Characterization of D-Arabitol as Newly Discovered Carbon Source of Bacillus methanolicus

Marina Gil López1, Marta Irla1,2, Luciana F. Brito1,2 and Volker F. Wendisch1*

1 Genetics of Prokaryotes, Faculty of Biology and CeBiTec, Bielefeld University, Bielefeld, Germany; 2 Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway

Bacillus methanolicus is a Gram-positive, thermophilic, methanol-utilizing bacterium. As a facultative methylo troph, B. methanolicus is also known to utilize D-mannitol, D-glucose and, as recently discovered, sugar alcohol D-arabitol. While metabolic pathways for utilization of methanol, mannitol and glucose are known, catabolism of arabitol has not yet been characterized in B. methanolicus. In this work we present the elucidation of this hitherto uncharted pathway. In order to confirm our predictions regarding genes coding for arabitol utilization, we performed differential gene expression analysis of B. methanolicus MGA3 cells grown on arabitol as compared to mannitol via transcriptome sequencing (RNA-seq). We identified a gene cluster comprising eight genes that was up-regulated during growth with arabitol as a sole carbon source. The RNA-seq results were subsequently confirmed via qRT-PCR experiments. The transcriptional organization of the gene cluster identified via RNA-seq was analyzed and it was shown that the arabitol utilization genes are co-transcribed in an operon that spans from BMMGA3_RS07325 to BMMGA3_RS07365. Since gene deletion studies are currently not possible in B. methanolicus, two complementation experiments were performed in an arabitol negative Corynebacterium glutamicum strain using the four genes discovered via RNA-seq analysis as coding for a putative PTS for arabitol uptake (BMMGA3_RS07330, BMMGA3_RS07335, and BMMGA3_RS07340 renamed to atlABC) and a putative arabitol phosphate dehydrogenase (BMMGA3_RS07345 renamed to atlD). C. glutamicum is a natural D-arabitol utilizer that requires arabitol dehydrogenase MtlD for arabitol catabolism. The C. glutamicum mtlD deletion mutant was chosen for complementation experiments. Heterologous expression of atlABCD as well as the arabitol phosphate dehydrogenase gene atlD from B. methanolicus alone restored growth of the C. glutamicum ΔmtlD mutant with arabitol. Furthermore, D-arabitol phosphate dehydrogenase activities could be detected in crude extracts of B. methanolicus and these were higher in arabitol-grown cells than in methanol- or mannitol-grown cells. Thus, B. methanolicus possesses an arabitol inducible operon encoding, amongst others, a putative PTS system and an arabitol phosphate dehydrogenase for uptake and activation of arabitol as growth substrate.

Keywords: Bacillus methanolicus, differential transcriptome analysis, mannitol metabolism, arabitol metabolism, monophasic growth, operon organization
INTRODUCTION

Bacillus methanolicus is an aerobic, Gram-positive, thermophilic, methanol-utilizing bacterium originally isolated from freshwater marsh soil (Schendel et al., 1990; Arfman et al., 1992). Methyloptrophs, such as B. methanolicus, utilize carbon sources without C-C bonds also called C1 substrates. The key intermediate for biological C1 fixation is formaldehyde, and B. methanolicus belongs to the group of facultative methyloptrophs that fix formaldehyde via the ribulose monophosphate (RuMP) cycle (Anthony, 1982; Arfman et al., 1992). What makes methanol an attractive feedstock is the fact that it is abundant and cheap, and that addition of methanol to fermentation broth reduces the risk of microbial contamination in fermentative processes due to toxicity of its derivative – formaldehyde (Irla et al., 2015; Müller et al., 2015a). Furthermore, methanol presents a non-food alternative to conventional feedstock generally used in biotechnological processes. The ability to utilize methanol as carbon source, in addition to its high growth temperature, makes B. methanolicus MGA3 a promising candidate for biotechnological amino acid production. It has been successfully used for methanol-based production of the amino acids L-lysine and L-glutamate (Brautaset et al., 2007), and has been engineered for production of the compounds cadaverine (Naerdal et al., 2015; Irla et al., 2016) and γ-aminobutyric acid (GABA) (Irla et al., 2017). Furthermore, tools for gene expression have been recently developed, comprising gene co-expression from two different plasmids and controlled inducible gene expression systems with both rolling circle and theta replicating plasmids (Irla et al., 2016).

Bacillus methanolicus methylotrophy has been extensively studied and characterized in recent years. This contributed to a broader understanding of methanol metabolism and its regulation by fully sequencing the MGA3 genome (Heggeset et al., 2012; Irla et al., 2014), achieving a comprehensive analysis of the transcriptional landscape using RNA-seq (Irla et al., 2015) and accomplishing proteome (Müller et al., 2014) and metabolome (Müller et al., 2015b; Carnicer et al., 2016) studies. These studies not only increased our understanding of methanol metabolism in B. methanolicus, but also yielded insight into catabolic pathways of alternative carbon sources. B. methanolicus is known to utilize D-mannitol and D-glucose as sole carbon and energy sources and metabolic pathways for the utilization of these substrates have already been described (Heggeset et al., 2012). Both mannitol and glucose enter the cells via a phosphotransferase system (PTS) as mannitol 1-phosphate and glucose 6-phosphate, respectively, and are converted to fructose 6-phosphate. Here, we describe and characterize utilization of the pentose sugar alcohol D-arabitol for growth of B. methanolicus MGA3. Two alternative pathways for arabitol utilization have been described in bacteria (Figure 1): uptake of arabitol via a permease followed by intracellular oxidation and phosphorylation to yield xylulose 5-phosphate, route described for proteobacteria and actinobacteria as in e.g., Corynebacterium glutamicum (Laslo et al., 2012), Enterobacter aerogenes (Charnetzky and Mortlock, 1974), Klebsiella pneumoniae (Heuel et al., 1997), Rhizobium trifolii (Primrose and Ronson, 1980), and Pseudomonas fluorescens (Brünker et al., 1998), or PTS-mediated uptake and phosphorylation followed by oxidation to pentose phosphates as described for the firmicutes Listeria monocytogenes (Kentache et al., 2016) and Enterococcus avium (Povelainen et al., 2003).

