The complete genome sequence of the rumen bacterium *Butyrivibrio hungatei* MB2003

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### Abstract

*Butyrivibrio hungatei* MB2003 was isolated from the plant-adherent fraction of rumen contents from a pasture-grazed New Zealand dairy cow, and was selected for genome sequencing in order to examine its ability to degrade plant polysaccharides. The genome of MB2003 is 3.39 Mb and consists of four replicons; a chromosome, a secondary chromosome or chromid, a megaplasmid and a small plasmid. The genome has an average G+C content of 39.7%, and encodes 2983 putative protein-coding genes. MB2003 is able to use a variety of monosaccharide substrates for growth, with acetate, butyrate and formate as the principal fermentation end-products, and the genes encoding these metabolic pathways have been identified. MB2003 is predicted to encode an extensive repertoire of CAZymes with 78 GHs, 7 CEs, 1 PL and 78 GTs. MB2003 is unable to grow on xylan or pectin, and its role in the rumen appears to be as a utilizer of monosaccharides, disaccharides and oligosaccharides made available by the degradative activities of other bacterial species.

### Keywords:

Rumen, Bacteria, Hemicellulose, Pectin, Degradation, *Butyrivibrio*, Genome

### Introduction

*Butyrivibrio* are important rumen bacteria [1], and are among the small number of rumen genera capable of utilizing the complex plant structural polysaccharides xylan and pectin [2, 3]. They are classified as anaerobic, monotrichous, butyrate-producing, curved rods and have been isolated from the gastrointestinal tracts and feces of various ruminants, monogastric animals and humans [4, 5]. *Butyrivibrio* are metabolically versatile and are capable of growing on a range of carbohydrates, from simple mono- or oligosaccharides to complex plant polysaccharides such as pectins, mannans, starch and hemicelluloses [6]. Furthermore, xylans of diverse chemical and physical properties, from a range of forages are degraded by *Butyrivibrio* species [7]. Some *Butyrivibrio* species show strong proteolytic activity [8], and *Butyrivibrio* are thought to be the main butyrate producers in the rumen [9, 10]. The genus *Butyrivibrio* is classified within the family Lachnospiraceae, order Eubacteriales, and is phylogenetically diverse. The *Butyrivibrio* genus originally consisted of only one species, *Butyrivibrio fibrisolvens* [2]. In addition to phenotypic characterisations [11, 12], studies have utilized DNA-DNA hybridization [13, 14], 16S rRNA gene sequencing [15, 16] and 16S rRNA-based hybridization probes [17], to differentiate these organisms. To accommodate the observed diversity amongst the newly discovered bacterial strains, a new genus, *Pseudobutyrivibrio*, was described [18]. Four species are currently recognized: *B. fibrisolvens*, *B. hungatei*, *B. proteoclasticus* and *B. crosotus* [6], although *B. crosotus* is more distantly related to the other three. *B. hungatei* are common anaerobic rumen bacteria found in domestic and wild ruminants and the type strain is JK615T [19]. *Butyrivibrio hungatei* JK615T is non-proteolytic and non-fibrolytic, but is able to utilize oligo- and monosaccharides as substrates for growth. Gaining an insight into the role of these secondary degrader species in microbial plant polysaccharide breakdown is important for understanding rumen function. Here we present the...
complete genome sequence of Butyrivibrio hungatei MB2003, a strain isolated from a pasture-grazed dairy cow in New Zealand [20], and describe its comparison with genomes of closely related B. hungatei strains.

Organism information
Classification and features
MB2003 was isolated from the plant-adherent fraction of rumen contents from a New Zealand dairy cow grazing fresh forage [20, 21]. MB2003 cells are Gram positive, short rods, occurring singly or in pairs (Fig. 1). The morphological features of MB2003 cells were determined by electron microscopy of cells grown on RM02 medium [22], negatively stained with 1% phosphotungstic acid, mounted on Formvar-coated copper grids, and examined using a Philips model 201C electron microscope (Eindhoven, The Netherlands). MB2003 cells were observed to have a single polar flagellum (Fig. 2), although cells in growing cultures were non-motile. A phylogenetic analysis of the full-length 16S rRNA gene sequence placed MB2003 within the B. hungatei species, being 98% similar to the Butyrivibrio hungatei type strain JK615T [19] (Fig. 3). Additional characteristics of B. hungatei MB2003 are shown in Table 1.

Strain MB2003 grew to highest optical density (OD) at pH values of 6.1 to 6.5 and at a temperature of 39 °C, conditions which are typical of its rumen environment. VFA production was determined from triplicate broth cultures grown overnight in RM02 medium with cellobiose as substrate and analysed for formate, acetate, propionate, n-butyrate, iso-valerate and lactate on a HP 6890 series GC (Hewlett Packard) with 2-ethylbutyric acid (Sigma-Aldrich, St. Louis, MO, USA) as the internal standard. To derivatize formic, lactic and succinic acids, samples were mixed with HCl ACS reagent (Sigma-Aldrich, St. Louis, MO, USA) and diethyl ether, with the addition of N-methyl-N-t-butyldimethylsilyltri-fluoroacetamide (MTBSTFA) (Sigma-Aldrich, St. Louis, MO, USA) [23]. Under these conditions MB2003 produced 16.4 mM formate, 3.6 mM acetate and 4.7 mM butyrate. MB2003 was able to grow in CO2-containing media with various soluble carbon sources and the semi-soluble inulin (all tested at 0.5% w/v final concentration). Growth on soluble substrates was assessed as an increase in culture density OD600nm compared to cultures without carbon source added, whereas total VFA production was used as an indicator of substrate utilization and growth for insoluble polymers (Table 2). All strains tested were net producers of formate, acetate and n-butyrate, which is characteristic of Butyrivibrio. Cellobiose and glucose supported the growth of MB2003, JK615T and B316T to high cell densities. Therefore, cellobiose was used to examine the growth of MB2003 over a 24 h period. The exponential phase of growth was between 4 and 8 h, with the maximum cell density reached at 8 to 10 h, and stationary phase between 10 to 24 h (Fig. 4).

