Combining of transcriptome and metabolome analyses for understanding the utilization and metabolic pathways of Xylo-oligosaccharide in *Bifidobacterium adolescentis* ATCC 15703

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**Abstract**
A combination of transcriptome and metabolome analyses was applied to understand the utilization and metabolism of Xylo-oligosaccharide (XOS) in *Bifidobacterium adolescentis* ATCC 15703 as well as identifying the key regulatory-related genes and metabolites. Samples of cultures grown on either XOS or xylose were collected. The transcript and metabolite profiles were obtained from high-throughput RNA-sequencing data analysis and UHPLC system. Compared with xylose, XOS highly promoted the growth of *B. adolescentis* 15703 and resulted in a growth yield about 1.5-fold greater than xylose. The transcriptome analysis showed that XOS could enhance genes, including ABC transporters, galactosidase, xylosidase, glucosidase, and amylase, which were involved in transport and metabolism of carbohydrate compared with xylose. Furthermore, the expression profile of 16 candidate genes using qRT-PCR has validated the accuracy of the RNA-seq data. Also, the metabolomic analyses, particularly those related to metabolic biomarkers of fatty acids, amino acids, and sugars showed a similar trend of result and approved the advantages of XOS as growth medium for *B. adolescentis* 15703 compared with xylose. The expression and abundance of specific genes and metabolites highlighted the complex regulatory mechanisms involved in utilization of XOS by *B. adolescentis* 15703. These results are useful in the understanding of the metabolic pathway of XOS in *B. adolescentis* 15703 and contribute to the optimization of XOS probiotic effects as a food additive.

**KEYWORDS**
*Bifidobacterium adolescentis*, metabolome, transcriptome, Xylo-oligosaccharide

1 | INTRODUCTION

*Bifidobacterium* is a genus of gram-positive bacteria that colonize in the human gastrointestinal tract and provide health benefits. Several studies verified a wide range of positive effects for *Bifidobacterium*, including the protection against pathogenic bacteria, alleviation of allergic disease symptoms (Casaro et al., 2018), immune regulation, reduction in intestinal inflammations, and the potential of bifidobacteria to prevent and/or treat colorectal cancer (Le Leu, Hu, Brown, Woodman, & Young, 2010). Due to claimed health benefits, bifidobacteria has been incorporated into many functional foods (O’Callaghan & van Sinderen, 2016). Therefore, more health benefits are expected if the amount of bifidobacteria could be increased in the body.
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Xylo-oligosaccharides (XOSs) are hydrolysates of xylan and consist of a backbone of xylose, which are noncaloric and indigestible by humans. XOSs are believed to exert bifidogenic effects and are increasingly used as prebiotics. XOS may be beneficial in stimulating the intestinal Bifidobacterium without significant effect on lactobacillus (Li, Summanen, Komoriya, & Finegold, 2015; Falck et al., 2013). Also, it was found that XOS increases bifidobacteria, but not lactobacilli in human gut microbiota (Finegold et al., 2014). Due to potential bifidobacteria proliferation effects, XOSs have attracted increasing interest.

Carbohydrate metabolism may vary among bifidobacterial strains considerably (Pokusaeva, Fitzgerald, & Sinderen, 2011). Bifidobacterium adolescentis has the ability to utilize XOS efficiently (Amaretti et al., 2013). Bifidobactera lack a number of key enzymes involved in the Embden–Meyerhof–Parnas (EMP) pathway; therefore, bifidobacteria metabolize carbohydrates through a metabolic pathway named the "bifid shunt," which is centered on the key enzyme fructose-6-phosphoketolase (De Vries & Stouthamer, 1967). In a previous study, we have found that the growth rate of B. adolescentis was higher in the presence of XOS than xylose (unpublished). However, the underlying molecular regulation mechanisms of XOS metabolism are not fully understood. In XOS utilization process, xylose is not neatly consumed and remaining unfermented (Amaretti et al., 2013). Although it has been established that XOSs confer positive benefits to bifidobacteria, there is a lack of knowledge regarding the molecular mechanisms that explain the metabolic pathway of XOS in B. adolescentis. Meanwhile, a recent study performed on the genome sequences from 47 Bifidobacterium (sub) species found that 5.5% of the core bifidobacterial genomic coding sequences are associated with carbohydrate metabolism (Pokusaeva et al., 2011). Therefore, an in-depth study on these functional genes has significance for understanding mechanisms of probiotic effects of Bifidobacterium. In this work, a combination of transcriptome and metabolome analyses was applied to elucidate the molecular mechanism for utilizing and metabolism of xylose and XOS in B. adolescentis 15703. Understanding of basic mechanisms may help in finding of novel ways to optimize the use of prebiotics and probiotics in the food industry.

2 | MATERIALS AND METHODS

2.1 | Materials

Bifidobacterium adolescentis ATCC 15703 was purchased from China General Microbiological Culture Collection Center. XOS extracted from corncob, 95% purity, DP of 2-7 and containing 22.76% xylobiose, 31.45% xylotriose, 20.37% xylotetraose, 10.89% xylopentaose, 4.68% xylolhexaose, and 6.37% wood seven sugar was obtained from LongLive Biotechnology. All other chemicals were of analytical grade.

2.2 | Bacterial cultivation and carbohydrates fermentation

Bifidobacterium adolescentis 15703 was resuscitated and precultivated twice using MRS broth. Cells were harvested and suspended as 2% inoculate into MRS medium containing xylose or XOS as well as a control medium without carbohydrate and incubated at 37°C under anaerobic conditions. Aliquots of cultures were drawn at regular intervals and cell growth was determined by measuring the optical density at 600 nm (Lei et al., 2018).

