCTTGACCTTTCA	Test primer
	LrP1_testR	CCCATGTGCTTACCAGTCAA	
	LrP1_RTf2	GCTTGGTTACGGTGGTAGTT	RT-qPCR
	LrP1_RTr2	ACAAGGGTGTATGAAAACAATC	
Mfs2 (Lreu23DRAFT_4938)	LrP2_mutF	TGACTGGATCCTAATTAACATGGTGGGGTGTATCA	Insert primer
	LrP2_mutR	TGACTGAATTCATCAAAACGAAGGAGAAGATA	
	LrP2_testF	TCGCCTTCCTTGATCTTTCA	Test primer
	LrP2_testR	CCCATGTGTTTACCAGACAA	
	LrP2_RTr1	GAGACAGGTGATAACTAGAATAA	RT-qPCR
MalA (LreuDRAFT_4917)	LrH_mutF	TGACTGGATCCTAAGAAAAGCACGCTTACAACAC	Insert
	LrH_mutR	TGACTGAATTCGTCAGCAACCCCAAGGTAAA	
	LrH_testF	ACCACGTGGATGACGGTACT	Test primer and RT-qPCR
	LrH_testR	TGGGAATAATGCACCATCAA	
Plasmid vector pORI28	pORI28F2	TGGTGATTTCAGAATCGCTAGT	Test primer
	pORI28R2	AATTATAGCACGGTCGAGATCTAT	
16S rRNA	HDA1	ACTCCTACGGGAGGCAGCAGT	RT-qPCR
	Lr468R	TCACGCACGTTCTTCTCCAA	
Alpha amylase catalytic region (Lreu23DRAFT_4936)	Amy_F	GCCAAATAATTGGGGTTCCT	RT-qPCR
	Amy_R	CGAAAACCATCGACACCTTT	

Abbreviations: rRNA, ribosomal RNA; RT-qPCR, reverse-transcription quantitative PCR.

the normaliser for transcript abundance (Livak and Schmittgen, 2001; Marco and Kleerebezem, 2008). Statistical comparisons were made by two-way analysis of variance.

*Inactivation of the major facilitator superfamily (*mfs1*,* mfs2*) and maltose phosphorylase (*malA*) genes of strain 100–23*

To inactivate the *mfs1* (Lreu23DRAFT_4916), *mfs2* (Lreu23DRAFT_4938) and maltose phosphorylase genes (*malA*; Lreu23DRAFT_4917) of strain 100–23C, an internal region of each of the genes was amplified by PCR with the applicable primers listed in Table 1 and cloned into pORI28 by directional cloning as described by Russell and Klaenhammer (2001), resulting in plasmids pORIdmfs1, pORIdmfs2 and pORIdmalA. Insertional mutagenesis of *mfs1*, *mfs2* and *malA* was achieved by site-specific integrations of these plasmids into the chromosome of *L. reuteri* 100–23C using the temperature-sensitive plasmid pVE6007 as a helper plasmid as described previously (Walter *et al.*, 2005). The correct integration of pORI28 was confirmed by PCR using primers flanking the target regions and primers pORI128F2 and pORI128R2 (Table 2).

In vitro *comparisons of utilisation of maltose and glucose by lactobacilli*

The doubling times of strains 100–23 and 100–33 were determined by culture of the lactobacilli in Lactobacilli MRS medium containing either maltose (2% w/v), glucose (2% w/v), sucrose (2%) or glucose/maltose (1% each) at 37 °C under anaerobic conditions. Aliquots of culture were removed at hourly intervals over an 8-h incubation period. Triplicate samples were collected per sampling time and the A_{600} was determined. The A_{600} values were converted to Natural Logarithims and plotted against sampling times. Doubling times were calculated by log(2)/slope of the linear part of the graph.

The utilisation of maltose and glucose by strains 100–23 and 100–33 (pure and mixed) in batch cultures was measured. Lactobacilli MRS medium containing maltose and glucose was inoculated with strain 100–23 or 100–33 or 1:1 mixture of the two strains and incubated anaerobically at 37 °C. One ml samples were removed at hourly intervals during a 13-h period. The samples were centrifuged to remove bacterial cells, and the supernatants were filtered and freeze dried. Determinations of carbohydrate concentrations were carried out on reconstituted samples using high-performance liquid chromatography as described above.

Maltose and glucose transport assays

L. reuteri 100–23 and *L. johnsonii* 100–33 were grown in Lactobacilli MRS medium containing either 2% maltose or glucose according to experimental requirements. Cells were harvested by centrifugation (14 500 × *g*, 10 min, 4 °C) and washed twice in 100 mM potassium phosphate buffer (pH 7.0) containing 5 mM magnesium chloride. The bacterial cell pellets were suspended to give a suspension of A_{600} 1.0–1.5. Before initiating transport experiments, the cell suspensions were energised with arginine (10 mM final concentration)