Arabitol is ubiquitous in nature and has been found in plants and fungi, often alongside mannitol, being involved in osmoprotection and carbohydrate storage (Daly et al., 1967; Plemenitaö et al., 2008; Wang et al., 2019). It has additionally been reported that arabitol conferred drought tolerance when provided by a lichenous fungi to the green algae Trebouxia sp. (Kosugi et al., 2013). Yeast or yeast-like fungi produce extracellular glycolipids, some of which have been reported to exceptionally contain mannitol and arabitol residues (Kulakovskaya and Kulakovskaya, 2014b). Their role in metabolism includes promotion of solubilization and absorption of hydrophobic substrates, extracellular reserve of carbon sources and antibiotic activity (Kulakovskaya and Kulakovskaya, 2014a). Roselipins, consisting of C20 fatty acids with three hydroxyl groups, mannos and arabitol residues, are extracellular glycolipids synthesized by Clonostachys rosea (Tabata et al., 1999). Mannosylerythritol lipids are major extracellular glycolipids of the Pseudozyma genera. In Pseudozyma parantarctica the rarely occurring mannosylarabitol lipids and mannosylmannitol lipids have been described (Morita et al., 2009, 2012). The natural ecological niches of these extracellular glycolipid yeast producers include soil, nectaries and leaves of plants (Kulakovskaya and Kulakovskaya, 2014a), coexisting together with B. methanolicus in similar habitats. Additionally, some yeasts possess the ability of transforming glucose into arabitol (Kordowska-Wiater, 2015). It has previously been reported that methanol is a by-product of pectin metabolism during cell wall synthesis and pathogen
attack in plants, which in turn assists in plant immunity (Fall and Benson, 1996; Komarova et al., 2014). Bacterial isolates from leaf surfaces showed the presence of \textit{B. methanolicus} on \textit{Citrus paradisi} plants (Izhaki et al., 2013). These findings indicate that methanol, mannitol, glucose and arabitol might be present in the natural habitat of \textit{B. methanolicus}.

In the present study, we characterized growth of \textit{B. methanolicus} MGA3 on arabitol. Based on our differential transcriptome analysis, the genes coding for proteins involved in arabitol utilization were identified, and their functionality confirmed by genetic complementation and enzyme assays.

### MATERIALS AND METHODS

#### Bacterial Strains, Media and Cultivation Conditions

The strains used in this study are listed in Table 1. \textit{C. glutamicum} ATCC 13032 was used as the expression host and \textit{Escherichia coli} DH5α was used as the general cloning host. \textit{E. coli} strains were routinely cultivated at 37°C and 180 rpm in Lysogeny Broth (LB) media or on LB plates [1% (w/v) agar] supplemented with 25 μg mL⁻¹ kanamycin if relevant. \textit{B. methanolicus} strains were cultivated at 50°C and 200 rpm in minimal MVcMY media for pre-cultures or MVcM for main cultures as previously described (Brautaset et al., 2003) with 200 mM methanol, 5, 10, 15, and 50 mM mannitol or 5, 10, 15, 30, and 60 mM arabitol. For co-consumption experiments, 15 mM mannitol and 15 mM arabitol were added to the media. Main cultures of all \textit{B. methanolicus} experiments were inoculated at a start optical density (OD₆₀₀) of 0.2. \textit{C. glutamicum} strains were routinely cultivated at 30°C and 120 rpm in LB media with 30 mM glucose for pre-cultures and in minimal CGXII media (Eggeling and Bott, 2005) for main cultures with 30 mM glucose or 30 mM arabitol. Media were supplemented with 25 μg g⁻¹ kanamycin when necessary and 1 mM IPTG was added for induction of gene expression at inoculation of the main cultures, which was done at an initial OD₆₀₀ of 0.5. Cultivations were performed in 500 mL baffled shake flasks with 50 mL media volume and in biological triplicates in all cases.

#### Recombinant DNA Work

The description of all plasmids constructed in this study is presented in Table 1. Molecular cloning was performed as described by Sambrook and Russell (2001). Primer sequences used in this study were obtained from Metabion (Planegg/Stein kirchen, Germany) and are listed in Supplementary Table 1. Total DNA isolation from \textit{B. methanolicus} MGA3 was performed as previously described (Eikmanns et al., 1994). Inserts were amplified by polymerase chain reactions (PCRs) using ALLIn™ HiFi DNA Polymerase (HighQ, Kraichtal, Germany) and purified with the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). For plasmid isolation, the GenelJet Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, MA, United States) was used. Ends of DNA fragments (PCR-amplified fragments and plasmid pVWEx1 cut with restriction enzymes) were joined by means of the isothermal DNA assembly method (Gibson et al., 2009). Transformation of chemically competent \textit{E. coli} cells was done following the procedure of Mandel and Higa (1970). Colony PCRs were performed using the Taq polymerase (New England Biolabs, Ipswich, England) with primers acF1, acR1 and acR2 (Gibson et al., 2009). Transformation of chemically competent cells and electroporation were prepared as previously described (Eggeling and Bott, 2005).

#### Table 1 | Strains and plasmids used in this study.

| Strain or plasmid | Relevant characteristics | References |
|-------------------|-------------------------|------------|
| **Strains**       |                         |            |
| \textit{E. coli} DH5α | F⁻ thi-1 endA1 hsdR17(r−, m−) supE44 ΔlacI169 (q80lacZΔM15) recA1 gyrA96 relA1 | Hanahan, 1983 |
| \textit{C. glutamicum} ATCC 13032 | Wild type strain | Abe et al., 1967 |
| \textit{C. glutamicum} RES167 ΔmttD | mttD deletion mutant of \textit{C. glutamicum} RES167 | Laslo et al., 2012 |
| \textit{B. methanolicus} MGA3 | Wild type strain (ATCC 53907) | Schendel et al., 1990 |
| \textit{B. methanolicus} PB1 | Wild type strain (ATCC 51375, NCIMB13113) | Arfman et al., 1992 |
| **Plasmids**      |                         |            |
| pVWEx1 | Km⁰; \textit{E. coli}/
\textit{C. glutamicum} shuttle vector for regulated gene expression (Pus., acrR, pCG1 oriV<sub>Cop</sub>) | Peters-Wendisch et al., 2001 |
| pVWEx1-atlABCD | pVWEx1 derivative for IPTG-inducible expression of BMMGA3_RS07330, BMMGA3_RS07335, BMMGA3_RS07340 and BMMGA3_RS07345 (atlABCDEF) from \textit{B. methanolicus} MGA3 | This study |
| pVWEx1-atlD | pVWEx1 derivative for IPTG-inducible expression of BMMGA3_RS07345 (atlD) from \textit{B. methanolicus} MGA3 | This study |
| pVWEx1-atlABCDEF | pVWEx1 derivative for IPTG-inducible expression of BMMGA3_RS07330, BMMGA3_RS07335, BMMGA3_RS07340, BMMGA3_RS07345, and BMMGA3_RS07355 (atlABCDEF) from \textit{B. methanolicus} MGA3 | This study |

Km⁰, kanamycin resistance.