2.3 | RNA extraction

Cells were harvested from triplicate cultures at the estimated early midexponential growth phase by centrifugation at 4,000 g for 10 min at 4°C for RNA isolation and purification. The samples were used for RNA extraction following the manufacturer’s recommendations of QIAGEN 74524 kit. RNA concentration was determined with a Qubit RNA Assay Kit in a Qubit 2.0 fluorometer (Life Technologies). RNA purity and integrity were assessed by a Nanodrop spectrophotometer (IMPLEN).

2.4 | Library construction and sequencing

After total RNA extraction, prokaryotic mRNA was enriched by removing rRNA using Ribo-Zero™ Magnetic Kit (Epicentre). Then the short fragments were obtained from the enriched mRNA by fragmentation buffer and were reverse transcribed into cDNA. Under the action of DNA polymerase I, RNase H and dNTP, second-stand cDNA was synthesized. Then, the cDNA fragments were purified, end repaired, poly (A) added, and ligated to Illumina sequencing adapters (Bellieny-Rabelo et al., 2019). The ligaton products size were chosen, amplified, and sequenced using illumina HiSeq™ 2500.

2.5 | Transcriptomic analysis

Raw reads were filtered to remove some adapters and low-quality reads, and the remaining reads were mapped to a reference genome by TopHat2 (Kim et al., 2013). The reconstruction of transcripts was carried out with software Cufflinks (Trapnell et al., 2012), then the transcripts were merged from multiple groups into a finally comprehensive set of transcripts for further downstream differential expression analysis. Gene abundances were quantified by software RSEM (Li & Dewey, 2011). The gene expression level was normalized with FPKM method, and the edgeR package was used to identify DEGs across groups. In comparison as significant DEGs, FDR <0.01 and fold change (FC) ≥2 were used as screening criteria. We conducted gene expression differences between xylose and XOS treatments using the DEseq package. DEGs were then subjected to enrichment analysis of COG functions and KEGG pathways.

2.6 | Confirmation of transcriptomic results by quantitative real-time PCR

Total RNA was isolated as described above. Using a Revert Aid Premium Reverse Transcriptase, the cDNA synthesis was performed. qRT-PCR primers are listed in Table 1 and each reaction (20 μl mixture) contained 2 μl cDNA, 10 μl 2 × sybrGreen qPCR Master Mix, 0.4 μl the forward and
reverse primers and 7.6 μl water. All qRT-PCR were performed in ABI Stepone plus and performed in two steps: Firstly, predenaturation for 3 min and 45 cycles of denaturation for 3 s at 95°C, then annealing/extension for 30 s at 60°C. Gene expression was normalized by the 2^{ΔΔCt} method, and the 16S rRNA gene was used as the normalized standard.

### 2.7 | Metabolites extraction

The sample of 100 μl was accurately removed and placed in EP tube, and 300 μl methanol was added to start extraction, add 20 μl internal standard substances and followed by vortex for 30 s. Then, the mixture tube was immersed into the ultrasonic bath with ice water and ultrasonically incubated in ice water for 10.0 min and incubated for 1 hr at −20°C to precipitate proteins. Then, the mixture was centrifuged at 11,390 g for 15 min at 4°C. About 200 μl of supernatant sample was transferred to a fresh 2 ml LC/MS glass vial, 20 μl from supernatant of each sample was marked as QC samples, and another supernatant was used for the UHPLC-QTOF-MS analysis. All experiments were carried out in triplicate.

### 2.8 | LC-MS/MS analysis

The UHPLC system (1290, Agilent Technologies) with a UPLC BEH Amide column (1.7 μm 2.1 × 100 mm, Waters) coupled with Triple
TOF 5600 (Q-TOF, AB Sciex) was used for LC-MS/MS analyses. 25 mM NH₂OAc and 25 mM NH₄OH in water (pH = 9.75) (A) and acetonitrile (B) were used as the mobile phase. The elution gradient was as follows: 0 min, 95% B; 7 min, 65% B; 9 min, 40% B; 9.1 min, 95% B; and 12 min, 95% B. The flow rate of the mobile phase was 0.5 ml/min. The injection volume of analytical solution was 3 μl. The Triple-TOF-MS was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria were surveyed by the acquisition software (Analyst TF 1.7, AB Sciex; Fraga, Clowers, Moore, & Zink, 2010). In each cycle, 12 precursor ions with intensity greater than 100 were chosen for fragmentation. ESI source conditions were set as following: ion source gas 1 as 60 Psi, ion source gas 2 as 60 Psi, accumulation time of 50 msec each. ESI source conditions were set as collision energy (CE) of 30 V (15 MS/MS events with product ion ions with intensity greater than 100 were chosen for fragmentation). Fraga, Clowers, Moore, & Zink, 2010). In each cycle, 12 precursor ions were surveyed by the acquisition software (Analyst TF 1.7, AB Sciex; Fraga, Clowers, Moore, & Zink, 2010). In each cycle, 12 precursor ions with intensity greater than 100 were chosen for fragmentation. ESI source conditions were set as following: ion source gas 1 as 60 Psi, ion source gas 2 as 60 Psi, Curtain gas as 35 Psi, source temperature 650°C, ion spray voltage floating (ISVF) 5,000 or −4,000 V in positive or negative modes, respectively.

**3.2 | RNA-seq analysis and differential gene expression**

From the RNA-seq analysis data, it can be seen that over 99% of the reads were aligned to encoding regions of the *B. adolescentis* Genes were assigned to 25 functional groups, which were annotated in COG database (Figure 2). Among these classifications, the largest group was amino acid transport and metabolism (191, 13.45%), followed by carbohydrate transport and metabolism (160, 11.27%) and general function prediction (151, 10.63%).