for 15 min at 37 °C. The aliquots of cell suspensions (200 µl) were incubated at 37 °C in 5 ml polystyrene test-tubes and transport was initiated by addition of either ([U-^{14}C]maltose 679 mCi mmol^{-1}, 100 nCi used per assay) or [^{14}C]glucose (D-[1-^{14}C]glucose, 3.96 mCi mmol^{-1}, 100 nCi used per assay). At various time intervals (0–60 s), the reactions were stopped by the addition of 2 ml of cold 0.1 M LiCl and rapid filtration through 0.45 µm HA MF membrane filters (Millipore, Billerica, MA, USA), using a vacuum manifold (Millipore) with an applied vacuum of approximately 80 p.s.i. Filters were washed with 2 ml 0.1 M LiCl, air-dried in 4 ml scintillation vials and covered in 2 ml scintillation fluid (Amersham, Piscataway, NJ, USA). The amount of radioactivity taken up by the cells was determined with a 1214 Rackbeta liquid scintillation counter (LKB Wallac, Mount Waverley, Victoria, Australia) using the [^{14}C]-window and counting each vial for 1 min. The amount of radiolabelled substrate taken up by the cells was calculated from the counts on the filters of each time-point relative to a t_0 control, the total counts initially added to the assay and the excess of cold substrate over radiolabelled substrate. Rates of solute uptake were expressed as nmol substrate min^{-1} mg protein^{-1}. V_{max} values of solute transport were determined from Michaelis–Menten plots, K_m values were determined from double-reciprocal plots of the same data. To control for nonspecific binding of radiolabelled solute, cells were pre-incubated cells with nigericin and valinomycin (15 µM each) for 15 min to dissipate both the membrane potential and pH gradient as described previously (Tannock *et al.*, 2005). Protein from NaOH-hydrolysed cells (0.2 M NaOH, 100 °C, 20 min) was assayed by the method of (Markwell *et al.*, 1978). A cell suspension of $A_{600} = 1.0$ had a protein content of 130 ± 18 mg l^{-1} for *L. reuteri* 100–23 and 137 ± 17 mg l^{-1} for *L. johnsonii* 100–33.

Mathematical model of growth of two species of Lactobacillus *in the mouse forestomach*

A mathematical model was devised that describes competition between two strains (100–23 and 100–33) in the presence of two substrates (maltose and glucose). For simplicity, it is assumed that the mouse forestomach acts as a continuous fermentor so that explicit expressions for the steady-state concentrations of substrate and bacteria could be obtained. These were used to derive conditions for which the two strains could co-exist. Descriptions of the model are provided in Appendix A, B and C of Supplementary Material.

Results

Co-existence of strains 100–23 and 100–33 in the mouse forestomach

Strains 100–23 and 100–33 independently colonised the gut of *Lactobacillus*-free mice, producing populations of similar sizes ($P > 0.05$; Figure 1a). The strains shared the forestomach habitat (on average 62% strain 100–23, 38% strain 100–33 after 5 weeks cohabitation) when a mixture of 100–23 and 100–33 was used to inoculate the mice. The populations were not statistically different in composition at 2, 3, 4 and 5 weeks after inoculation of the mice (Figure 1b; $P > 0.05$). Total *Lactobacillus* numbers in the forestomach were similar in all experiments, averaging 1.02×10^9 per gram (range 5.86×10^8–1.45×10^9).

Concentrations of mono- and disaccharides in stomach contents

Mono- and disaccharide concentrations were measured in stomach contents to determine the fermentable substrates available to bacteria inhabiting that site. Glucose (6.37 µg per mg dry weight of stomach contents (s.e.m., 2.63), maltose (12.2 (3.75)), sucrose (2.65 (1.71)) and fructose (2.00 (0.95)) were detected in stomach contents. The main carbohydrate

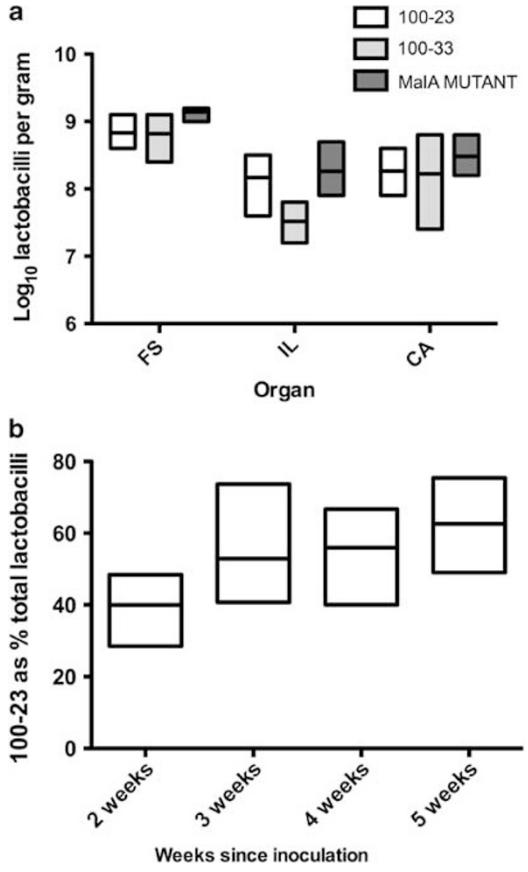

Figure 1 *Lactobacillus* populations in the gut of mice. (**a**) Populations of strains 100–23, 100–33 and 100–23C *malA* mutant when inhabiting the gut of mice alone. (**b**) Proportions of the total *Lactobacillus* population comprised of strain 100–23 in the forestomach of mice in cohabitation with 100–33. In both (**a** and **b**), horizontal line = mean, box = range. Six mice were used per experimental group. Abbreviations: CA, caecum; FS, forestomach; IL, ileum.

substrates available to the lactobacilli were therefore glucose and maltose in approximately equimolar quantities. The glucose and maltose were likely to be the hydrolysis products from dietary starch catalysed by salivary amylase activity.

There was considerable variation in carbohydrate concentrations between pooled samples of stomach contents. Statistically significant differences in the concentrations of these carbohydrates were not obtained between groups of mice colonised by lactobacilli relative to *Lactobacillus*-free (data not shown). There was a confounding trend for higher concentrations of glucose (14.40 μg per mg (s.e.m. 2.81)) and maltose (26.10 (6.76)) to be present in the stomachs of mice colonised by strain 100–23. This may have been due to amylase activity of strain 100–23, which may be inducible *in vivo*. Thus, it was not possible to obtain accurate measurements of the utilisation of glucose and maltose by the *Lactobacillus* strains when inhabiting the mouse stomach.