Genome sequencing information
Genome project history
Butyrivibrio hungatei MB2003 was selected for genome sequencing as a NZ strain of B. hungatei. A summary of the genome project information is shown in Table 3 and in Additional file 1: Table S1.

Growth conditions and genomic DNA preparation
MB2003 was grown in RM02 medium [22] with 10 mM glucose and 0.1% yeast extract but without rumen fluid. Culture purity was confirmed by Gram
stain and sequencing of the 16S rRNA gene. Genomic DNA was extracted from freshly grown cells by a modification of the standard cell lysis method of Saito and Miura [24], using lysozyme, proteinase K and sodium dodecyl sulphate, followed by phenol-chloroform extraction, and purification using the Qiagen Genomic-Tip 500 Maxi kit (Qiagen, Hilden, Germany). Genomic DNA was precipitated by the addition of a 0.7 volume of isopropanol, and collected by centrifugation at 12,000×g for 10 min at room temperature. The supernatant was removed, and the DNA pellet was washed in 70% ethanol, re-dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and stored at −20 °C until required.

Genome sequencing and assembly
The complete genome sequence of MB2003 was determined by pyrosequencing 3 kb mate paired-end sequence libraries using the 454 GS FLX platform with Titanium chemistry (Macrogen, Korea). Pyrosequencing reads provided 234× coverage of the genome and were assembled using the Newbler assembler (version 2.7, Roche 454 Life Sciences, USA) which resulted in 31 contigs across 7 scaffolds. Gap closure was managed using the Staden package [25] and gaps were closed using additional Sanger sequencing by standard and inverse PCR techniques. In addition, MB2003 genomic DNA was sequenced using shotgun sequencing of 2 kb paired-end sequence libraries using the Illumina MiSeq platform (Macrogen, Korea) which provided 800-fold sequencing coverage. Illumina reads were analysed using the Galaxy web-based platform [26] and de novo assembly was performed using the Velvet assembler, version 3.0 [27]. The Velvet assembled MB2003 genome MiSeq sequences were combined with the Newbler assembly using the Staden package and Geneious, version 8.1 [28]. Genome assembly was confirmed by pulsed-field gel electrophoresis.

Genome annotation
Annotation of the MB2003 genome was performed as described previously [29]. The MB2003 genome sequence was prepared for NCBI submission using Sequin [30], and the adenine residue of the start codon of the chromosomal replication initiator protein DnaA1 (bhn_I0001, bhn_RS00450) gene was chosen as the first base for the MB2003 genome.

Genome properties
The genome of B. hungatei MB2003 consists of four replicons [21, 31]; a single chromosome (3,143,784 bp, %G + C 39.91), a chromid or secondary chromosome (Bhull, 91,776 bp, %G + C 37.71), a megaplasmid (pNP144, 144,470 bp, %G + C 36.86) and a plasmid (pNP6, 6284 bp, %G + C 35.71). The total size of the closed genome is 3,386,314 bp with an overall %G + C content of 39.71%. A total of 3064 genes were predicted, of which 2983 (97.4%) were protein-coding genes. A putative function was assigned to 2225 of the protein-coding genes, while 775 protein coding genes were annotated as hypothetical proteins. The MB2003 chromosome encodes 2758 genes, and Bhull, pNP144 and pNP6 encode 89, 147 and 6 genes, respectively. The properties and statistics of the MB2003 genome are included in the supplementary material.
The nucleotide sequences of the MB2003 chromosome, chromid (BhuII), megaplasmid (pNP144) and plasmid (pNP6) have been deposited in Genbank under accession numbers CP017831, CP017830, CP017832 and CP017833. The genome atlas for *B. hungatei* MB2003 is shown in Fig. 5.

**Table 1** Classification and general features of the rumen bacterium *B. hungatei* MB2003 in accordance with the MIGS recommendations [58]