A total number of 302 DEGs were identified for *B. adolescentis* grown on xylose and XOS, including 158 upregulated genes and 144 downregulated genes (Figure 3). The top 10 upregulated genes and 10 downregulated genes of xylose and XOS treatments are presented in Table 2. Four genes of the top 10 upregulated genes encode ABC and MFS transporters. Among the remaining genes, two genes encode hsp20/alpha crystallin family protein and ATP-dependent chaperone CipB, two genes encode RNA polymerase sigma factor and death-on-curing protein, other two genes encode enzyme proteins belonging to multiple sugar-binding transport system permease and shikimate kinase. Five genes of the top 10 downregulated genes encode structure protein, including penicillin-binding protein, von willebrand factor type A domain protein, fhaA protein, arginine repressor DUF4956, domain-containing protein, three genes are associated with membrane transport, including peptide ABC transporter ATP-binding protein, ABC transporter permease, and membrane spanning polysaccharide biosynthesis protein, while two genes encode O-antigen polymerase and hypothetical protein.

**3.3 | KEGG pathway mapping of DEGs**

The DEGs involved in biological functions were further analyzed by KEGG pathways, and 20 pathways were predicted (Figure 4). ABC transporters, galactose metabolism, peptidoglycan biosynthesis pyrimidine metabolism, starch, and sucrose metabolism are the highly represented categories.

The DEGs involved in the ABC transporters are shown in Table 3. In the ABC transporter pathway (ko02010), 27 genes were significantly upregulated. Genes 07405, 07410, 02260, 08210, 08205, 00815, 00810, 08280, 03705, 08275, 06685, 08210 encoded ABC transporter permease, Genes 07415, 02255, 01495, 00390, 00805, 08285, 00990, 06680 encoded ABC transporter substrate-binding protein, Gene 02265, 04090, 00495, 03325, 08375 encoded ABC transporter ATP-binding protein, 07050 and 00340 encoded ABC transporter, while nine genes (02355, 02470 and 03935), which are ABC transporter-related genes, significantly downregulated after XOS treatment.
The DEGs involved in carbohydrate metabolism are shown in Table 4. Compared with xylose treatment, five genes (08325, 07400, 07395, 06400, 08455) encoded beta-galactosidase and two genes (08195, 08270) encoded alpha-amylase related to galactose metabolism pathway (ko00052) significantly upregulated after XOS treatment. Also, three genes (02270, 08480, 02400) expressed key enzymes (beta-xylosidase, beta-glucosidase) involved in starch and sucrose metabolism (ko00500) significantly upregulated after XOS treatment. Gene 05480 coded mannan endo-1,4-beta-mannosidase involved in fructose and mannose metabolism (ko00051). 01050 coded shikimate kinase, 01040 coded 6-phosphogluconate dehydrogenase, 02150 coded lactaldehyde reductase, 07445 coded L-ribulose-5-phosphate 4-epimerase, 01580 coded UDP-N-acetylenolpyruvoylglucosamine reductase, which involved in biosynthesis of antibiotics (ko01130), microbial metabolism in diverse environments (ko01120) carbon metabolism (ko01200), pentose phosphate pathway (ko00030), glyoxylate and dicarboxylate metabolism (ko00630), propanoate metabolism (ko00640), and pentose and glucuronate interconversions (ko00040).

### 3.4 Validation of transcript abundance using qRT-PCR

To verify the RNA-Seq results, the mRNA expression of 16 selected candidate genes (eight upregulated and eight downregulated) was measured by qRT-PCR. The expression levels of 16 DEGs with qRT-PCR were compared with those of DEGs with RNA-Seq by the linear fitting. A significant correlation ($R^2 = 0.96345$) was found between the RNA-Seq and qRT-PCR (Figure 5). The qRT-PCR results are consistent with their transcript abundance in RNA-seq, which verified the accuracy of the DEGs from RNA-seq analyses.
3.5 | Metabolite profile

The metabolites profiling of B. adolescentis 15703 was performed using LC-MS. The primary metabolites are amino acids, organic acids, fatty acid, polyhydroxy acids, sugars, phosphates, polyols, and N-compounds. A total number of 157 different metabolites (MS2) were identified (p < .05, log2 FC >1) for xylose and XOS treatments, including 79 upregulated metabolites and 78 downregulated metabolites. The top 10 upregulated and 10 downregulated metabolites for xylose and XOS treatments are presented in Table 5. Six metabolites of the top 10 upregulated metabolites are acids, including linolenic acid, epoxy stearic acid, myristic acid, uric acid, palmitoleic acid, and oleic acid. The remaining metabolites are D-sorbitol 6-phosphate, 3-prenyl-4-Hydroxyacetophenone, L-threonine, and L-phenylalaninol-L-proline. Three metabolites of the top 10 downregulated metabolites are 3-Hydroxymandelic acid, 3-Dehydroshikimic acid, vanillylmandelic acid, and other remaining metabolites are flu-tamide, dihydroxyfumarate, hydroxyhydroquinone, quinone, 3′-O-Methylinosine, N-acetyl-L-alanine, and norepinephrine.

3.6 | KEGG pathway mapping of metabolites

A total number of 50 enriched KEGG pathways were predicted, which were associated with different metabolites (Figure 6). The 50 pathways were classified as environmental information processing, genetic information processing, and metabolism. The environmental information processing included ABC transporters and phospho-transferase system. In metabolism processing, microbial metabolism in diverse environments and biosynthesis of unsaturated fatty acids are the most highly represented (Figure 6).