Microarray comparisons of transcriptomes of glucose and maltose-grown cultures of strain 100–23

Transcriptome comparisons were made to identify candidate genes for mutation in strain 100–23 so that the importance of maltose utilisation in forestomach colonisation could be shown. A total of 669 genes (27% of the genome) were differentially expressed between the two conditions by greater than twofold ($P<0.001$). In all, 156 genes (6% of genome) were differentially expressed by more than fourfold. These differentially expressed genes, grouped according to predicted protein function, were highly represented in the Clusters of Orthologous Groups categories 'amino acid transport and metabolism' (54), and 'nucleotide transport and metabolism' (34), 'carbohydrate transport and metabolism' (39) and 'cell wall/membrane/envelope biogenesis' (34) (Tatusov *et al.*, 2003).

The transcription levels of two putative maltose transporters (major facilitator superfamily; *mfs1* and *mfs2*) were greater when the bacteria were grown in maltose medium relative to glucose medium (Table 3). *Mfs1* was located immediately upstream of the maltose phosphorylase (*malA*) and β-phosphoglucomutase genes. *Mfs2* was located elsewhere in the genome sequence. Therefore it appeared that strain 100–23, in the presence of maltose, increased transcription of transporters to facilitate entry of maltose into cells, maltose phosphorylase to transform maltose to β-D-glucose plus β-D-glucose-1-phosphate, and β-phosphoglucomutase to convert the latter to β-D-glucose-6-phosphate.

The genomic locus containing *malA,* β-phosphoglucomutase and *mfs1* genes that had increased transcription in cells grown in maltose is depicted in Figure 2. A BLAST search of the *L. reuteri* 100–23 genomic sequence (NCBI NZ_AAPZ00000000) using the *mfs1* gene as a query revealed *mfs2*. This gene was located 28 kb upstream of *mfs1*. This second locus also revealed a gene (Lreu23DRAFT_4936) whose product was annotated by the Joint Genome Institute as including the catalytic region of α-amylase. Our analysis by Interproscan (http://www. ebi.ac.uk/interpro) confirmed the presence of a Protein Family (Pfam) motif corresponding to

Table 3 Differential expression levels of selected maltose utilisation genes

Genetic locus	*Function*	*Fold change in transcription (maltose grown relative to glucose grown): microarray*	*Fold change in transcription (maltose grown relative to glucose grown): RT-qPCR*
Lreu23DRAFT_4916	*mfs1*; Major facilitator superfamily_1	41.64	114.56
Lreu23DRAFT_4917	*malA*; Maltose phosphorylase	14.22	77.71
Lreu23DRAFT_4918	*β*-Phosphoglucomutase	13.9	Not tested
Lreu23DRAFT_4938	*mfs2*; Major facilitator superfamily_2	7.2	10.12

Abbreviation: RT-qPCR, reverse-transcription quantitative PCR.

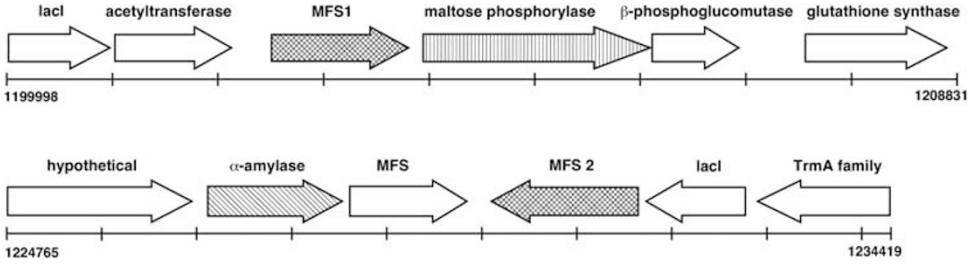

Figure 2 Annotation of the maltose utilisation locus of strain 100–23. The upper map shows strain 100–23 genome positions 1199998–1208831 and includes the maltose phosphorylase (*malA*) gene (vertical bars) and the *mfs1* gene (hashed). The lower map shows genome positions 1224765–1234419 and includes the α-amylase sequence (diagonal bars) and the *mfs2* gene (hashed). Arrows indicate the direction of transcription. Genome positions and annotations were from NCBI genome sequence NZ_AAPZ00000000.

α-amylase in this gene product (Pfam PF00128), and a Panther Family assignment to α-amylases (PTHR10357). Growth of strain 100–23 on plates of Lactobacilli MRS medium containing starch (1% w/v) was negligible. However, quantitative PCR of complementary DNA prepared from stomach contents of mice colonised with strain 100–23 showed that the α-amylase sequence was transcribed *in vivo* (16S ribosomal RNA normalised $2^{-\Delta Ct}$ values of 0.0096 in 100–23 colonised mice; 4.15×10^{-7} in *Lactobacillus*-free mice). Therefore, if the bioinformatic prediction that Lreu23DRAFT_4936 is correct, strain 100–23 had the potential to hydrolyse starch in the stomach habitat to release maltose and glucose that could be used for growth. A lack of growth of strain 100–23 on starch-containing medium, but evidence of *Lactobacillus* amylase gene transcripts in the forestomach, indicated that the enzyme might be inducible only under *in vivo* conditions.

Mutants of *mfs1* and *mfs2* were generated separately. Both mutants were able to grow on maltose-containing medium, which suggested redundancy with regard to maltose uptake in strain 100–23.