| MIGS ID | Property                  | Term                                    | Evidence code<sup>a</sup> |
|---------|---------------------------|-----------------------------------------|---------------------------|
|         | Current classification    | Domain: *Bacteria*                      | TAS [59]                  |
|         |                           | Phylum: *Firmicutes*                    | TAS [60, 61]              |
|         |                           | Class: *Clostridia*                     | TAS [62]                  |
|         |                           | Order: *Eubacteriales*                  | TAS [63]                  |
|         |                           | Family: *Lachnospiraceae*               | TAS [64]                  |
|         |                           | Genus: *Butyrivibrio*                   | TAS [4]                   |
|         |                           | Species: *hungatei*                     | TAS [19]                  |
|         | Type strain: No           |                                         |                           |
|         | Strain: MB2003            |                                         | TAS [20, 21]              |
|         | Gram stain               | Positive                                | TAS [21, 31]              |
|         | Cell shape               | Rod                                     | TAS [21, 31]              |
|         | Motility                 | Non-motile                              | IDA                       |
|         | Sporulation              | Not reported                            | NAS                       |
|         | Temperature range         | 37–39 °C                                | IDA                       |
|         | Optimum temperature      | 39 °C                                   | IDA                       |
|         | pH range; Optimum        | 6.0–7.0; 6.4                            | IDA                       |
|         | Carbon source            | Variety of carbohydrates                | IDA                       |
|         | Energy metabolism        | Fermentative metabolism                 | IDA                       |
|         | Habitat                  | Bovine rumen                            | TAS [20]                  |
|         | Salinity                 | Not reported                            |                           |
|         | Oxygen requirement       | Anaerobic                               | IDA                       |
|         | Biotic relationship      | Symbiont of ruminants                   | TAS [20]                  |
|         | Pathogenicity            | Non-pathogen                            | NAS                       |
|         | Geographic location      | Ruakura, Hamilton, New Zealand          | TAS [20]                  |
|         | Sample collection time   | May 2009                                | TAS [20]                  |
|         | Latitude                 | −37.77 (37°46′28″S)                     | IDA                       |
|         | Longitude                | +175.31 (175°18′31″E)                   | IDA                       |
|         | Altitude                 | 40 m                                    | IDA                       |

<sup>a</sup>Evidence codes - IDA, Inferred from Direct Assay, NAS, Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [65]

**Insights from the genome sequence**

**Comparison of the MB2003, *B. hungatei* JK615<sup>T</sup>, and *B. proteoclasticus* B316<sup>T</sup> genomes**

A comparison of the *B. hungatei* MB2003 genome with the draft genome of *B. hungatei* JK615<sup>T</sup> [32] and the complete *B. proteoclasticus* B316<sup>T</sup> genome is shown in Table 7. The MB2003 genome is 8633 bp smaller than
JK615$^T$ and contains 27 fewer protein-coding genes. Although several plasmid replication genes have been identified in the JK615$^T$ draft genome, the presence of extrachromosomal elements requires experimental validation.

A novel feature of both the MB2003 and B316$^T$ genomes is the presence of chromids or secondary chromosomes [33]. Chromids are replicons that have %G + C content similar to that of their main chromosome, but have plasmid-type maintenance and replication systems, are smaller than the chromosome, but are usually larger than any other plasmids present. Chromids contain genes essential for growth and maintenance of the organism along with several core genus-specific genes that can be found on the chromosome in other species of bacteria [33]. The Bhu II replicon has most of these characteristics and therefore has been designated as a chromid of MB2003. In B316$^T$, almost 10% of the genes encoding enzymes that have a role in carbohydrate metabolism and transport are found on the chromid [29]. The Bhu II chromid of MB2003 is smaller than the BPc2 chromid of B316$^T$ (186,325 bp), it is now the smallest chromid reported for bacteria. Comparison of MB2003, JK615$^T$ and B316$^T$ genomes based on COG category (Table 8) and synteny analysis (Fig. 6), show that these

| Table 2 Carbon source utilization of the Butyrivibrio strains |
|----------------|----------------|----------------|
| Substrate       | MB2003 | JK615$^T$ | B316$^T$ |
|----------------|--------|----------|----------|
| **Monosaccharides** |        |          |          |
| Arabinose       | ++     | ++       | ++       |
| Fructose        | –      | –        | ++       |
| Galactose       | ++     | –        | ++       |
| Glucose         | ++     | ++       | ++       |
| Mannose         | –      | ++       | ++       |
| Rhamnose        | –      | –        | ++       |
| Ribose          | –      | –        | –        |
| Xylose          | ++     | ++       | ++       |
| **Disaccharides** |        |          |          |
| Cellobiose      | ++     | ++       | ++       |
| Lactose         | ++     | ++       | ++       |
| Maltose         | ++     | ++       | ++       |
| Melibiose       | –      | –        | +        |
| Sucrose         | ++     | ++       | ++       |
| **Trisaccharides** |        |          |          |
| Melezitose      | –      | –        | ++       |
| Raffinose       | –      | ++       | ++       |
| Trehalose       | –      | –        | ++       |
| **Sugar Alcohols** |        |          |          |
| myo-Inositol    | –      | –        | –        |
| Mannitol        | –      | –        | +        |
| Sorbitol        | –      | –        | –        |
| **Glycosides**  |        |          |          |
| Amygdalin       | +      | –        | ++       |
| Esculin         | –      | ++       | ++       |
| Rutin           | –      | ++       | ++       |
| Salicin         | ++     | ++       | ++       |
| **Insoluble substrates** | | | |
| Cellulose       | –      | –        | –        |
| Dextrin         | –      | –        | ++       |
| Inulin          | +      | –        | ++       |
| Starch          | –      | –        | ++       |
| Pectin          | –      | –        | ++       |
| Xylan           | –      | –        | ++       |

$\Delta$O$_{600nm}$ readings of 0.5–1.0 were scored as ++, 0.2–0.5 scored as +, and 0–0.2 scored as –. Results for B. hungatei JK615$^T$ and B. proteoclasticus B316$^T$ are adapted from Kopečný et al. [19] and Moon et al. [6], respectively.
Butyrivibrio species and strains are genetically similar. Although the MB2003 and B316 genome sizes differ, the basic metabolism of these two rumen bacterial species is indicated to be similar.