Different metabolites involved in carbohydrate transport and metabolism are shown in Table 6. Compared with xylose treatment,

### Table 2

| Gene no. | Log₂ (Fc) | Annotation | Linear FMPK value | XOS | Xylose |
|----------|-----------|------------|-------------------|-----|--------|
| BAD_RS07405 | 3.63↑ | Multiple sugar-binding transport system permease | 160.73 | 12.98 |
| BAD_RS05940 | 3.51↑ | MFS transporter | 6.71 | 0.59 |
| BAD_RS01050 | 3.47↑ | Shikimate kinase | 173.6 | 15.68 |
| BAD_RS07410 | 3.27↑ | ABC transporter permease | 159.79 | 16.53 |
| BAD_RS07415 | 3.23↑ | ABC transporter, solute-binding protein | 486.74 | 51.88 |
| BAD_RS08735 | 3.18↑ | RNA polymerase sigma factor | 18.24 | 2.01 |
| BAD_RS00260 | 3.01↑ | hsp20/alpha crystallin family protein | 13,180.79 | 1,634.87 |
| BAD_RS02255 | 2.85↑ | Sugar ABC transporter substrate-binding protein | 7,109.23 | 989.28 |
| BAD_RS05015 | 2.83↑ | Death-on-curing protein | 89.91 | 12.64 |
| BAD_RS07715 | 2.83↑ | ATP-dependent chaperone ClpB | 3,432.39 | 484.32 |
| BAD_RS00210 | 2.08↓ | Penicillin-binding protein | 120.14 | 505.44 |
| BAD_RS07300 | 2.14↓ | O-antigen polymerase | 28.92 | 127.77 |
| BAD_RS02985 | 2.20↓ | Hypothetical protein | 32.4 | 148.91 |
| BAD_RS02975 | 2.22↓ | von Willebrand factor type A domain protein | 44.03 | 204.58 |
| BAD_RS08925 | 2.25↓ | fhaA protein | 22.97 | 109.11 |
| BAD_RS03210 | 2.35↓ | Peptide ABC transporter ATP-binding protein | 97.09 | 496.58 |
| BAD_RS07325 | 2.41↓ | Membrane spanning polysaccharide biosynthesis protein | 22.11 | 117.21 |
| BAD_RS04925 | 2.53↓ | Arginine repressor | 43.27 | 250.28 |
| BAD_RS02140 | 2.69↓ | DUF4956 domain-containing protein | 3.07 | 19.86 |
| BAD_RS03215 | 2.99↓ | ABC transporter permease | 60.43 | 480.91 |

Gene number referenced as B. adolescentis 15703 being alphabet and a five-digit number.
Significance of fold change data is judged by having a p value of no more than .01.
Gene annotations were blasted against Swiss prot.
FPKM (fragments per kilobase of exon per million fragments mapped) values for cultures on media with Xylose or XOS.
metabolites (58, 911, 166, 1695, 82, 651, 15, 81, 348, 376), which are L-threonine, cellobiose, D-Mannose, maltotriose, L-isoleucine, D-biotin, glycerol, L-leucine, N-acetyl-D-glucosamine, D-ribose are significantly different in the ABC transporters pathway (ko02010) for XOS treatment. Also, five metabolites, including D‐sorbitol 6-phosphate, cellobiose, D-mannose, L-ascorbic acid, and N-acetyl-D-glucosamine are significantly different in the phosphotransferase system (PTS; ko02060) for XOS treatment compared with xylose treatment. Isocitrate, citrate, and pyruvate are significantly different in the Citrate cycle (TCA; ko00020). Sedoheptulose, isocitrate, tetrahydrofolate are significantly different in carbon fixation pathways in prokaryotes (ko00720). The remaining metabolites, which are galactinol, L-ribulose, D-glucose 6-phosphate, 2-keto-D-gluconic acid, and N-acetyl-D-glucosamine are significantly different in the ABC transporters pathway (PTS; ko02010) for XOS treatment compared with xylose treatment. Isocitrate, citrate, and pyruvate are significantly different in the Citrate cycle (TCA; ko00720). Sedoheptulose, isocitrate, tetrahydrofolate are significantly different in carbon fixation pathways in prokaryotes (ko00720). The remaining metabolites, which are galactinol, L-ribulose, D-glucose 6-phosphate, 2-keto-D-gluconic acid, and L-threonine involved in galactose metabolism (ko00052), starch and sucrose metabolism (ko00500), pentose phosphate pathway (ko00030), biosynthesis of amino acids (ko01230), biosynthesis of secondary metabolites (ko01110), and so on.

4 | DISCUSSION

4.1 | B. adolescentis responses to xylose and XOS

To investigate the growth performance of B. adolescentis on xylose and XOS as carbon sources, growth curves of strain were determined. Bifidobacterium adolescentis showed a strong capacity in utilizing of XOS to proliferate, which may indicate that most genes and metabolites in B. adolescentis are related to XOS transport and metabolism. XOS needs to be degraded into xylose before it can be metabolized (Broekaert et al., 2011). Therefore, degradation of XOS is complicated, resulting in a relatively longer lag phase when used as a substrate compared with xylose.

4.2 | Gene prosperities of B. adolescentis ATCC 15703

Bifidobacterium adolescentis ATCC 15703, the predominant species of Bifidobacterium, was isolated from the human GIT (Pokusaeva et al., 2011). Currently, more than 40 bifidobacterial genomes including those of B. adolescentis 15703 strain have been completely sequenced and annotated in the NCBI database (Sayers et al., 2019; Schell et al., 2002). The complete genome size of the current B. adolescentis ATCC 15703 is 2,089,645 bp, with gene number 1701, protein 1631, and G-C content of 59% (Bondue & Delcenserie, 2015).