Plasmids for inducible gene expression in \textit{C. glutamicum} were constructed on the basis of pVWEx1 (Peters-Wendisch et al., 2001). The \textit{atlABCD} and \textit{atlABCDEF} genes were PCR-amplified from \textit{B. methanolicus} MGA3 genomic DNA using the primers acF1 and acR1 or acF1 and acR2, respectively, and the \textit{atlD} gene

#### Heterologous Expression of \textit{B. methanolicus} Genes in \textit{C. glutamicum}

Plasmids for inducible gene expression in \textit{C. glutamicum} were constructed on the basis of pVWEx1 (Peters-Wendisch et al., 2001). The \textit{atlABCD} and \textit{atlABCDEF} genes were PCR-amplified from \textit{B. methanolicus} MGA3 genomic DNA using the primers acF1 and acR1 or acF1 and acR2, respectively, and the \textit{atlD} gene
was amplified using primers aPDF and aPDR (Supplementary Table 1). The resulting PCR product was joined with BamHI digested pVWEx1 via Gibson assembly.

**Isolation of Total RNA**

In order to perform total RNA extraction from *B. methanolicus* MGA3 cells, the NucleoSpin RNA isolation Kit (Machery-Nagel, Düren, Germany) and the RNase-free DNase set (Qiagen, Hilden, Germany) were used according to the manufacturer's instructions. *B. methanolicus* cultures were grown in minimal MVcM media containing 200 mM methanol, 15 mM mannitol or 15 mM arabitol. Cells were harvested in the middle of the exponential growth phase at an OD\textsubscript{600} of 1.0 followed by total RNA isolation individually for each cultivation condition. The RNA material was tested for contaminating DNA using primers PRIF and PRIR for the amplification of the *proI* gene and primers MRF1 and MRR1 for the amplification of the *mtlR* gene (Supplementary Table 1). No product was obtained for any of the tested samples (data not shown). The quality of the samples was subsequently verified by capillary gel electrophoresis (Agilent Bioanalyzer 2100 system using the Agilent RNA 6000 Pico kit; Agilent Technologies, Böblingen, Germany) and the concentration checked by DropSense\textsuperscript{TM} 16 (Trinean, Ghent, Belgium). The RNA material was subsequently used either for RNA-seq analysis, qRT-PCR or RT-PCR analysis of operon structure.

**cDNA Library Preparation, RNA-Seq and Mapping of Generated RNA-Seq Data**

Isolated RNA samples from *B. methanolicus* MGA3 were pooled in equal parts and the total RNA was subsequently used for the cDNA library preparation. The library was prepared and sequenced on a single flow cell of a MiSeq Desktop Sequencer system (Illumina, San Diego, CA, United States) in paired-end mode following a protocol that allowed for the analysis of the whole transcriptome (Mentz et al., 2013). Previous to mapping of the generated reads onto the reference genome, the sequences were trimmed using the tool Trimmomatic version 0.33 (Bolger et al., 2014) to a minimal length of 35 base pairs. The trimmed reads were mapped to the *B. methanolicus* MGA3 reference sequences of the chromosome as well as the two plasmids pBM19 and pBM69 (GenBank accession numbers CP007739, CP007741, and CP007740, respectively) using the software for short read alignment Bowtie (Langmead et al., 2009). For the visualization of the mapped reads the ReadXplorer software was used (Hilker et al., 2014). The differential gene expression analysis was performed with the statistical method DESeq (Anders and Huber, 2010) using the same software. In order to designate a gene as differentially expressed, the cut-off values were set to a change in expression level higher than 30, for which the P-value was adjusted to be equal to or less than 0.01. Sequences of differentially expressed genes that coded for proteins of unknown function were subjected to BLASTx analysis for identification of protein family conservations (Altschul et al., 1990).

**Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)**

qRT-PCR was performed in order to validate the generated data in RNA-seq analysis. Isolated RNA samples from *B. methanolicus* MGA3 grown on either mannitol, arabitol or a combination of both were used as template. The optimization of a qRT-PCR protocol included a series of standard PCRs using different primer concentrations (250, 400, and 600 mM) and different annealing temperatures (48 to 65°C). For each gene to be analyzed, a pair of primers were designed for the amplification of about 200 bp using the primer design tool of Clone Manager 9 (Scientific & Educational Software, Denver, CO, United States) (Supplementary Table 1). The experiments were performed with the LightCycler\textsuperscript{®} 96 System (Roche Diagnostics, Penzberg, Germany) using the SensiFAST\textsuperscript{TM} SYBR\textsuperscript{®} No-ROX One-Step Kit (Bioline, Luckenwalde, Germany) according to the manufacturer's instructions. After the optimization process, each reaction mixture contained 400 mM of each primer and 50 ng of RNA in a final volume of 20 µL. The qRT-PCR profile was chosen to be performed as follows: the reverse transcription was performed at 45°C for 10 min, the polymerase activation at 95°C for 2 min followed by 40 cycles of a three-step amplification composed of a denaturation step at 95°C for 5 s, annealing at 55°C for 10 s and extension at 72°C for 5 s and, lastly, dissociation curve analyses were done from 65°C up to 95°C in 0.5°C increments for 5 s each step. Amplification of *repB*, the pBM19 replication initiator gene, was used for sample normalization following the recommendations of Jakobsen et al. (2006). They could confirm by qRT-PCR that the *repB* transcript levels were similar in cells utilizing mannitol and methanol and, additionally, our RNA-seq data showed similar *repB* expression between mannitol and arabitol, too (data not shown). Relative quantification was done by means of the comparative threshold cycle method: the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All measurements were performed in technical replicates.

**Reverse Transcription PCR (RT-PCR) Analysis of Operon Structure**

Analysis of the transcriptional organization of the arabitol gene cluster was done via RT-PCR. Isolated RNA from *B. methanolicus* MGA3 grown on arabitol was used as template for cDNA synthesis using the BioScript\textsuperscript{TM} Reverse Transciptase kit (Bioline, Luckenwalde, Germany) according to the manufacturer's instructions with gene specific primer RT03, which hybridizes in BMGMA3_RS07365 (Supplementary Table 1). In order to detect if the referred gene cluster was transcribed as a single mRNA molecule, the resulting cDNA was then used as template for PCR using primers spanning gene borders of all genes putatively present in the arabitol gene cluster (Supplementary Table 1).