Butyrate production
For the production of butyrate and H2 from glucose, the MB2003 genome possesses a pyruvate:ferredoxin oxidoreductase gene, nifJ (bhn_I2528) required for pyruvate conversion to acetyl-CoA, as well as a butyryl-CoA dehydrogenase/electron transferring flavoprotein bcd-etfAB (bhn_I2225, bhn_I2221 and bhn_I2222) to generate ATP by classic substrate level phosphorylation. In addition, MB2003 possesses genes that encode all six subunits of the Rnf (rfnA, rfnB, rfnC, rfnD, rfnE, rfnF) and Ech (echA, echB, echC, echD, echE, echF) hydrogenases. These pathways involve the transmembrane ion pumps Ech [34] or Rnf [35–38], that generate a transmembrane proton and/or sodium electrochemical potential from redox cofactors for ATP synthesis by ETP [34, 36]. The MB2003 genome does not possess genes for Por-ABDG, a pyruvate ferredoxin oxidoreductase similar in function to NifJ or genes for EhaA-R, EhbA-P, HydA-C, MbhLKJ, or MvhADG/HdrABC similar in function to the Fd-dependent Ech hydrogenase. In addition, an alternative pathway exists where formate is predicted to be the end product and involves the decarboxylation of acetyl-CoA by a pyruvate formate lyase pflB (bhn_I0124) instead of NifJ. It has been proposed that Ech and Rnf work in concert with NifJ and Bcd-Etf complex to drive ATP synthesis by ETP during glucose fermentation to butyrate [34, 36, 39]. Interestingly, the vast majority of anaerobic prokaryotes appear to possess either an Ech or Rnf but not both [40, 41]. However, a recent analysis of rumen prokaryotic genomes identified Butyrivibrio and Pseudobutyrivibrio as a rare group of bacteria that possess genes for both Ech and Rnf [42]. These findings warrant further biochemical investigation to determine the activity of Ech and Rnf in Butyrivibrio.

The MB2003 pathways for butyrate production presume the possession of a complete Embden-Meyerhof-Parnas glycolytic pathway. Enolase (eno, EC4.2.1.11), converts 2-phospho-D-glycerate to phosphoenolpyruvate in the second to last step of

Table 3 MB2003 genome project information

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS-31 | Finishing quality | High-quality, closed genome |
| MIGS-28 | Libraries used | 454 3 kb mate paired-end library |
| MIGS-29 | Sequencing platforms | 454 GS FLX Titanium chemistry |
| MIGS-31.2 | Fold coverage | 234x |
| MIGS-30 | Assemblers | Newbler version 2.3 |
| MIGS-32 | Gene calling method | Glimmer and BLASTX |
| Locus Tag | bhn and bhn_RS |
| Genbank ID | CP017830, CP017831, CP017832, CP017833 |
| Genbank Date of Release | 31 October 2016 |
| GOLD ID | Ga0074201 |
| BIOPROJECT ID | PRUNA349214 and PRUNA224116 |
| BIOSAMPLE ID | SAMN05928573 |
| MIGS-13 | Source Material Identifier | Butyrivibrio hungatei MB2003 |
| Project relevance | Ruminant plant-fibre degradation |

Table 4 Summary of MB2003 genome replicon features

| Replicon type | Size (bp) | Topology | INSDC identifier | RefSeq ID |
|---------------|-----------|----------|------------------|----------|
| Chromosome | 3,143,784 | circular | CP017831 | NZ_CP017831 |
| Chromid_Bhull | 91,776 | circular | CP017830 | NZ_CP017830 |
| Megaplasmid_pNP144 | 144,470 | circular | CP017832 | NZ_CP017832 |
| Plasmid_pNP6 | 6284 | circular | CP017833 | NZ_CP017833 |
the EMP pathway. Previous work has shown that B316T lacks a detectable enolase [29], and the Methylglyoxal Shunt was proposed as a possible alternative to the EMP pathway. In this pathway the dihydroxyacetone phosphate is transformed to pyruvate via methylglyoxal and D-lactate dehydrogenase, encoded by \(\text{ldhA} \) [43]. The MB2003 genome possesses two methylglyoxal synthase genes, \(\text{mgsA} \) (bhn_I1328 and bhn_I1996), glyoxalase gene \(\text{gloA} \) (bhn_I1783) and an alternative L-lactate dehydrogenase, encoded by \(\text{ldh} \) (bhn_I0363). MB2003 has the same set of genes as B316T for the production of butyrate, formate, acetate and lactate, but also is the only \(B.\) hungatei reported to date that lacks a detectable enolase gene. Genome sequences from a wider range of \(B.\) hungatei and \(B.\) proteoclasticus strains are required to determine if these are common features in these organisms.

### Polysaccharide degradation

The Carbohydrate-Active enZYmes database was used to identify glycoside hydrolases, glycosyl transferases, polysaccharide lyases, carbohydrate esterases and carbohydrate-binding protein module families within the MB2003 genome. MB2003 has a similar CAZyme profile to B316T [21, 31], and analysis of the functional domains of enzymes involved in the breakdown or synthesis of complex carbohydrates, has revealed the polysaccharide-degrading potential of this rumen bacterium.