A recent study performed on the genome sequences from 47 Bifidobacterium species found that 5.5% of the core bifidobacterial genomic coding sequences were associated with carbohydrate metabolism (Milani et al., 2015). The bifidobacterial genome encode a variety of carbohydrate-modifying enzymes, such as glycosyl hydrolases, sugar ABC transporters, and PEP-PTS system components, all of which are required for the metabolism of carbohydrates (Chen et
Majority of these genes are devoted to carbohydrate uptake, by means of ABC transporters and permeases (Table 3). According to the KEGG and COG classifications, most of genes in *B. adolescentis* 15703 are associated with carbohydrate metabolism and could imply relative importance of carbohydrate utilization.

**TABLE 3** DEGs involved in related ABC transporter during growth of *Bifidobacterium adolescentis* 15703 on XOS compared with xylose assessed by RNAseq

| Gene no.     | Log$_2$ (Fc) | Symbol | Annotation                                                      | Linear FMPK value |
|--------------|--------------|--------|----------------------------------------------------------------|-------------------|
| BAD_RS07405  | 3.02↑        | amyC   | Multiple sugar-binding transport system permease                | 105.08            |
| BAD_RS07410  | 3.27↑        | amyD   | ABC transporter permease                                        | 159.79            |
| BAD_RS07415  | 2.80↑        | mdxE   | ABC transporter, solute-binding protein                         | 360.4             |
| BAD_RS02255  | 3.00↑        | yurO   | Sugar ABC transporter substrate-binding protein                 | 7,940.17          |
| BAD_RS01495  | 2.69↑        | TP_0034| ABC transporter substrate-binding protein                       | 2,060.19          |
| BAD_RS00390  | 2.33↑        | BR1785 | Branched-chain amino acid ABC transporter substrate-binding protein | 30.52             |
| BAD_RS02265  | 2.38↑        | yurM   | Thiamine ABC transporter ATP-binding protein                    | 2,679.25          |
| BAD_RS02260  | 2.34↑        | malF   | Sugar ABC transporter permease                                  | 2,356.22          |
| BAD_RS00385  | 2.28↑        | livF   | ABC-type branched-chain amino acid transport systems ATPase component | 25.2              |
| BAD_RS08210  | 1.99↑        | amyD   | Permease of ABC transporter possibly for oligosaccharides      | 3,704.78          |
| BAD_RS08085  | 1.95↑        | yurO   | Solute-binding protein of ABC transporter system                | 2,384.03          |
| BAD_RS08205  | 1.88↑        | amyC   | Sugar ABC transporter permease                                  | 2,233.03          |
| BAD_RS00495  | 1.76↑        | MT1311 | Multidrug ABC transporter ATP-binding protein                   | 276.15            |
| BAD_RS07050  | 1.58↑        | lipO   | ABC transporter                                                 | 10,020.43         |
| BAD_RS08285  | 1.52↑        | ugpB   | ABC transporter, solute-binding protein                         | 1,971.94          |
| BAD_RS0815   | 1.48↑        | araQ   | Sugar ABC transporter permease                                  | 911.12            |
| BAD_RS08280  | 1.46↑        | msM    | Sugar ABC transporter permease                                  | 1,353.65          |
| BAD_RS00810  | 1.45↑        | yurN   | Sugar ABC transporter permease                                  | 762.04            |
| BAD_RS03705  | 1.35↑        | –      | ABC transporter permease                                        | 91.4              |
| BAD_RS00990  | 1.33↑        | –      | ABC transporter substrate-binding protein                       | 34.99             |
| BAD_RS03325  | 1.29↑        | MJ1508 | ABC transporter ATP-binding protein                             | 209.24            |
| BAD_RS08275  | 1.27↑        | amyC   | ABC transporter permease                                        | 1,000.47          |
| BAD_RS04090  | 1.14↑        | TM_0352| Macrolide ABC transporter ATP-binding protein                   | 33.93             |
| BAD_RS00340  | 1.05↑        | Pip    | ABC transporter                                                 | 228.58            |
| BAD_RS06680  | 1.27↑        | yxeM   | Amino acid ABC transporter substrate-binding protein             | 552.97            |
| BAD_RS06685  | 1.05↑        | tcyL   | ABC transporter permease                                        | 409.27            |
| BAD_RS00370  | 1.04↑        | livH   | Branched-chain amino acid ABC-type transport system permease components | 17.92            |
| BAD_RS08375  | 1.02↑        | msmX   | ABC transporter ATP-binding protein                             | 38,386.82         |
| BAD_RS03070  | 1.01↓        | artQ   | Glutamine ABC transporter permease                              | 89.94             |
| BAD_RS02355  | 1.03↓        | braC   | Solute-binding protein of ABC transporter for branched-chain amino acids | 83.57            |
| BAD_RS02470  | 1.05↓        | ftsX   | ABC transporter permease                                        | 240.08            |
| BAD_RS03935  | 1.07↓        | rbsA1  | ABC transporter ATP-binding protein                             | 10.55             |
| BAD_RS04785  | 1.22↓        |yclH    | ATP-binding protein of ABC transporter similar to Vex2          | 40.66             |
| BAD_RS05605  | 1.28↓        | –      | Sugar ABC transporter substrate-binding protein                 | 101.96            |
| BAD_RS03930  | 1.33↓        | –      | Cobalt ABC transporter permease                                 | 12.88             |
| BAD_RS03210  | 2.35↓        | lolD   | Peptide ABC transporter ATP-binding protein                     | 97.09             |
| BAD_RS03215  | 2.76↓        | macB   | ABC transporter permease                                        | 71.17             |
Comparison of transport pathways of *B. adolescentis* grown on xylose and XOS