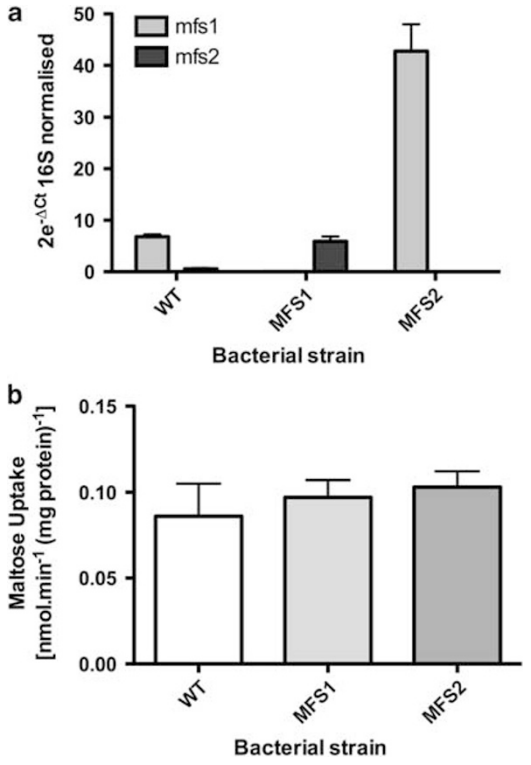

Figure 3 Effect of mutations of *mfs1* and *mfs2* in strain 100–23C. (**a**) Comparison of transcription of *mfs1* and *mfs2* in wild-type 100–23C (WT), and *mfs* mutants (MFS1, MFS2). Data ($n=4$ replicates) were normalised relative to transcription of the 16S ribosomal RNA (rRNA) sequences. (**b**) Uptake of radioactive maltose by cells harvested from cultures containing 2% (w/v) maltose. WT, wild-type strain 100–23C; MFS1 and MFS2, 100–23C derivatives with mutations in respective genes. In both (**a** and **b**), means and s.e.m. are shown. *Mfs1* transcription in mutant MFS2 was greater than in the WT ($P<0.001$).

Transcriptional analysis (reverse-transcription, quantitative PCR) of the wild type and mutants cultured in maltose medium and harvested in exponential growth phase ($A_{600}=0.43$) showed that higher level transcription of *mfs1* occurred when the *mfs2* gene had been mutated (Figure 3a), suggesting an associated alteration in the control of *mfs1* expression. Both *mfs* mutants transported maltose into their cells at about the same rates as the wild-type strain (Figure 3b).

Comparison of glucose and maltose utilisation by strains 100–23 and 100–33

To investigate the trophic basis for the co-existence of these strains in the forestomach habitat, strains 100–23 and 100–33 were grown in medium containing either glucose or maltose (final concentration 1% each). The doubling times of strain 100–23 grown in either glucose or maltose-containing media were 88 and 48 min, respectively, suggesting maltose was the preferred carbon and energy source. To test this hypothesis, strain 100–23 was grown on a mixture of maltose and glucose (Figure 4a). Under these conditions, the doubling time was 47 min and both substrates were utilised simultaneously, but the rate of glucose utilisation was approximately twofold faster than maltose (Figure 4a). When the glucose concentration was depleted to just below 20% (10 mM glucose), of the initial concentration, glucose utilisation slowed considerably suggesting that the glucose permease operating in these cells had a low affinity for glucose. In contrast, maltose was consumed to depletion suggesting a high-affinity permease was operating for maltose uptake.

The doubling time of strain 100–33 in either glucose or maltose-containing medium was 38 and 53 min, respectively. When grown on a mixture of glucose and maltose, glucose was used preferentially over maltose at a fast rate (high affinity) (Figure 4b) and the doubling time was 38 min. Maltose utilisation by 100–33 was slow and the rate was proportional to the external maltose concentration suggested a low-affinity mechanism was mediating maltose consumption in this strain (Figure 4b).

Carbohydrate utilisation in mixed cultures of strain 100–23 and 100–33 reflected the combined nutritional preferences of the two strains with the rates of glucose and maltose utilisation being comparable to the monocultures (Figure 4c). To determine if this was a general property of these strains, doubling times in sucrose-containing medium were measured and found to be similar for the two strains (100–23, 39 min; 100–33, 40 min) suggesting that the substrate preferences observed were unique to glucose and maltose.

The glucose and maltose utilisation experiments suggested that strains 100–23 and 100–33 had different affinities for glucose and maltose.