Approximately 3% of the MB2003 genome (90 CDSs) is predicted to encode either secreted or intracellular proteins dedicated to polysaccharide degradation, similar to that found in B316T. The MB2003 genome is predicted to encode 19 secreted (16 GHs, two CEs and one CBP) and 65 intracellular (59 GHs, 5 CEs and one PL) proteins involved in polysaccharide breakdown (Table 9). The enzymatic profiles of MB2003 and JK615T are almost identical, as both possess the same genes encoding predicted secreted and intracellular CAZymes in their genomes (Table 9). Out of the 19 genes predicted to encode secreted polysaccharide-degrading enzymes, only two, lysozyme \(\text{lcy25B} \) (bhn_III074) and feruloyl esterase \(\text{est1A} \) (bhn_III076), are encoded by the MB2003 chromid (Bhu II). MB2003 has no secreted enzyme larger than 1000 aa in size, with the average size secreted enzymes being 510 aa. The majority (59) of MB2003 genes involved in polysaccharide breakdown (excluding GTs), had corresponding homologues in B316T and

| Table 5 MB2003 genome statistics |
|----------------------------------|
| Attribute                        | Value       | % of total |
| Genome size (bp)                 | 3,386,314   | 100        |
| DNA coding (bp)                  | 3,064,986   | 90.51      |
| DNA G + C (bp)                   | 1,344,683   | 39.71      |
| DNA scaffolds                     | 4           | 100        |
| Total genes                      | 3064        | 100        |
| Protein coding genes             | 2983        | 97.36      |
| RNA genes                        | 60          | 1.96       |
| Pseudogenes                      | 17          | 0.56       |
| Genes in internal clusters       | 160         | 5.22       |
| Genes with function predicted    | 2247        | 73.34      |
| Genes assigned to COGs           | 1842        | 61.34      |
| Genes with Pfam domains          | 2350        | 78.26      |
| Genes with signal peptides       | 148         | 4.93       |
| Genes with transmembrane helices | 881         | 29.34      |
| CRISPR repeats                   | 2           |            |

*The total is based on either the size of the genome in base pairs or the total number of genes or protein-coding genes in the annotated genome.

| Table 6 Number of genes associated with the general COG functional categories |
|----------------------------------|
| Code   | Value | % of total | Description                                      |
| J      | 194   | 9.52      | Translation, ribosomal structure and biogenesis |
| A      | 0     | 0         | RNA processing and modification                 |
| K      | 149   | 7.31      | Transcription                                   |
| L      | 88    | 4.32      | Replication, recombination and repair           |
| B      | 0     | 0         | Chromatin structure and dynamics                |
| D      | 32    | 1.57      | Cell cycle control, Cell division, chromosome partitioning |
| V      | 65    | 3.19      | Defense mechanisms                              |
| T      | 139   | 6.82      | Signal transduction mechanisms                  |
| M      | 155   | 7.61      | Cell wall/membrane biogenesis                   |
| N      | 61    | 2.99      | Cell motility                                   |
| U      | 22    | 1.08      | Intracellular trafficking and secretion         |
| O      | 78    | 3.83      | Posttranslational modification, protein turnover, chaperones |
| C      | 69    | 3.39      | Energy production and conversion                |
| G      | 243   | 11.93     | Carbohydrate transport and metabolism           |
| E      | 177   | 8.69      | Amino acid transport and metabolism             |
| F      | 80    | 3.93      | Nucleotide transport and metabolism             |
| H      | 79    | 3.88      | Coenzyme transport and metabolism               |
| I      | 72    | 3.53      | Lipid transport and metabolism                  |
| P      | 79    | 3.88      | Inorganic ion transport and metabolism          |
| Q      | 16    | 0.79      | Secondary metabolites biosynthesis, transport and catabolism |
| R      | 158   | 7.76      | General function prediction only                |
| S      | 73    | 3.58      | Function unknown                                |
| –      | 1245  | 40.33     | Not in COGs                                    |

*The total is based on the total number of protein coding genes in the genome.
Three of the genes encoding intracellular proteins were found in the Bhu II chromid: a β-glucosidase bgl3A (bhn_III062), a β-galactosidase bga42A (bhn_III010) and a polysaccharide deacetylase est4A (bhn_III070). The analysis of the Pfam domains from the most abundant GH families (GH2, GH31, GH3, GH13 and GH43) showed they did not contain signal sequences and hence were predicted to be located intracellularly. Similarly, CAZymes with predicted roles in xylan and pectin degradation, the GH8, GH28, GH51, GH67, GH88, GH105, GH115, CE2 and CE10 families were also predicted to be intracellular. Of these, MB2003 contains CAZymes with homologues in B316T except for the α-L-arabinofuranosidase arf51C (bhn_I1509). These findings suggest that a variety of complex oligosaccharides resulting from extracellular hydrolysis are metabolized within the cell.