Bifidobacteria internalize carbohydrates by ATP-dependent ABC transporters and PEP-PTS systems (Degnan & Macfarlane, 1993; Turroni et al., 2012). However, a minority of sugars utilized by bifidobacteria are believed to be internalized via a PEP-PTS (Degnan & Macfarlane, 1993; Maze, O’Connell-Motherway, Fitzgerald, Deutscher, & Sinderen, 2007). Compared with xylose treatment, PTS beta-glucoside transporter subunit EIIBCA (encoded by BAD_RS01940) and phosphoenolpyruvate-protein phosphotransferase (encoded by BAD_RS00875) were downregulated in *B. adolescentis* 15703 grown on XOS. Meanwhile, metabolites including upregulated D-Sorbitol 6-phosphate (meta_761), D-mannose (meta_166) and downregulated L-ascorbic acid (meta_312), D-Glucose 6-phosphate (meta_533), N-acetyl-D-glucosamine (meta_348), pyruvate (meta_8) were involved in PTS system (Tables 4 and 6). Related downregulated genes and metabolites are more than upregulated ones. Therefore, uptake of the most complex sugars is possibly facilitated by specific ABC transporters.

**ABC transporters couple ATP hydrolysis to efficient internalization of sugars and appear to represent the primary carbohydrate transport systems for bifidobacteria. Compared to xylose treatment, genes including the sugar transporter permease protein (encoded by BAD_RS00815, BAD_RS08280, BAD_RS00810, BAD_RS08205, BAD_RS03705, BAD_RS02260, BAD_RS07410) and transporter ATP-binding protein (encoded by BAD_RS02265, BAD_RS00495, BAD_RS04090, BAD_RS08375) were upregulated (Table 3), the same situation occurs in metabolites, including L-threonine (meta_58), cellobiose (meta_991), D-mannose (meta_166), L-isoleucine (meta_82), maltotriose (meta_1695), D-biotin (meta_651), and glycerol (meta_15) involved in ABC transporters pathway (ko02010). Thus, XOS may enhance the sugar transport process by ABC transporters system.**

| Gene no.          | Log₂ (Fc) | Symbol | Annotation                                | Linear FMPK value | KEGG pathway                  |
|-------------------|-----------|--------|-------------------------------------------|--------------------|--------------------------------|
| BAD_RS01050       | 3.47↑     | Idnk   | Shikimate kinase                          | 173.6              | ko01100                        |
| BAD_RS07400       | 2.44↑     | BGAL16 | Beta-galactosidase                        | 70.88              | ko01100/ko00052/ko00600/ko00511|
| BAD_RS01040       | 2.42↑     | gnd    | 6-phosphogluconate dehydrogenase         | 240.52             | ko01100/ko01110/ko01130/ko01120/ko1220/ko00030/ko00480|
| BAD_RS08195       | 2.20↑     | malL   | Alpha-amylase                             | 3,359.55           | ko01100/ko00500/ko00600/ko00092|
| BAD_RS02270       | 2.08↑     | xynB   | Beta-xyllosidase                          | 1,066.15           | ko01100/ko00500/ko00052         |
| BAD_RS08325       | 1.97↑     | LacZ   | Beta-galactosidase                        | 98.72              | ko01100/ko00052/ko00600/ko00511|
| BAD_RS02150       | 1.68↑     | fucO   | Lactaldehyde reductase                    | 5,736.74           | ko01120/ko00600/ko00460         |
| BAD_RS08455       | 1.38↑     | lacZ   | Beta-galactosidase                        | 329.16             | ko01100/ko00052/ko00600/ko00511|
| BAD_RS08270       | 1.34↑     | malL   | Alpha-amylase                             | 1,248.09           | ko01100/ko00500/ko00052         |
| BAD_RS07445       | 1.26↑     | ulaF   | L-ribulose-5-phosphate 4-epimerase        | 549.87             | ko01100/ko00400                 |
| BAD_RS06365       | 1.23↑     | exgA   | Beta-glucosidase                          | 1,112.17           | ko00500                        |
| BAD_RS06400       | 1.19↑     | bgaB   | Beta-galactosidase                        | 45.11              | ko00052                        |
| BAD_RS01695       | 1.18↑     | —      | Sulfurtransferase                        | 20.92              | ko01100/ko00052                 |
| BAD_RS02400       | 1.16↑     | malL   | Alpha-glucosidase                         | 76.25              | ko01100/ko00500/ko00052         |
| BAD_RS06090       | 1.14↑     | cscA   | Beta-(1-2)-fructofuranosidase             | 47.64              | ko01100/ko00500/ko00052         |
| BAD_RS05480       | 1.11↑     | BAD_1030 | Mannan endo-1,4-beta-mannosidase         | 29.85              | ko00051                        |
| BAD_RS05180       | 1.08↑     | murB   | UDP-N-acetylenolpyruvoylglucosamine reductase | 198.47             | ko01100/ko00500/ko00400         |
| BAD_RS08480       | 1.06↑     | bgIB   | Beta-glucosidase                          | 137.7              | ko01100/ko01110/ko00500/ko00460|
| BAD_RS07395       | 1.05↑     | bgaB   | Beta-galactosidase l                      | 175.7              | ko00052                        |
| BAD_RS05595       | 1.05↓     | acn    | Aconitate hydratase                       | 137.1              | ko01100/ko01110/ko01130/ko01230/ko0120/ko0120/ko00630/ko00020|
| BAD_RS07575       | 1.25↓     | glgE   | Alpha-1,4-glucan-maltose-1-phosphate maltoyltransferase | 461.18             | ko01100/ko00500                 |
Comparison of carbohydrate metabolism pathways of *B. adolescentis* grown on xylose and XOS

After internalization, carbohydrates can then be hydrolyzed, phosphorylated, deacetylated, and/or transglycosylated by dedicated intracellular enzymes. Glycosyl hydrolases appear to be the most critical group of enzymes for bifidobacteria. β-glucosidases (EC3.2.1.21) are pivotal enzymes for the metabolism and homeostasis of *Bifidobacterium* because they hydrolyze small and soluble saccharides (Kelly et al., 2016; Maria, Margarita, Iliia, & Iskra, 2014).