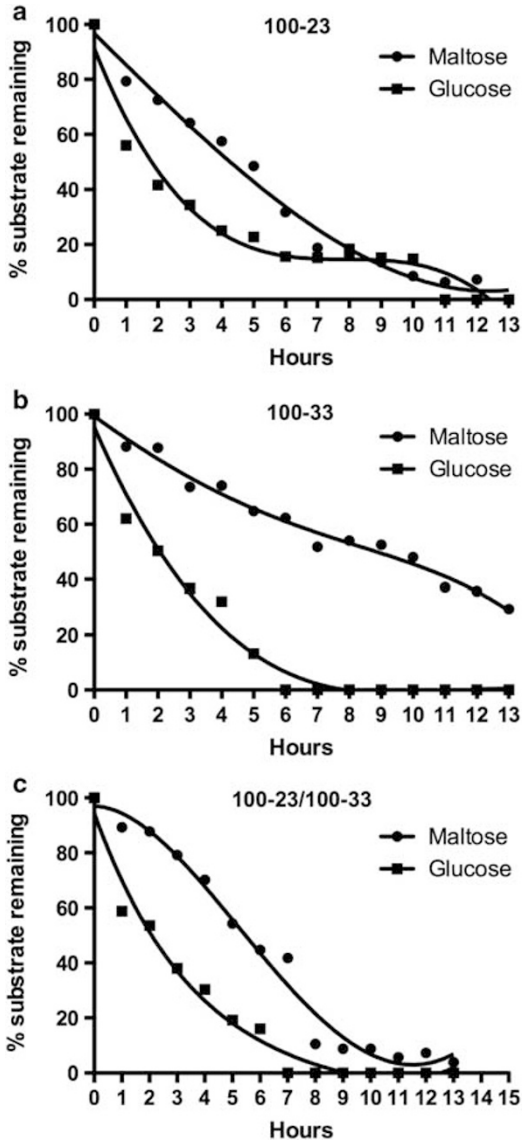

Figure 4 Utilisation of maltose and glucose by pure cultures of strains 100–23 (**a**) or 100–33 (**b**), or by a mixed culture of 100–23 and 100–33 (**c**). Means of triplicate samples per sampling time were plotted.

Substrate utilisation is the sum of transport and metabolism so experiments were carried out to determine the substrate affinities for maltose and glucose transport. Cells of strain 100–23 grown on either glucose or maltose were constitutive for [^{14}C]maltose (4 μM final concentration) transport, but the rate was 2.5-fold higher in maltose grown cells compared with glucose grown cells (Figure 5a). While glucose did not appear to repress maltose uptake, we cannot rule out the operation of a second maltose permease (glucose-insensitive) that may be mediating maltose transport in glucose-grown cells. In contrast, [^{14}C]glucose (6 μM final concentration) uptake could not be detected in strain 100–23 (that is, glucose or maltose grown) indicating the absence of a high-affinity transporter for glucose uptake (Figure 5b). To further characterise glucose and maltose transport in strain 100–23, we determined the kinetics of glucose and maltose uptake at different external substrate concentrations (5 μM–600 μM; Figures 5c and d). Initial rates of transport were determined and plots of rates of transport versus substrate concentration showed that maltose uptake followed saturation kinetics (Figure 5c). The apparent K_m and V_{max} values for maltose uptake were 366 μM and 72 nmol min^{-1} (mg protein)$^{-1}$. In contrast, the rate of glucose uptake was low with increasing concentrations of glucose, suggesting the operation of a low-affinity (non-saturable) transporter (Figure 5d).

Cells of strain 100–33 grown on either glucose or maltose were constitutive for [^{14}C]glucose (6 μM final concentration) transport (Figure 5b). In contrast, low levels of [^{14}C]maltose (4 μM final concentration) uptake could be detected in the same cells (that is, glucose or maltose grown; Figure 5a). The kinetics of glucose and maltose transport were studied in strain 100–33 (Figures 5c and d). Glucose transport by strain 100–33 showed typical Michaelis–Menten kinetics and the apparent K_m and V_{max} values for glucose uptake were 4 μM and 15.2 nmol min^{-1} (mg protein)$^{-1}$ indicating the operation of a high-affinity transporter for glucose (Figure 5d). In contrast, the rate of maltose uptake was low with increasing concentrations of maltose suggesting the operation of a low-affinity transporter (Figure 5c).

Effect of mutation of malA *of strain 100–23*

Mutation of the *malA* gene was carried out to prevent utilisation of maltose by strain 100–23. The *malA* mutant did not grow in MRS medium containing maltose but grew in glucose medium to the same extent as the wild type (A_{600} at 24 h in glucose medium, both wild type and mutant = 3.7; doubling time 90 min). [^{14}C]maltose (4 μM) was transported into the mutant cells but the rate (0.36 nmol min^{-1}(mg protein)$^{-1}$) was approximately eightfold less than the wild type. Mutation of *malA* would have prevented transformation of the maltose to β-D-glucose + β-D-glucose-1-phosphate and non-metabolised substrate would have accumulated intracellularly. The mutant formed a small proportion (average 11.42% (s.e.m. 5.09)) of the total *Lactobacillus* population when cohabiting the stomach with strain 100–33. The mutant was almost eliminated (average 0.40% (0.18)) in competition with wild-type 100–23C. Yet, alone in the mouse gut, the *malA* mutant attained populations similar ($P > 0.05$) to those of the wild type (Figure 1a).

Mathematical model

Cohabitation of the mouse forestomach could be affected by multiple, as yet unknown, factors in addition to trophism. Therefore, we derived a mathematical model (Appendices A–C in Supplementary Material) to investigate whether resource