**Arabitol Phosphate Dehydrogenase Enzymatic Assay of *B. methanolicus***

**Crude Extracts**

*Bacillus methanolicus* MGA3 cells were grown in minimal media with methanol, mannitol or arabitol and collected by
centrifugation at 4°C and 4,000 rpm for 10 min in the middle of the exponential growth phase at an OD_{600} of around 1.0. The pelleted cells were re-suspended in Tris-HCl buffer (pH 7.2) and disrupted by sonication (UP 200 S, Dr. Hielscher GmbH, Teltow, Germany) on ice at an amplitude of 55% and a duty cycle of 0.5 for 9 min with a 30 s pause in between. To obtain lysates, the samples were centrifuged for 60–90 min at 4°C and 14,000 rpm to remove cell debris, the supernatants were then tested conditions except when 60 mM was used, since about 20 mM remained in the supernatant when growth stopped (Supplementary Figure 1). Although arabinol supported growth of MGA3 as a sole source of carbon and energy, the strain grew at a lower growth rate than with its preferred sugar alcohol substrate mannitol: the growth rate using arabinol was 0.20 ± 0.01 h^{-1} as compared to 0.37 ± 0.01 h^{-1} when mannitol was used (Table 2). Accordingly, the substrate consumption rate was higher for mannitol than for arabinol (7.4 ± 0.5 vs. 5.7 ± 0.1 mmol g CDW^{-1} h^{-1}), which was also the case for the biomass yield (0.28 ± 0.01 vs. 0.24 ± 0.01 g CDW g^{-1} or carbon normalized biomass yield 0.70 ± 0.02 vs. 0.60 ± 0.02 g CDW g carbon^{-1}) (Table 2). As shown in Figure 2A, a relation between growth rate and substrate concentration according to Monod kinetics indicated that 2.9 ± 0.9 mM arabinol supported growth with a half-maximal growth rate.

In order to test if arabinol and mannitol are utilized sequentially or simultaneously, growth of MGA3 in minimal media containing a mixture of 15 mM arabinol and 15 mM mannitol was analyzed (Figure 2B). MGA3 did not show biphasic growth in that experiment, and the maximum OD_{600} of 6.34 ± 0.41 was reached. Mannitol was utilized faster than arabinol and co-consumption of both sugar alcohols was observed between 6 and 12 h, at which point the growth stopped and both substrates were fully consumed (Figure 2B). As expected, the uptake rates for mannitol and arabinol during co-consumption (2.3 ± 0.1 and 1.8 ± 0.0 mmol g CDW^{-1} h^{-1}, respectively) were lower than when either carbon source was provided as sole substrate (7.4 ± 0.5 and 5.7 ± 0.1 mmol g CDW^{-1} h^{-1}, respectively) and the biomass yield using both mannitol and arabinol was the same as when only mannitol was provided to the media (0.28 ± 0.01 g CDW g^{-1}) (Table 2).

### Comparative Analysis of Global Gene Expression Profiles of B. methanolicus MGA3 During Growth With Arabinol or Mannitol

In order to elucidate the genetic background of arabinol utilization, a differential gene expression analysis of B. methanolicus MGA3 cultivated with either 15 mM mannitol or 15 mM arabinol as sole carbon source was performed by RNA-seq. Sequencing of the prepared cDNA libraries from RNA isolated under the two chosen conditions resulted in 3,200,444 raw reads for cDNA library of mannitol grown cells and 2,728,707 raw reads for cDNA library of arabinol grown

### RESULTS

#### Growth of B. methanolicus MGA3 on Arabinol as Single Carbon Source and as Co-substrate to Mannitol

In search of gratuitous inducer of an mtlR promoter described by Irla et al. (2016), arabinol was tested as one of the potentially feasible compounds. While it was shown that addition of arabinol to cultivation broth does not lead to induction of expression of genes controlled by P_{mtlR}, the authors have discovered that arabinol serves as sole carbon and energy source for B. methanolicus (Irla et al., 2016). Due to the fact that at that time only two alternative carbon sources had been described for this facultative methylotroph, it was interesting to investigate the physiology and genetic background of arabinol utilization in B. methanolicus. For that purpose, B. methanolicus MGA3 was cultivated in MVcM minimal media containing 10, 15, 30, or 60 mM arabinol. Arabinol was completely consumed in all

### Table 2 | Growth rates, uptake rates, biomass yields, and other characteristics of B. methanolicus MGA3 grown on arabinol, mannitol, or a combination of both.

| Carbon source(s) (15 mM each) | Growth rate (h^{-1}) | Biomass yield (g CDW g^{-1}) | Biomass yield (g CDW g carbon^{-1}) | Uptake rate (mmol g CDW^{-1} h^{-1}) |
|-----------------------------|----------------------|-----------------------------|-----------------------------------|-----------------------------|
| Arabinol                    | 0.20 ± 0.01          | 0.24 ± 0.01                 | 0.60 ± 0.02                       | 5.7 ± 0.1                   |
| Mannitol                    | 0.37 ± 0.01          | 0.28 ± 0.01                 | 0.70 ± 0.02                       | 7.4 ± 0.5                   |
| Arabinol + mannitol         | 0.31 ± 0.01          | 0.28 ± 0.01                 | 0.70 ± 0.03                       | 1.8 ± 0.0 (arabinol); 2.3 ± 0.1 (mannitol) |
TABLE 3 | Key genes of mannitol and arabitol metabolism with altered expression in B. methanolicus MGA3 cultivated with arabitol in comparison to mannitol as sole carbon source.

| Locus tag          | Gene                  | Annotation                                                                 | Log2 fold change of relative RNA levels (arabitol/mannitol) |
|--------------------|-----------------------|------------------------------------------------------------------------------|-------------------------------------------------------------|
| BMMGA3_RS01065      | mtlA<sup>B</sup>      | PTS system mannitol-specific EIICB component<sup>B</sup>                     | −4.22                                                       |
| BMMGA3_RS01070      | mtlR<sup>B</sup>      | Transcriptional regulator<sup>B</sup>                                        | −4.62                                                       |
| BMMGA3_RS01075      | mtlF<sup>B</sup>      | Mannitol-specific phosphotransferase enzyme<sup>B</sup>                      | −3.62                                                       |
| BMMGA3_RS01080      | mtlD<sup>B</sup>      | Mannitol-1-phosphate 5-dehydrogenase<sup>B</sup>                            | −3.87                                                       |
| BMMGA3_RS07325      |                      | Transcriptional antiterminator<sup>B</sup>                                   | 2.99                                                        |
| BMMGA3_RS07330      | atlC<sup>B</sup>      | IIA arabitol PTS component<sup>C</sup>                                      | 3.06                                                        |
| BMMGA3_RS07335      | atlB<sup>B</sup>      | IIB arabitol PTS component<sup>C</sup>                                      | 3.41                                                        |
| BMMGA3_RS07340      | atlC<sup>B</sup>      | IIC arabitol PTS component<sup>C</sup>                                      | 2.73                                                        |
| BMMGA3_RS07345      | atlD<sup>B</sup>      | Arabitol phosphate dehydrogenase<sup>C</sup>                               | 2.90                                                        |
| BMMGA3_RS07350      |                      | Hypothetical protein                                                         | 3.00                                                        |
| BMMGA3_RS07355      | galactitol-1-phosphate| 5-dehydrogenase                                                              | 2.97                                                        |
| BMMGA3_RS07360      | S-methyl-β-thiuronine-1-phosphate isomerase                                | 2.15                                                        |