Growth experiments showed MB2003 to be a metabolically versatile bacterium able to grow on a wide variety of monosaccharides, disaccharides and glycosides (Table 2). However, unlike B316T, MB2003 and JK615T were unable to utilize the insoluble substrates pectin and xylan for growth (Table 2). In addition, MB2003, JK615T and B316T are unable to degrade cellulose, however among these organisms, only B316T is able to utilize a range of other insoluble plant polysaccharides. The ability of B316T to breakdown pectin, starch and xylan is predicted to be based on nine large (>1000 aa) cell-associated proteins shown to be significantly up-regulated in B316T cells grown on xylan [44]. These are: α-
amylase *amy13A* (bpr_I1087), arabinogalactan endo-1,4-β-galactosidase *agn53A* (bpr_I2041), carbohydrate esterase family 12 est12B (bpr_I1204), endo-1,3(4)-β-glucanase *lic16A* (bpr_I2326), pectate lyase *pel1A* (bpr_I2372), pectin methylesterase *pme8B* (bpr_I2473), and the cell wall binding domain-containing protein (bpr_I0264). These proteins contain multiple cell wall binding repeat domains (CW-binding domain, Pfam01473) at their C-termini that are predicted to anchor the protein to the peptidoglycan cell membrane. Among these secreted polysaccharidases, some contain single or combinations of catalytic activities: GH10 (endo-1, 4-β-xylanase, xyn10B), GH43 (xyllosidase/arabinofuranosidase, xsa43J (bpr_I12935), endo-1,4-β-xylanase xyn10B (bpr_I0026), and the cell wall binding domain-containing protein (bpr_I0264). These proteins contain multiple cell wall binding repeat domains (CW-binding domain, Pfam01473) at their C-termini that are predicted to anchor the protein to the peptidoglycan cell membrane. Among these secreted polysaccharidases, some contain single or combinations of catalytic activities: GH10 (endo-1, 4-β-xylanase, xyn10B), GH43 (xyllosidase/arabinofuranosidase, xsa43J), PL1 (pectate lyase, pel1A), CE8 and PL9 (pectin methylesterase, pme8B) [45, 46]. Neither MB2003 nor JK615T contain any genes encoding CW-binding domains and are thus are markedly different from B316T.

A curious feature of MB2003 was the presence of a single large (983 aa) carbohydrate binding protein (CBP, bhn_I1848), also present in JK615T (EJ23DRAFT_00192). The domain structures of bhn_I1848 and EJ23DRAFT_00192 are unusual, containing six CBM6 (Pfam03422) domains towards the N-terminus and a single C-terminal CBM2a (Pfam00553) domain. In contrast, B316T encodes two CBPs (bpr_I0736 and bpr_I1599) where both contain two CBM2a domains, and bpr_I1599 also contains two CBM6 domains [29]. CBM6 non-catalytic modules characteristically bind xylose and are associated with xylanase activity with ligand specificity for xylan [47, 48]. CBM2 domains, are divided into two sub-families: 2a, that bind to crystalline cellulose even when associated with xylanases [49], and 2b, that bind to xylan [50]. Recent studies have shown that in discrete regions of plant cell walls, initial enzymatic attack of pectin increases the access of CBMs to cellulose [51], effectively loosening the polysaccharide interactions to expose the xylan and xyloglucan substrates [52, 53]. This initial stage in enzymatic saccharification of plant cell walls termed amorphogenesis [54], and is a possible role for such CBPs containing multiple non-catalytic domains.

In the rumen, MB2003, B316T and JK615T may secrete these non-catalytic CBPs synergistically with
polysaccharide-active enzymes as a mechanism to disrupt the interface between polysaccharides to enhance the rate and extent of plant cell wall degradation.

Conclusion

The *B. hungatei* MB2003 genome sequence adds valuable information regarding the polysaccharide-degrading potential present in the genus *Butyrivibrio*. Genomic comparisons revealed that *B. hungatei* MB2003 shows a high level of similarity with *B. hungatei* JK615T and *B. proteoclasticus* B316T type strains, including genes involved in production of butyrate, formate, acetate and lactate. While MB2003 and JK615T encode a large repertoire of enzymes predicted to metabolize insoluble polysaccharides such as xylan and pectin, they are unable to grow on these substrates and instead appear to be equipped to utilize mainly oligo- and monosaccharides as substrates for growth. Although MB2003 has similar phenotypic characteristics and occupies the same habitat as other *Butyrivibrio* species, its genome encodes fewer extracellular polysaccharide-degrading enzymes, in particular, those that contain multiple cell wall binding repeat domains. The overall genome similarities, metabolic versatility and differences in the abundance of CAZymes observed in *B. proteoclasticus* and *B. hungatei* offers a new view of the genes required for polysaccharide degradation in the rumen. MB2003 appears to occupy a ruminal niche as a secondary degrader of oligosaccharides, in order to coexist with fibre-degrading organisms in this dynamic and competitive environment.