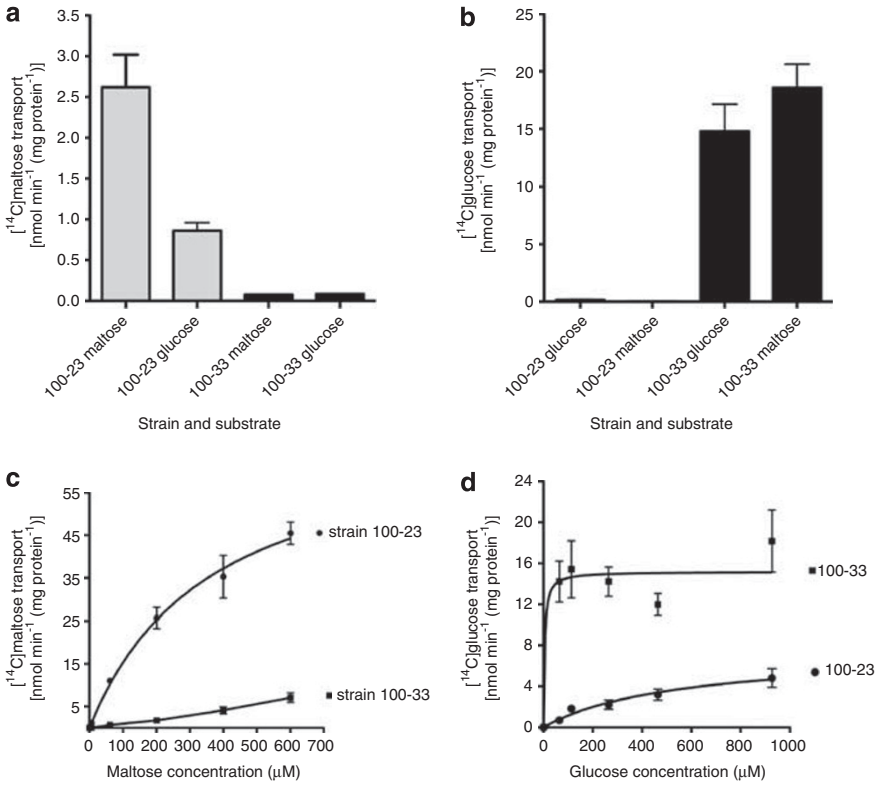

Figure 5 Measurements of uptake of radioactive maltose (**a** and **c**) or glucose (**b** and **d**) by cells of 100–23 and 100–33 harvested from medium containing 2% (w/v) glucose or maltose. Means and s.d. of triplicate assays are shown.

partitioning alone is sufficient to explain long-term cohabitation. The mouse forestomach was modelled as a continuous fermentor vessel with two bacterial strains and two substrates, with growth rates and affinity constants derived from *in vitro* observations of substrate concentrations, substrate uptake and utilisation, and bacterial growth parameters. The model assumed that glucose and maltose were growth-limiting substrates because they were detected in stomach contents and were fermented by both strains. The steady-state solution of the model showed that the two strains co-existed in numbers of similar magnitude. Although in steady state, both strains fed on both substrates. Strain 100–23 predominantly fed on maltose while 100–33 predominantly fed on glucose (full details are given in Appendix B in Supplementary Material). Therefore, resource partitioning can be invoked as a major factor in cohabitation of the two strains of lactobacilli in the mouse forestomach.

Discussion

Lactobacillus species belong to the lactic acid bacteria, a broad group defined as gram-positive organisms that ferment hexoses to produce primarily lactic acid (Hammes and Vogel, 1995). Complete genome sequences of many lactic acid bacteria have been determined and these show a prominence of genes associated with the metabolism of simple carbohydrates, including oligosaccharides, and energy conversion systems (Makarova *et al*., 2006). *L. reuteri* 100–23 and *L. johnsonii* 100–33 persist in the gut of mice following a single inoculation and have an equal capacity to inhabit the forestomach of the animals. Of the four carbohydrates detected in mouse stomach contents, strain 100–23 ferments glucose and maltose but not fructose. Strain 100–33 ferments fructose in addition to glucose and maltose. Both strains ferment sucrose and have similar doubling times when utilising this substrate. As fructose was present in stomach contents in very low concentration relative to glucose and maltose, it seemed likely that the two *Lactobacillus* strains would compete for the same ecological niche. This niche might be defined as 'glucose and maltose trophism'. Therefore, the cohabitation of the forestomach by the two strains required explanation with regard to bacterial nutrition.

Ecological niches are multi-dimensional, conceptual entities that help to define where and how a population lives. Experiments conducted by Gause and Witt (1935) with two closely related species of ciliated protists showed that one species, *Paramecium caudatum*, was eliminated when co-cultured with *Paramecium auriola*. Gause and Witt concluded that two species competing for the same limiting resources cannot co-exist in the same place. In the absence of disturbance, one population will

use the habitat resources more efficiently and thus reproduce more rapidly than the other. Even a slight reproductive advantage would eventually lead to the elimination of the inferior competitor, an outcome known as 'competitive exclusion'. However, ecologically similar species could co-exist in a habitat if sharing resources rather than competing for them resolved the competition. This sharing of resources is referred to as 'resource partitioning'.

Pathogenic and commensal strains of *Escherichia coli* were shown, in a series of *in vitro* and *in vivo* experiments conducted by Fabich *et al*. (2008) to share carbohydrate resources in gut mucus. Although both strains of *E. coli* utilised arabinose, fucose and *N*-acetylglucosamine for growth, the pathogenic strain also used galactose, hexuronates, mannose and ribose. In contrast, the commensal strain used gluconate and *N*-acetylneuraminic acid. In this example of resource sharing, the bacterial strains had the capacity to use different carbohydrates for growth. In the case of *L. reuteri* 100–23 and *L. johnsonii* 100–33, however, there was a major overlap in fermentative abilities in that both strains could utilise the two major carbohydrates present in the habitat. Our data show that the two *Lactobacillus* strains could co-exist in the habitat because of physiological adaptations governing the utilisation of glucose and maltose. These adaptations involved differences in the relative rates of transport, and subsequent growth on, the carbohydrates by the bacterial cells. When faced with a mixture of the two sugars, strain 100–23 could utilise both sugars simultaneous at high concentrations, albeit the initial rate for glucose was significantly faster. However, once the glucose concentration reached 10 mM, glucose utilisation stopped, but maltose utilisation continued. This pattern of substrate consumption was reflected in the affinity of the glucose and maltose permeases operating in strain 100–23; 100–23 had a high-affinity maltose transporter and a low-affinity glucose transporter. Strain 100–33 utilised glucose at a faster rate than maltose and this was reflected in the doubling times for growth on these substrates. The pattern of maltose and glucose consumption was consistent with the operation of a high-affinity glucose transporter and a low-affinity maltose transporter in strain 100–33. When the two strains were co-cultured, maltose and glucose were consumed rapidly and simultaneously and the kinetics of utilisation were indicative of the high-affinity permeases operating in strain 100–23 and 100–33 for maltose and glucose respectively.