<sup>A</sup>Cut-off values set to a change in expression level higher than 30; P ≤ 0.01, determined by Student’s t-test. <sup>B</sup>Annotation according to Irla et al. (2016). <sup>C</sup>Annotation according to this work’s findings.

The cluster comprises four genes annotated as coding for a PTS for galactitol uptake (BMMGA3_RS07330, BMMGA3_RS07335 and BMMGA3_RS07340) and a sorbitol dehydrogenase gene (BMMGA3_RS07345). However, neither galactitol nor sorbitol supported growth of B. methanolicus MGA3 (data not shown). Therefore, it was concluded that these genes may, in fact, be involved in arabitol metabolism.

To validate the RNA-seq results, qRT-PCR experiments were performed. As shown in Figure 3A, the expression levels of the targeted genes detected by qRT-PCR were in accordance with the gene expression patterns obtained by RNA-seq analysis (Table 3). Additionally, RNA levels of mtlD, mtlR, atlC, and atlD were determined by qRT-PCR during growth on the mixture of mannitol and arabitol as combined carbon sources. Differential expression was observed for genes mtlD and atlD, which code for catabolic enzymes, whereas RNA levels of the regulatory gene mtlR and the transport gene atlC did not change significantly (Figure 3B).

Complementation of the Arabitol-Negative C. glutamicum Mutant ΔmtlD by Heterologous Expression of B. methanolicus MGA3 atlABCD or atlD

In order to verify the hypothesis that the atlABCD genes code for a PTS and an arabitol phosphate dehydrogenase and...
support arabitol catabolism, the arabitol-negative *C. glutamicum* ΔmtlD mutant (Laslo et al., 2012) was used for genetic complementation experiments. This experiment could not have been performed with an arabitol-negative *B. methanolicus* because gene deletion studies are currently not possible in this bacterium. *C. glutamicum* ΔmtlD is unable to grow with arabitol as sole carbon source (Laslo et al., 2012). Therefore, *atlABCD* genes from MGA3 were cloned into *C. glutamicum* expression vector pVWEx1, and the resulting vector pVWEx1-*atlABCD* was used to transform *C. glutamicum* ΔmtlD with the aim of restoring growth on arabitol. While the ΔmtlD mutant transformed with pVWEx1 empty vector was unable to grow on arabitol, heterologous expression of *B. methanolicus* MGA3-derived *atlABCD* genes from vector pVWEx1 allowed for growth with arabitol as sole carbon source and led to complete arabitol consumption (Figure 4A). However, since *C. glutamicum* ΔmtlD still possesses the arabitol transporter *rbtT* (Laslo et al., 2012), the possibility that the strain could still import arabitol via the native permease and that AtlD would subsequently take over its oxidation to xylulose could not be excluded. Therefore, we performed a complementation experiment in *C. glutamicum* ΔmtlD heterologously expressing the *B. methanolicus* atlD gene alone. Indeed, *atlD* could complement the deficiency of the ΔmtlD strain as efficiently as when the four *atlABCD* genes were heterologously expressed (Figure 4B and Supplementary Table 4). As expected, growth of *C. glutamicum* wild type (WT) with pVWEx1 empty vector on arabitol (Figure 4) and growth of *C. glutamicum* ΔmtlD on glucose was unaffected (Supplementary Figure 2). Thus, the *atlD* gene was shown to be sufficient to restore growth on arabitol of the arabitol-negative *C. glutamicum* ΔmtlD strain and provided evidence that *atlD* functions in arabitol utilization in *B. methanolicus* MGA3. Although the functionality of the *atlABC* genes could not be shown, their genetic organization and proximity to *atlD* in addition to the results obtained from the differential gene expression analysis strongly support their involvement in arabitol uptake, hence the genes were re-annotated to *atlABCD* (Table 3).

In an additional experiment, the *C. glutamicum* ΔmtlD mutant strain was complemented with the *atlABCD* and the two consecutive BMMGA3_RS07350 and BMMGA3_RS07355 genes. BMMGA3_RS07350 codes for a hypothetical protein and BMMGA3_RS07355 is annotated as coding for a galactitol-1-phosphate 5-dehydrogenase. However, growth and uptake rates weren’t significantly different than when only *atlD* or *atlABCD* from *B. methanolicus* MGA3 were used for complementation (Supplementary Table 4).

**Transcriptional Organization of the Arabitol Operon**

The *atlABCD* genes are clustered on the *B. methanolicus* genome and are arranged in the same transcriptional orientation as several neighboring genes (Figure 5A). Previous transcription analyses indicated that genes BMMGA3_RS07325 to BMMGA3_RS07355 might be organized in an operon (Irla et al., 2015). In addition to the previously described genes showing higher RNA levels during growth on arabitol in comparison to mannitol (Table 3), our RNA-seq data suggested that BMMGA3_RS07365 might also be co-transcribed with the preceding genes based on reads spanning two genes. For independent confirmation of that assumption, RT-PCR experiments were performed. RNA obtained during growth on arabitol was reverse transcribed with primer RT03 annealing downstream of BMMGA3_RS07365 and used as template for PCRs with primer pairs spanning the gene borders (Figure 5B). The observed amplification products indicated that indeed genes
prepared and arabitol phosphate dehydrogenase activities were increased during growth with arabitol, crude extracts of MGA3 cells grown on arabitol, mannitol or methanol were assayed. Instead, reduction of xylulose 5-phosphate was assayed and arabitol phosphate dehydrogenase activity was shown in B. methanolicus crude extracts. As expected, the highest enzyme activity (0.05 ± 0.01 U mg⁻¹) was detected in extracts of cells grown on arabitol (Table 4). Surprisingly, mannitol grown cells showed, albeit reduced, arabitol phosphate dehydrogenase activity (0.02 ± 0.00 U mg⁻¹), while methanol grown cells lacked detectable arabitol phosphate dehydrogenase activity (Table 4). Using the crude extracts prepared from arabitol grown cells, the $K_M$ value for the substrate xylulose 5-phosphate was determined to be 0.03 ± 0.02 mM. Although this is a rough estimate obtained with crude extracts rather than with the purified enzyme, the sub-millimolar $K_M$ value indicates high affinity of the arabitol phosphate dehydrogenase from B. methanolicus for the substrate xylulose 5-phosphate and is in line with the arabitol concentration supporting half-maximal growth ($K_S$ of 1.2 ± 0.3 mM).