**TABLE 5** Top 10 significantly upregulated and downregulated metabolites during growth of *Bifidobacterium adolescentis* 15703 on XOS compared with xylose assessed by metabolome

| Meta ID   | log₂FC | MS₂ name                  | nzmed  | rtmed  |
|-----------|--------|----------------------------|--------|--------|
| meta_736  | 9.127↑ | All cis-(6, 9, 12)-Linolenic acid | 277.222 | 45.101 |
| meta_761  | 5.107↑ | D-Sorbitol 6-phosphate     | 283.128 | 44.744 |
| meta_428  | 3.978↑ | 3-Prenyl-4-Hydroxyacetophenone | 220.130 | 250.897 |
| meta_827  | 3.251↑ | Nname, cis-9, 10-Epoxysearic acid | 297.248 | 62.159 |
| meta_468  | 3.091↑ | Myristic acid              | 227.205 | 45.097 |
| meta_458  | 2.915↑ | Uric acid                  | 227.036 | 88.723 |
| meta_58   | 2.848↑ | L-Threonine                 | 118.053 | 239.084 |
| meta_607  | 2.675↑ | cis-9-Palmitoleic acid     | 253.221 | 44.427 |
| meta_741  | 2.566↑ | L-phenylalanyl-L-proline   | 278.144 | 114.569 |
| meta_753  | 2.533↑ | Oleic acid                  | 281.253 | 43.288 |
| meta_721  | 3.087↓ | Flutamide                   | 275.064 | 118.82 |
| meta_459  | 3.105↓ | 3-Hydroxymandelic acid     | 227.061 | 47.247 |
| meta_192  | 3.107↓ | Dihydroxyfumarate          | 169.043 | 48.585 |
| meta_65   | 3.131↓ | Hydroxyhydroquinone        | 125.027 | 206.664 |
| meta_182  | 3.220↓ | Quinone                     | 167.039 | 48.545 |
| meta_750  | 3.387↓ | 3′-O-Methylinosine          | 281.088 | 26.536 |
| meta_1000 | 4.285↓ | 3-Dehydroshikimic acid     | 343.068 | 166.570 |
| meta_622  | 4.393↓ | Vanillylmandelic acid      | 257.071 | 192.365 |
| meta_289  | 4.855↓ | N-Acetyl-L-alanine          | 190.075 | 104.461 |
| meta_473  | 6.446↓ | Norepinephrine             | 228.092 | 67.598 |
Compared with xylose, XOS upregulated genes involved in KEGG pathway (ko00052), including beta-galactosidase (encoded BAD_RS07400, BAD_RS08325, BAD_RS08455, BAD_RS06400, BAD_RS07395). XOS was hydrolyzed by xylosidase to produce xylose, which was furtherly characterized to 5-P-xylulose with the action of xylose isomerase and xylulose kinase. The beta-xylosidase (encoded BAD_RS02270) and alpha-amylase (encoded BAD_RS08195) involved in KEGG pathway (ko00500 and ko00052) were upregulated. The upregulated genes were associated with some metabolites, including xylulose kinase, xylosidase, xylose proton symportor, which may pertain to the efficient utilization of XOS by *B. adolescentis*.

Carbohydrates were ultimately transformed to phosphoenolpyruvate through glycolysis and pentose conversions during the fermentation by *Bifidobacterium* and furtherly involved in the TCA cycle (Louis, Hold, & Flint, 2014). L-ribulose-5-phosphate 4-epimerase involved in 5-P-xylulose production of pentose and glucuronate interconversion pathways (ko00040) were significantly upregulated in *B. adolescentis* 15703 grown on XOS compared with that grown on xylose. However, critical DEGs related to pyruvate metabolism and the TCA cycle (ko00620; ko00020) were significantly downregulated in *B. adolescentis* 15703 grown on XOS compared with that grown on xylose. Abundance of specific genes and metabolites highlighted the complex regulatory mechanisms involved in *B. adolescentis* 15703 in the presence of XOS.

**5 | CONCLUSION**

To gain insights into the regulatory networks related to XOS metabolism *B. adolescentis*, a combination of transcriptome and metabolome analyses was applied to understand the utilization and metabolism of XOS in *B. adolescentis* 15703 as well as identifying the key regulatory-related genes and metabolites. Compared with xylose, XOS highly promoted the growth of *B. adolescentis* 15703 and the fermentation performance. XOS could enhance genes involved in transport and metabolism of carbohydrate compared with xylose. Also, the metabolomic analyses, particularly those related to metabolic biomarkers of fatty acids, amino acids, and sugars showed a similar trend of results and approved the advantages of XOS as a growth medium for *B. adolescentis* 15703 compared with xylose. These results indicated that XOS was preferable to be proliferated by *B. adolescentis* than xylose.