Strain 100–23 had two genes encoding transporters (*mfs1* and *mfs2*) that we considered were involved in maltose transport because they had increased transcription in cells grown in maltose medium compared with glucose medium. Mutation of each of these genes resulted in greater transcription of the non-mutated paralogous gene. We are unable to derive double mutants in *L. reuteri*, therefore the importance of maltose utilisation to strain 100–23 *in vivo* was shown by mutation of *malA*. This prevented growth of the strain in maltose medium, did not impair colonisation of the mouse forestomach in pure culture, but greatly reduced the ability of the bacteria to cohabit with 100–33. It would have been desirable to investigate the competitive attributes of a complemented *malA* mutant strain to negate the possibility of downstream effects of the *malA* mutation. However, past experience has shown that instability of complementing constructs *in vivo*, and altered physiological kinetics compared with the wild type, make this impractical (Tannock *et al*., 2005). The ability of the *malA* mutant to colonise the murine gut in the absence of competition suggested that possible impairment of glucose utilisation because of polar effects of the *malA* mutation had not actually occurred.

The genetic basis of physiological adaptations has been little studied in gut commensals. Giraud *et al*. (2008) have suggested that, with respect to *E. coli*, mutations in global regulators allow physiological modifications to occur when bacteria must adapt to altered environments. Regulation of catabolic pathways in gut commensals must be extremely important in bowel habitats where available substrates may change from day to day because of alterations in dietary intake of the host. Transcriptional analysis of large bowel contents of diassociated gnotobiotic mice (*Bacteroides thetaiotaomicron* and *Eubacterium rectale*) has shown that major alterations occurred in the transcriptomes of the bacterial inhabitants when living together in comparison with when mice were monoassociated with each strain (Mahowald *et al*., 2009). As the gut of vertebrates is home to hundreds of bacterial species, there is much scope for *in vivo* studies of the mechanisms of gene regulation in commensals in response to community structure and substrate availability.

Members of the *Lactobacillus acidophilus* complex (such as *Lactobacillus crispatus* and *Lactobacillus gallinarum*) produce surface-associated, crystalline protein layers, termed S-layers, which have been suggested to be mediators of adherence to epithelial surfaces in chicken crops. Hagen *et al*. (2005) screened 38 crop isolates of *L. gallinarum* for the presence of genes encoding S-layer proteins. All of the isolates had two S-protein genes (*lgs*). One gene in each isolate was either *lgsA* or *lgsB*. The second gene in each of eight strains (differentiated by genotyping) was sequenced and shown to differ among strains. Thus, the genome of each strain encoded a species-specific S-protein (*lgsA* or *lgsB*) and a strain-specific S-protein. Proteomic and transcriptional studies showed that each strain produced, *in vitro* and *in vivo*, a single S-protein that was always encoded by the strain-specific *lgs* gene. Mutation is considered to be the ultimate source of genetic variation. Therefore, the presence of two similar genes in a genome such as observed in

this example may reflect gene duplication followed by conservation of the ancestral gene while mutations accrue in the duplicate, which is transcribed. The mutations in the duplicated gene may result in a product that enhances the ecological fitness of the strain for a particular environment. The genes encoding the putative maltose transporters (*mfs1* and *mfs2*) in strain 100–23 have 75% identity in DNA sequence and 90% for protein sequences. This may indicate an instance of gene duplication and modification that might confer increased ecological fitness in the forestomach of mice.

The fundamental niche that a species occupies can be identified by testing the range of conditions in which it grows in the absence of competitors. As indicated previously, the fundamental niche ('glucose and maltose trophism') of strains 100–23 and 100–33 overlap because both of the strains can utilise the two major mono- and disaccharides present in their habitat. In the absence of other lactobacilli, each strain achieved a population level of about 10^8–10^9 cells per gram of forestomach. The part of the fundamental niche that a species actually occupies in the ecosystem is called the 'realised niche'. It is generally thought of as 'smaller' than the fundamental niche and includes the attributes of the population that make it competitively superior as well as physiologically capable (Hutchinson, 1965). When sharing the forestomach habitat with 100–33, we reason that the realised niche of strain 100–23 is 'maltose trophism'.

Unknown factors in addition to those concerning bacterial nutrition are likely to be involved in sharing habitats. To investigate the concept of 'resource partitioning' more formally, a mathematical model was developed that described two strains, each of which could grow on either of two substrates. Regarding the mouse forestomach as a continuous fermentor vessel, mathematical conditions were derived showing that the two strains could co-exist if, compared with 100–33, 100–23 had a relative advantage on maltose, while 100–33 had a relative advantage on glucose compared with 100–23. *In vitro* measurements (transport rates, doubling times) supported these requirements, which then allowed us to predict the steady-state bacterial populations of the two strains in the forestomach. These were predicted to be similar in size, in agreement with *in vivo* observations. Furthermore, the model also provided insight into the relative contributions of maltose and glucose to the growth of each of the two strains: 98% of growth of 100–23 was obtained from maltose (with the remaining 2% from glucose). For 100–33, the majority of growth was achieved from glucose (77%, versus 23% from maltose). These findings imply that co-existence in the forestomach is achieved through 'maltose trophism' by strain 100–23. Mathematical modelling in conjunction with our observations therefore supports the view that preferential utilisation of a substrate (niche differentiation) by one strain will result in co-existence of two strains with overlapping fundamental niches. Resource sharing alone seems to explain the real-life outcome.