**DISCUSSION**

Growth of B. methanolicus MGA3 on the sugar alcohol D-arabitol is characterized for the first time. Based on an RNA-seq analysis of global gene expression during growth on this sugar alcohol, genetic and biochemical investigation of a role of atlABCD encoding a PTS and arabitol phosphate dehydrogenase in uptake and activation of arabitol is demonstrated. Figure 6 depicts the proposed pathway for arabitol uptake and catabolism next to the known pathways for methanol, mannitol and glucose utilization operating in B. methanolicus MGA3.

The finding that B. methanolicus MGA3 is able to catabolize low arabitol concentrations effectively ($K_S$ value of about 3 mM) is in line with our prediction that arabitol is taken up by the cells via arabitol PTS encoded by arabitol inducible genes atlABCD. High substrate affinity has been reported in several organisms in relation to PTS-mediated uptake (Nothaft et al., 2003; Lindner et al., 2011; Opačić et al., 2012), as is also the case for mannitol. Thus, genes atlA, atlB, atlC, and atlD are part of a larger operon that ranges from BMMGA3_RS07325 to BMMGA3_RS07365.

### Arabitol Phosphate Dehydrogenase Activity of B. methanolicus MGA3 Crude Extracts

In order to confirm whether B. methanolicus MGA3 possesses an arabitol phosphate dehydrogenase and to assay its activity is increased during growth with arabitol, crude extracts of MGA3 cells grown on arabitol, mannitol or methanol were prepared and arabitol phosphate dehydrogenase activities were determined. The enzyme assays were carried as described by Povelainen et al. (2003) with xylulose 5-phosphate as substrate. Since arabitol 1-phosphate and arabitol 5-phosphate were not available, arabitol phosphate oxidation could not be assayed. Instead, reduction of xylulose 5-phosphate was assayed and arabitol phosphate dehydrogenase activity was shown in B. methanolicus crude extracts. As expected, the highest enzyme activity (0.05 ± 0.01 U mg⁻¹) was detected in extracts of cells grown on arabitol (Table 4). Surprisingly, mannitol grown cells showed, albeit reduced, arabitol phosphate dehydrogenase activity (0.02 ± 0.00 U mg⁻¹), while methanol grown cells lacked detectable arabitol phosphate dehydrogenase activity (Table 4). Using the crude extracts prepared from arabitol grown cells, the $K_M$ value for the substrate xylulose 5-phosphate was determined to be 0.03 ± 0.02 mM. Although this is a rough estimate obtained with crude extracts rather than with the purified enzyme, the sub-millimolar $K_M$ value indicates high affinity of the arabitol phosphate dehydrogenase from B. methanolicus for the substrate xylulose 5-phosphate and is in line with the arabitol concentration supporting half-maximal growth ($K_S$ of 1.2 ± 0.3 mM).
its ecological niche, since *B. methanolicus* cannot utilize arabitol efficiently at concentrations exceeding 30 mM, as biomass yields with 60 mM arabitol, for example, were only moderately higher than with 30 mM and residual arabitol was observed when growth stopped (Supplementary Figure 1).

Co-consumption of arabitol with mannitol and monophasic growth were observed with an equimolar mixture of both sugar alcohols as combined carbon source (Table 2). Monophasic growth with simultaneous substrate consumption has been previously reported for e.g., *C. glutamicum* in a mixture of glucose and pyruvate (Cocaing et al., 1993), glucose and fructose (Dominguez et al., 1997) or glucose and acetate (Wendisch et al., 2000), *Mycobacterium tuberculosis* in a mixture of glucose, acetate, and/or glycerol (de Carvalho et al., 2010) and *Bacillus subtilis* in a mixture of glucose and malate (Kleijn et al., 2010), although consumption of single carbon substrates in a preferred order displaying diauxic growth, consequence of catabolite repression, is a more widespread mechanism in most bacteria (Kovárová-Kovar and Egli, 1998). The fact that both arabitol and mannitol uptake rates were more than three-fold lower during growth with the mixture than with either sugar alcohol alone (Table 2) indicated regulation of carbon source utilization. This assumption is confirmed by our qRT-PCR analysis (Figure 3B) where *mtID* transcript levels are lower in the cells cultivated on mixture of arabitol and mannitol in comparison to mannitol only. Inhibition of glucose uptake during growth on glucose in the presence of arabitol was previously reported by Laslo et al. (2012) both for the wild type and the *C. glutamicum* Δ*mtID* mutant strain. Co-consumption of glucose and xylose

### Table 4: Specific activities of arabitol phosphate dehydrogenase in cell extracts of *B. methanolicus* MGA3 grown in minimal medium containing different carbon sources.

| Carbon source | Mean specific activity (U mg⁻¹) |
|---------------|--------------------------------|
| Arabitol      | 0.05 ± 0.01                    |
| Mannitol      | 0.02 ± 0.00                    |
| Methanol      | <0.01                          |
led, too, to lower consumption rates for glucose in the *E. coli* ΔptsG mutant (Matsuoka and Shimizu, 2013), while glucose was shown to inhibit pentose uptake in *Saccharomyces cerevisiae* (Subtil and Boles, 2012).