Additional file

**Additional file 1: Table S1.** Associated MIGS record for *B. hungatei* MB2003, which links to the SIGS supplementary content website. (DOCX 17 kb)
| Locus tag | Name   | Annotation                                      | Size (aa) | CAZy | Binding domains |
|-----------|--------|-------------------------------------------------|-----------|------|-----------------|
| bhn_I2518 | bga2A  | β-galactosidase<sup>b</sup>                     | 1034      | GH2  |                 |
| bhn_I0827 | bga2C  | β-galactosidase<sup>b</sup>                     | 714       | GH2  |                 |
| bhn_I11587| bga2B  | β-galactosidase<sup>b</sup>                     | 825       | GH2  |                 |
| bhn_I0200 | gh2B   | glycoside hydrolase family 2<sup>b</sup>       | 641       | GH2  |                 |
| bhn_I1127 | gh2A   | glycoside hydrolase family 2<sup>b</sup>       | 912       | GH2  |                 |
| bhn_I1849 | gh2C   | glycoside hydrolase family 2<sup>b</sup>       | 776       | GH2  |                 |
| bhn_III062| bgI3A  | β-glucosidase<sup>b</sup>                      | 803       | GH3  |                 |
| bhn_I0707 | bgI3B  | β-glucosidase<sup>b</sup>                      | 808       | GH3  |                 |
| bhn_I0180 | bgI3C  | β-glucosidase<sup>b</sup>                      | 671       | GH3  |                 |
| bhn_I0706 | bgI3D  | β-glucosidase<sup>b</sup>                      | 982       | GH3  | C-terminal TMH  |
| bhn_I0189 | xyI3A  | β-xyllosidase<sup>b</sup>                      | 707       | GH3  |                 |
| bhn_I11640| bxI3A  | β-N-acetylhexosaminidase<sup>b</sup>           | 427       | GH3  |                 |
| bhn_I11693| celI5C | endo-1,4-β-glucanase<sup>b</sup>               | 543       | GH5  | CBM2a           |
| bhn_I0165 | celI5A | endo-1,4-β-glucanase/xylanase<sup>b</sup>     | 417       | GH5  |                 |
| bhn_I1756 | xynI8A | reducing end xylose-releasing exo-oligoxylanase<sup>b</sup> | 383 | GH8 | |
| bhn_I0834 | celI9B | cellobextrinase<sup>b</sup>                    | 552       | GH9  |                 |
|           |        |                                                 |           |      | CelD            |
| bhn_I0568 | xynI10B| endo-1,4-β-xylanase<sup>b</sup>                | 425       | GH10 |                 |
| bhn_I0169 | xynI10A| endo-1,4-β-xylanase<sup>b</sup>                | 451       | GH10 |                 |
| bhn_I1158 | glgI2  | 1,4-α-glucan branching enzyme<sup>b</sup>     | 824       | GH13 | CBM48           |
| bhn_I0053 | glgI1  | 1,4-α-glucan branching enzyme<sup>b</sup>     | 663       | GH13 | CBM48           |
| bhn_I2702 | amyI13A| α-amylose<sup>b</sup>                         | 697       | GH13 | CBM34           |
| bhn_I0634| amyI13B| α-amylose<sup>b</sup>                         | 536       | GH13 |                 |
| bhn_I1680 | amyI13C| α-amylose<sup>b</sup>                         | 434       | GH13 |                 |
| bhn_I0669 | amyI13D| α-amylose<sup>b</sup>                         | 511       | GH13 |                 |
| bhn_I1153 | glgX1  | glycogen debranching enzyme<sup>b</sup>       | 726       | GH13 | CBM48           |
| bhn_I1315 | glgX2  | glycogen debranching enzyme<sup>b</sup>       | 648       | GH13 |                 |
| bhn_I0652 | sucI3P | sucrose phosphorylase<sup>b</sup>             | 553       | GH13 |                 |
| bhn_I2526 | chiI18A| chitinase<sup>b</sup>                         | 567       | GH18 |                 |
| bhn_I11254| lyc25A | lysozyme<sup>b</sup>                          | 362       | GH25 |                 |
| bhn_III074| lyc25B | lysozyme<sup>b</sup>                          | 515       | GH25 |                 |
| bhn_I0191 | lyc25C | lysozyme<sup>b</sup>                          | 561       | GH25 |                 |
| bhn_I1763 | lyc25D | lysozyme<sup>b</sup>                          | 242       | GH25 |                 |
| bhn_I0527 | lyc25E | lysozyme<sup>b</sup>                          | 1213      | GH25 |                 |
|           |        |                                                 |           |      | Big2 (x2)       |
| bhn_I11287| aga27A | α-galactosidase<sup>b</sup>                    | 577       | GH27 |                 |
| bhn_I1002 | gh27A  | glycoside hydrolase family 2<sup>b</sup>       | 442       | GH27 |                 |
| bhn_I1152 | pgl28A | polygalacturonase<sup>b</sup>                  | 531       | GH28 |                 |
| bhn_I02679| pgl28B | polygalacturonase<sup>b</sup>                  | 519       | GH28 |                 |
| bhn_I11087| suc29A | α-L-fucosidase<sup>b</sup>                    | 475       | GH29 |                 |
| bhn_I2734 | gh30A  | glycoside hydrolase family 30<sup>b</sup>     | 575       | GH30 |                 |
| bhn_I11581| gh31A  | glycoside hydrolase family 31<sup>b</sup>     | 756       | GH31 |                 |
| bhn_I2191 | gh31C  | glycoside hydrolase family 31<sup>b</sup>     | 674       | GH31 |                 |
| bhn_I0283 | gh31B  | glycoside hydrolase family 31<sup>b</sup>     | 635       | GH31 |                 |
| bhn_I0582 | scr32A | sucrose-6-phosphate hydrolase<sup>b</sup>   | 493       | GH32 |                 |
| bhn_I0826 | bga35A | β-galactosidase<sup>b</sup>                    | 622       | GH35 |                 |
| Locus tag | Name | Annotation | Size (aa) | CAZy | Binding domains |
|-----------|------|------------|----------|------|-----------------|
| bhn_11817 | bga35B | β-galactosidase<sup>b</sup> | 735 | GH35 | |
| bhn_11044 | aga36A | α-galactosidase<sup>b</sup> | 782 | GH36 | |
| bhn_11583 | aga36B | α-galactosidase<sup>b</sup> | 620 | GH36 | |
| bhn_11945 | aga36C | α-galactosidase<sup>b</sup> | 730 | GH36 | |
| bhn_10086 | man38A | α-mannosidase<sup>b</sup> | 1053 | GH38 | |
| bhn_11010 | bga42A | β-galactosidase<sup>b</sup> | 673 | GH42 | |
| bhn_0167 | xso43A | xylosidase/arabinofuranosidase<sup>b</sup> | 543 | GH43 | CBM6 |
| bhn_01981 | xso43B | xylosidase/arabinofuranosidase<sup>b</sup> | 301 | GH43 | |
| bhn_12037 | xso43C | xylosidase/arabinofuranosidase<sup>b</sup> | 302 | GH43 | |
| bhn_12111 | xso43D | xylosidase/arabinofuranosidase<sup>b</sup> | 517 | GH43 | |
| bhn_12735 | xso43E | xylosidase/arabinofuranosidase<sup>b</sup> | 352 | GH43 | |
| bhn_10032 | xso43F | xylosidase/arabinofuranosidase<sup>b</sup> | 312 | GH43 | |
| bhn_0164 | xso43G | xylosidase/arabinofuranosidase and esterase<sup>b</sup> | 925 | GH43 | |
| bhn_11509 | arf51C | α-L-arabinofuranosidase<sup>b</sup> | 630 | GH51 | |
| bhn_12228 | arf51A | α-L-arabinofuranosidase<sup>b</sup> | 502 | GH51 | |
| bhn_00100 | arf51B | α-L-arabinofuranosidase<sup>b</sup> | 504 | GH51 | |
| bhn_01670 | agn53A | arabinogalactan endo-1,4-β-galactosidase<sup>b</sup> | 439 | GH53 | |
| bhn_0183 | agu67A | α-D-glucuronidase<sup>b</sup> | 662 | GH67 | |
| bhn_12177 | mal77A | 4-α-glucanotransferase<sup>b</sup> | 506 | GH77 | |
| bhn_01697 | ugl88A | unsaturated glucuronyl hydrolase<sup>b</sup> | 385 | GH88 | |
| bhn_12381 | ugl88B | unsaturated glucuronyl hydrolase<sup>b</sup> | 383 | GH88 | |
| bhn_12196 | cbp94A | cellobiose phosphorylase<sup>b</sup> | 814 | GH94 | |
| bhn_11582 | gh95A | glycoside hydrolase family 95<sup>b</sup> | 734 | GH95 | |
| bhn_12548 | gh105A | unsaturated rhamnogalacturonyl hydrolase<sup>b</sup> | 349 | GH105 | |
| bhn_10090 | gh105B | unsaturated rhamnogalacturonyl hydrolase<sup>b</sup> | 363 | GH105 | |
| bhn_12549 | gnpA | D-galactosyl-β-1,4-L-rhamnose phosphorylase<sup>b</sup> | 722 | GH112 | |
| bhn_0185 | gh115A | α-glucuronidase<sup>b</sup> | 947 | GH115 | |
| bhn_11083 | xyl120A | xylosidase<sup>b</sup> | 861 | GH120 | |
| bhn_11738 | xyl120B | xylosidase<sup>b</sup> | 664 | GH120 | |
| bhn_11076 | est1A | feruloyl esterase<sup>b</sup> | 351 | CE1 | |
| bhn_11244 | est2A | acetyl-xylan esterase<sup>b</sup> | 372 | CE2 | |
| bhn_11070 | est4A | polysaccharide deacetylase<sup>b</sup> | 207 | CE4 | |
| bhn_10843 | est4C | polysaccharide deacetylase<sup>b</sup> | 280 | CE4 | |
| bhn_08666 | nagA | N-acetylgalactosamine-6-phosphate deacetylase<sup>b</sup> | 371 | CE9 | |
| bhn_11609 | est12A | carbohydrate esterase family 12<sup>b</sup> | 584 | CE12 | |
| bhn_11927 | est12B | carbohydrate esterase family 12<sup>b</sup> | 244 | CE12 | |
| bhn_11926 | pil71A | polysaccharide lyase<sup>b</sup> | 746 | PL11 | |
| bhn_10657 | pilP1 | glycogen phosphorylase<sup>b</sup> | 769 | GT35 | |
| bhn_12673 | pilP2 | glycogen phosphorylase<sup>b</sup> | 824 | GT35 | |
| bhn_11848 | – | carbohydrate binding protein<sup>b</sup> | 983 | CBM2a (×1), CBM6 (×6) | |