We do not know if strains 100–23 and 100–33 originated in the same conventional animal. If they did, the strains may have evolved sympatrically with complementary abilities with regard to glucose and maltose utilisation. This niche differentiation would have solved the problem of competition. Even without this knowledge, our *in vivo* experimental system coupled with transcriptional and physiological data provide one of the few mechanistic explanations of how commensals with overlapping ecological niches can share a gut habitat.

Acknowledgements

CW was supported by a grant from the Marsden Fund. PWOT was supported by Science Foundation Ireland. GH received support from the Scottish Government Rural Environment Research and Analysis Directorate. We thank Wei Ping Tan for his assistance in the initial stages of the investigation.

References

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. (2009). Bacterial community variation in human body habitats across space and time. *Science* **326**: 1694–1697.

Fabich AJ, Jones SA, Chowdhury FZ, Cernosek A, Anderson A, Smalley D *et al.* (2008). Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. *Infect Immun* **76**: 1143–1152.

Garcia de al Nava J, Hijum van S, Trelles O. (2003). Pre*P*: gene expression data pre-processing. *Bioinformatics* **19**: 2328–2329.

Gause GF, Witt AA. (1935). Behavior of mixed populations and the problem of natural selection. *Amer Nat* **69**: 596–604.

Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS *et al.* (2006). Metagenomic analysis of the human distal gut microbiome. *Science* **312**: 1355–1359.

Giraud A, Arous S, de Parpe M, Gaboriau-Routhiau V, Bambou J-C, Rakotobe S *et al.* (2008). Dissecting the genetic components of adaptation of *Escherichia coli* in the mouse gut. *PLoS Genet* **4**(1): e2.doi10.1371/journal.pgen.0040002.

Hagen KE, Guan LL, Tannock GW, Korver DR, Allison GE. (2005). Detection, characterization, and *in vitro* and *in vivo* expression of genes encoding S-proteins in *Lactobacillus gallinarum* strains isolated from chicken crops. *Appl Environ Microbiol* **71**: 6633–6643.

Hammes WP, Vogel RF. (1995). The genus *Lactobacillus*. In: Wood BJB, Holzapfel WH (eds). *The Genera of Lactic Acid Bacteria*. Blackie: London, UK, pp 19–54. The lactic acid bacteria (volume 2).

Hardin G. (1960). The competitive exclusion principle. *Science* **131**: 1292–1297.

Hijum van SA, Jong de A, Baerends RJ, Karsens HA, Kramer NE, Larsen R *et al.* (2005). A generally applicable validation scheme for the assessment of factors involved in reproducibility and quality of DNA-microarray data. *BMC Genomics* **6**: 77.

Hutchinson RE. (1965). *The Ecological Theater and the Evolutionary Play*. Yale University Press: New Haven, CT, USA, 139 pp.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS *et al.* (2008). Evolution of mammals and their gut microbes. *Science* **320**: 1647–1651.

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-deltadelta C(T) method. *Methods* **25**: 402–408.

Long AD, Mangalam HJ, Chan BYP, Tolleri L, Hatfield GW, Baldi P. (2001). Improved statistical inference from DNA microarray data using analysis of variance and a Bayesian statistical framework. Analysis of global gene expression in *Escherichia coli* K12. *J Biol Chem* **276**: 19937–19944.

Louie T, Miller M, Donskey C, Mullane K, Goldstein EJC. (2009). Clinical outcomes, safety and pharmacokinetics of fidaxomicin in a phase 2 trial of patients with *Clostridium difficile* infection. *Antimicrob Agents Chemother* **53**: 223–228.

Mahowald MA, Rey FE, Seedorf H, Turnbaigh PJ, Fulton RS, Wollam A *et al.* (2009). Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc Natl Acad Sci USA* **106**: 5859–5864.

Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E *et al.* (2006). Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci USA* **103**: 15611–15616.

Marco ML, Kleerebezem M. (2008). Asssessment of real-time RT-PCR for quantification of *Lactobacillus plantarum* gene expression during stationary phase and nutrient starvation. *J Appl Microbiol* **104**: 587–594.

Markwell MA, Haas SM, Bieber LL, Tolbert NE. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87**: 206–210.

Rozen S, Skaletsky H. (2000). Primer 3 on the WWW for general users and for biological programmers. In: Krawetz S, Misener S (eds). *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Human Press: Totowa, NJ, USA, pp 365–386.

Russell WM, Klaenhammer TR. (2001). Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. *Appl Environ Microbiol* **67**: 4361–4364.

Tannock GW, Crichton C, Welling GW, Koopman JP, Midtvedt T. (1988). Reconstitution of the gastrointestinal microflora of lactobacillus-free mice. *Appl Environ Microbiol* **54**: 2971–2975.

Tannock GW, Ghazally S, Walter J, Loach D, Brooks H, Cook G *et al.* (2005). Ecological behavior of *Lactobacillus reuteri* 100–23 is affected by mutation of the *luxS* gene. *Appl Environ Microbiol* **71**: 8419–8425.

Tannock GW. (2004). A special fondness for lactobacilli. *Appl Environ Microbiol* **70**: 3189–3194.

Tannock GW. (2009). Research for the 21st century: can we draw a blueprint of the bowel ecosystem? *Bioscience Microflora* **28**: 75–80.

Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV *et al.* (2003). The COG database: an updated version includes eukaryotes. *BMC Bioinform* **4**: 41.

Walter J, Chagnaud P, Tannock GW, Loach D, Bello FD, Jenkinson HF *et al.* (2005). A high-molecular mass surface protein (Lsp) and methionine sulfoxide reductase B (MsrB) contribute to the ecological performance of *Lactobacillus reuteri* in the murine gut. *Appl Environ Microbiol* **71**: 979–986.

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