Differential gene expression analysis of *B. methanolicus* MGA3 grown on arabitol as compared to mannitol detected higher RNA levels for *mtlA, mtlR, mtlF*, and *mtlD* in mannitol-grown cells (Table 3) as expected (Heggeset et al., 2012; Müller et al., 2014; Irla et al., 2016). The higher RNA levels observed for the putative arabitol PTS genes *atlA, atlB*, and *atlC* as well as arabitol phosphate dehydrogenase gene *atlD* during growth on arabitol compared to growth with mannitol might be due to arabitol induction or lack of mannitol repression. In *C. glutamicum*, arabitol induces expression of *rbiT, mtlD, sixA, xyIB* and *atlR* and glucose represses expression of *mtlD* (Laslo et al., 2012). In *B. methanolicus*, RNA levels for *atlD* were higher while lower for *mtlD* during growth with both carbon sources as compared to growth with mannitol alone (Figure 3B). By contrast, the RNA levels of both *mtlR* and *atlC* were comparable under both conditions. This may indicate arabitol repression of *mtlR* and mannitol repression of *atlC* in the arabitol/mannitol mixture condition. Moreover, this observation is in line with the finding that both carbon sources are utilized faster when present alone as compared to growth with a mixture of mannitol and arabitol (Table 2). Contrarily, in *Pseudomonas fluorescens*

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**FIGURE 6** | Ribulose monophosphate cycle and proposed methylotrophic and carbohydrate assimilation pathways in *B. methanolicus* MGA3. Genes: *mdh*, methanol dehydrogenase (EC 1.1.1.244); *hps*, 3-hexulose-6-phosphate synthase (EC 4.1.2.43); *phi*, 6-phospho-3-hexuloseisomerase (EC 5.3.1.27); *pfk*, 6-phosphofructokinase, (EC 4.1.2.13); *tkt*, transketolase (EC 2.2.1.1); *glpX*, fructose-bisphosphatase (EC 3.1.3.1); *tal*, transaldolase (EC 2.2.1.2); *rpe*, ribulosephosphate 3-epimerase (EC 5.1.3.1); *rpi*, ribose-5-phosphate isomerase (EC 5.3.1.8); *tpiA*, triosephosphate isomerase (EC 5.3.1.1); *ptsG*, PTS-glucose-specific transporter subunit ICBA (EC 2.7.1.69); *pgi*, glucose-6-phosphate isomerase (EC 5.3.1.9); *mtlF*, mannitol-specific phosphotransferase enzyme IIA component (EC 2.7.1.69); *mtlA*, PTS system mannitol-specific enzyme IIBC component (EC 2.7.1.69); *mtlD*, mannitol-1-phosphate 5-dehydrogenase (EC 1.1.1.17); *atlA*, IIA arabitol PTS component; *atlB*, IIB arabitol PTS component; *atlC*, IIC arabitol PTS component; *atlD*, arabitol phosphate dehydrogenase. Metabolites: *H6-P*, 3-hexulose 6-phosphate; *F6-P*, fructose 6-phosphate; *FBP*, fructose-bisphosphate; *F6P*, fructose-6-phosphate; *TCA*, tricarboxylic acid. Superscript “C”: chromosomally encoded; superscript “P”: natural plasmid pBM19 encoded. Phosphoenolpyruvate–protein phosphotransferase [enzyme E] (EC 2.7.3.9) and phosphocarrier protein (HPr) are not depicted.
DSM 50106 mannitol, arabitol and glucitol are inducers for transcription of mtl operon coding for proteins involved in transport and utilization of those sugar alcohols and the gene expression is regulated by the transcriptional regulator MtlR (Hoffmann and Altenbuchner, 2015).

Here, the physiological role of the altABC genes in the utilization of arabitol by B. methanolicus was analyzed via complementation studies. Growth with arabitol of the arabitol growth-deficient C. glutamicum ΔmtlID strain was restored by heterologous expression of altABCD as well as atlID from B. methanolicus. However, involvement of altABC in arabitol uptake in B. methanolicus could not be confirmed via complementation experiments: the phenotypes of C. glutamicum ΔmtlID(pVWEx1-altABCD) and C. glutamicum ΔmtlID(pVWEx1-atlID) did not show significant differences (Figure 4 and Supplementary Table 4). The fact that the altABC genes were not required for arabitol uptake in the ΔmtlID mutant was additionally supported by the displayed substrate affinity: the arabitol affinity of the PTS in B. methanolicus was estimated at the level of 2.9 ± 0.9 mM, whereas the arabitol K_S for C. glutamicum ΔmtlID(pVWEx1-altABCD) was almost ten-fold higher, namely 9.4 ± 0.3 mM (Supplementary Figure 3A). This result is similar to arabitol K_S in C. glutamicum WT(pVWEx1) of 8.3 ± 2.0 mM (Supplementary Figure 3B) or wild type C. glutamicum 7.91 ± 0.52 mM (Laslo et al., 2012). The difference of K_S of B. methanolicus-derived PTS system in the genetic background of B. methanolicus and C. glutamicum ΔmtlID might be due to the presence of the native arabitol permease rbtT in the latter. The observation that only atlID was necessary to complement the C. glutamicum ΔmtlID deficiency implies that either AtlD has arabitol dehydrogenase activity besides the here determined arabitol phosphate dehydrogenase activity (Table 4) or that C. glutamicum can import arabitol via an additional uptake route, which would be supported by the fact that C. glutamicum ArbtT, although poorly, can still grow on arabitol (Laslo et al., 2012). Despite the fact that the altABC genes were not found necessary to complement C. glutamicum ΔmtlID, their participation in arabitol uptake in B. methanolicus cannot be excluded. As seen from the differential gene expression analysis, atlABCD were clearly up-regulated under arabitol conditions (Table 3). Moreover, transcriptional organization experiments revealed that said genes are part of the same operon and the genetic organization is in accordance to previously reported arabitol PTS and dehydrogenase genes (Povelainen et al., 2003; Kentache et al., 2016). BLASTp analyses recognized AtlABC as part of the multienzyme PTS complex involved in the transport and phosphorylation of carbohydrates. The PTS phosphorylation cascade involves the general PTS components phosphoenolpyruvate–protein phosphotransferase enzyme I (EI) and phosphocarrier protein (HPr), and the carbohydrate-specific permease enzyme II, consisting of two cytoplasmic domains (IIA and IIB) and a transmembrane channel domain (IIC, with or without IID depending on the system) (Saier, 2015; Kentache et al., 2016). Homology comparisons identified AtIA as PTS sugar transporter subunit IIA inside the family of fructose/mannitol specific IIA subunits (cd00211), AtIB as subunit IIB of enzyme II of the galactitol-specific PTS (cd05566) and AtlC as PTS galactitol-specific IIC component (COG3775). EI autophosphorylates using phosphoenolpyruvate (PEP) as phosphoryl donor, which in turn transfers the phosphoryl group to HPr (Saier, 2015; Kentache et al., 2016). Following this, we propose that HPr phosphorylates arabitol-specific EIIA AtlA, which subsequently transfers the phosphoryl group to EIIB component AtlB and, in the last step, donates its phosphoryl group to arabitol bound to EIIC transmembrane domain AtlC, releasing arabitol-phosphate into the cytoplasm. Sequence comparison of characterized transmembrane permease IIC for the arabitol AtlC from L. monocytogenes (Saklani-Jusforgues et al., 2001) with B. methanolicus AtlC showed identity at the level of 56%.