<sup>a</sup>CAZy descriptions and classifications compiled from the CAZy database [68]. <sup>b</sup>Indicates homologues in the B. hungatei JK615<sup>7</sup> draft genome. Genes encoding predicted secreted polysaccharide degrading enzymes are in bold.
Abbreviations
Bp: Base pair(s); C2Zymes: Carbohydrate-Active eZymes; CBMs: Carbohydrate-Binding Module(s); CE: Carbohydrate Esterase(s); Ech: Escherichia coli hydrogenase-3-type hydrogenase; Eta: Energy-convertling hydrogenase A; Etb: Energy-convertling hydrogenase B; EMP: Embden-Meyerhof-Parnas; ETP: Electron transport phosphorylation; Ghts: Glycoside Hydrolase(s); GTs: Glycosyl Transferase(s); Hyd: Ferredoxin hydrogenase; Mibh: Membrane-bound hydrogenase; MvhH/Drh: Methyl viologen hydrogenase/heterodisulfide reductase; Pl: Pyruvate formate lyase; PLS: Poly saccharide Lyase(s); Pr: Pyruvate ferredoxin oxidoreductase; Rnf: Rhodobacter nitrogen fixation

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Authors’ contributions
NP, WJK, GTA conceived and designed the experiments. NP performed the sequencing and assembly experiments. NP, WJK, GTA, SCL, EA performed the growth studies and polysaccharide utilization profiling. NP, WJK, GTA wrote the manuscript. All authors commented on the manuscript before submission. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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