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### TABLE 6  
Metabolites involved in related carbohydrate transport and metabolism in KEGG pathway during growth of *Bifidobacterium adolescentis* 15703 on XOS compared with xylose assessed by metabolome

| ID   | MS2 name                | mzmed  | rtmed  | log_{2}Fc | KEGG_pathway_annotation                                                                 |
|------|-------------------------|--------|--------|-----------|----------------------------------------------------------------------------------------|
| meta_761 | D-Sorbitol 6-phosphate | 283.128 | 44.744 | 5.11↑     | ko02060/ko00051                                                                        |
| meta_58  | L-Threonine             | 118.053 | 239.084 | 2.85↑     | ko000260/ko01130/ko01230/ko01110/ko02010/ko00860/ko00290/ko00970/ko01100/ko01120/ko00261 |
| meta_85  | D-Xylulose              | 131.038 | 357.043 | 2.45↑     | ko00040/ko01100                                                                        |
| meta_2004 | Galactinol             | 683.235 | 370.331 | 2.26↑     | ko00052                                                                                |
| meta_991 | L-Threonine             | 341.113 | 281.354 | 2.14↑     | ko02010/ko00500/ko02060/ko01100                                                        |
| meta_227 | 2-keto-D-Gluconic acid  | 175.028 | 242.935 | 2.08↑     | ko00030/ko01100/ko01120                                                                |
| meta_135 | Ribitol                 | 151.064 | 232.243 | 1.91↑     | ko00040/ko00054/ko01100                                                                |
| meta_166 | D-Mannose               | 161.048 | 418.573 | 1.41↑     | ko00520/ko02060/ko02010/ko01100/ko00052/ko00051                                          |
| meta_211 | Isocitrate              | 173.012 | 478.821 | 1.41↑     | ko01210/ko00720/ko01200/ko01100/ko01120/ko00020/ko00630/ko01230/ko01130/ko01110         |
| meta_2096 | Stachyose               | 725.246 | 370.331 | 2.26↑     | ko00052                                                                                |
| meta_1317 | Tetrahydrofolate        | 444.157 | 237.902 | 2.35↑     | ko00720/ko01100/ko01200/ko00970/ko00670/ko00790/ko00680/ko00260/ko00290/ko00970/ko01120 |
| meta_1695 | Maltotriose             | 563.190 | 430.279 | 1.29↑     | ko02010                                                                                |
| meta_295  | Sedoheptulose           | 191.060 | 73.835  | 1.16↑     | ko00710                                                                                |
| meta_82   | L-Isoleucine            | 130.090 | 221.442 | 1.15↑     | ko01110/ko00460/ko01130/ko01230/ko00280/ko02010/ko01100/ko00290/ko00970/ko01210         |
| meta_651  | D-Biotin                | 260.109 | 104.804 | 1.13↑     | ko02010/ko01100/ko00780                                                                 |
| meta_15   | Glycerol                | 91.042  | 107.553 | 1.05↑     | ko00520/ko02010/ko01100/ko00561/ko00052                                               |
| meta_84   | L-Ribulose              | 131.037 | 372.505 | 1.02↑     | ko00040/ko01100                                                                        |
| meta_312  | L-Ascorbic acid         | 197.006 | 45.633  | 1.05↓     | ko01120/ko01100/ko00053/ko00480/ko02060                                               |
| meta_81   | L-Leucine               | 130.090 | 180.456 | 1.08↓     | ko01110/ko01120/ko000280/ko02010/ko01100/ko00970/ko00290/ko01210/ko01120/ko00780/ko01210 |
| meta_1    | Dihydroxyacetone        | 71.016  | 198.357 | 1.21↓     | ko00561/ko01200/ko01100/ko01200/ko00680                                               |
| meta_533  | D-Glucose 6-phosphate   | 241.007 | 91.875  | 1.24↓     | ko00520/ko02060/ko05111/ko01130/ko02020/ko00521/ko00562/ko01100                        |
| meta_74   | Citraconic acid         | 129.022 | 73.135  | 1.29↓     | ko00630/ko00660/ko01210/ko01200/ko01100/ko00290                                         |
| meta_4    | Glycolate               | 75.010  | 262.283 | 1.33↓     | ko00361/ko00625/ko00630/ko01130/ko01110/ko01120/ko01200/ko01100/ko00261/ko00350/ko00362 |
| meta_348  | N-Acetyl-D-glucosamine  | 202.076 | 65.036  | 1.42↓     | ko02010/ko02060/ko00520/ko01100                                                        |
| meta_293  | Citrate                 | 191.022 | 376.386 | 2.50↓     | ko00020/ko00630/ko01230/ko00250/ko01130/ko01210/ko02020/ko00720/ko01200/ko01100/ko01120 |
| meta_376  | D-Ribose                | 209.070 | 204.675 | 2.61↓     | ko00300/ko02030/ko02010                                                               |
| meta_8    | Pyruvate                | 87.011  | 54.515  | 2.74↓     | ko01220/ko01110/ko00260/ko01130/ko00010/ko00770/ko00620/ko01100/ko01200/ko00730/ko01502/ko00622/ko00660/ko00270/ko00760/ko00710/ko00250/ko00020/ko00440/ko00040/ko01210/ko00430/ko00030/ko01230/ko00900/ko00680/ko00650/ko01120/ko00362/ko00360/ko00621/ko00290/ko00350/ko00053/ko00473/ko02060/ko00330/ko00261/ko00720/ko01502/ko00622/ko00660/ko00270/ko00760/ko00710/ko00250/ko00020/ko00440/ko00040/ko01210/ko00430/ko00030/ko01230/ko00900/ko00680/ko00650/ko01120/ko00362/ko00360/ko00621/ko00290/ko00350/ko00053/ko00473/ko02060/ko00330/ko00261/ko00720 |
CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing.

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