Furthermore, the comparison of the sequence of the putative arabitol phosphate dehydrogenase encoded by atlD gene with an amino acid sequence of characterized arabitol 1-phosphate dehydrogenase from E. avium (Povelainen et al., 2003) showed 51% identity between these proteins. Arabitol phosphate dehydrogenase (AtlD) activities were tested in crude extracts of MGA3 cells grown on arabitol, mannitol or methanol (Table 4). The results confirm that B. methanolicus MGA3 indeed possesses an arabitol phosphate dehydrogenase with increased activity during growth with arabitol, which is in accordance with the differential gene expression and the qRT-PCR analyses comparing arabitol- to mannitol-based growth (Table 3 and Figure 3). Additionally, the K_M value for xylulose 5-phosphate was determined to be 0.03 ± 0.02 mM using crude extracts from cells grown on arabitol, which shows high substrate affinity (Supplementary Figure 4). Despite the fact that the reaction was not assayed in the physiological direction (i.e., oxidation of arabitol 1-phosphate or arabitol 5-phosphate to xylulose 5-phosphate), the enzyme kinetics are in accordance with the K_M value of 2.9 ± 0.9 mM determined for arabitol. Although AtlD affinity for the substrate xylulose 5-phosphate was not determined using the purified enzyme, our results are in the range of previously characterized arabitol phosphate dehydrogenase in E. avium (Povelainen et al., 2003). Taken all together, these results strongly indicate the functionality of the atlABCD encoded proteins in the arabitol utilization in B. methanolicus MGA3.

Addition of arabitol to cultivation broth not only induced expression of atlABCD, but also of adjacent genes, i.e., eight genes from BMMGA3_RS07325 to BMMGA3_RS07360 and all part of the arabitol operon as established via RT-PCR analysis (Figure 5). BMMGA3_RS07325 codes for a putative transcriptional antiterminator BglG. The bgl-sac family of antiterminator proteins are effectors of substrate-induced antitermination of catabolic operons and include, e.g., SaCt and SaCy of B. subtilis (Debarbouille et al., 1990; Manival, 1997) and BglG of E. coli (Nussbaum-Shochat and Amster-Choder, 1999; Rapeh et al., 2009). These antiterminator proteins have been extensively characterized and act as RNA-binding proteins abrogating termination of transcription and allowing transcription elongation (Rutberg, 1997; Van Assche et al., 2015). A BLASTp analysis showed that the protein encoded by BMMGA3_RS07325 shared 30% identity to BglG of E. coli, 35% identity to SaCy and 37% identity to SaCy of B. subtilis, and 40% identity to BglG of L. monocytogenes (Gorski et al., 2003).
Additionally, both \textit{E. coli bglG} and \textit{B. subtilis sacT} are located upstream of genes coding for PTS components involved in the utilization of β-glucosides and sucrose, respectively (Rutberg, 1997). Similar gene order, where gene coding for antiterminator is upstream of genes encoding arabitol PTS, was observed in an arabitol gene cluster in \textit{L. monocytogenes} and \textit{E. avium} (Gorski et al., 2003; Povelainen et al., 2003). Interestingly, the other \textit{B. methanolicus} wild type strain PB1 (NCIMB13113) is unable to grow on arabitol (data not shown). Both strains have been previously reported to show physiological differences (Heggset et al., 2012). A BLAST analysis comparing the arabitol operon sequences of MGA3 and PB1 showed an incomplete BMMGA3_RS07325 gene sequence in the genome of the PB1 strain. These findings may indicate that BMMGA3_RS07325 functions as regulator of arabitol catabolism in \textit{B. methanolicus} MGA3, and that its truncated form in \textit{B. methanolicus} PB1 leads to absence of growth on arabitol.

RT-PCR and RNA-seq analysis revealed that genes BMMGA3_RS07325 to BMMGA3_RS07365 are co-transcribed as an operon (Figure 5). Accordingly, conserved promoter motifs were present upstream of BMMGA3_RS07325 (Irla et al., 2015). Using the ARNold tool for identification of transcriptional terminators (Naville et al., 2011), additional promoter motifs were found between BMMGA3_RS07360 and BMMGA3_RS07365 overlapping with a putative terminator structure (data not shown). These findings suggest the presence of a sub-operon starting at BMMGA3_RS07365 that would most likely not be involved in arabitol metabolism since it is not induced by arabitol. It is therefore remarkable that BMMGA3_RS07365 is co-transcribed alongside the arabitol inducible genes.

\textit{Bacillus methanolicus} MGA3 is a facultative methyloptroph with a narrow substrate spectrum. Here, we have identified and characterized growth with D-arabitol. RNA-seq analysis revealed evidence for arabitol inducible catabolism of this sugar alcohol via a PTS AtlABC and an arabitol phosphate dehydrogenase AtlD, and genetic complementation studies confirmed functionality of the latter during arabitol metabolism. The role of a second putative arabitol phosphate dehydrogenase co-transcribed with \textit{atlABCD} and metabolic fluxes during growth with arabitol remain to be studied. Once established for \textit{B. methanolicus}, gene deletion experiments combined with biochemical characterization of the enzymes and $^{13}$C labeling experiments will help to further our understanding on how this sugar alcohol is catabolized as sole or combined carbon source.

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**DATA AVAILABILITY**

All datasets generated for this study are included in the manuscript and/or the Supplementary Files. The data sets supporting the results of this article are available in the NCBI Gene Expression Omnibus database under the accession number GSE133849, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133849.

**AUTHOR CONTRIBUTIONS**

ML carried out the experimental procedures. ML, MI, and LB analyzed the data. ML prepared a draft of the manuscript. ML, MI, LB, and VW finalized the manuscript. VW coordinated the study. All authors read and approved the final version of the manuscript.

**FUNDING**

This work was supported by the ERASysAPP project MetAPP (No. 031A603) and the ERA CoBioTech project CiPro (No. 722361). Support for the Article Processing Charge by the Deutsche Forschungsgemeinschaft and the Open Access Publication Fund of Bielefeld University is acknowledged. The funding bodies had no role in the design of the study or collection, analysis, or interpretation of data or in writing of the manuscript.

**ACKNOWLEDGMENTS**

We thank Anika Winkler, Dr. Tobias Busche, and Prof. Dr. Jörn Kalinowski from the technology platform Genomics of CeBiTec for the kind assistance with the preparation and sequencing of the cDNA libraries and for the bioinformatics advice. We additionally thank Lara Paparić, Maximilian Schöne, and Lucas Riedel for the technical assistance.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01725/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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