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# Transcriptome analysis of xylo-oligosaccharides utilization systems in *Weissella confusa* XU1

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**Abstract: [Objective]** The prebiotic effect of xylo-oligosaccharides (XOS) has obtained much attention in recent years due to their fermentation properties. In this study, a kimchi-derived strain, *Weissella confusa* XU1 grew better on XOS than on glucose and xylose. The mechanism of XOS utilization by *W. confusa* XU1 was further explored. **[Methods]** Differential transcriptomes of *W. confusa* XU1, induced by XOS, glucose and xylose, were analyzed to identify the genetic loci involved in the uptake and catabolism of XOS. **[Results]** Transcriptome analysis reveals that several major facilitator superfamily (MFS) transporters and glycoside hydrolases were involved in the uptake and hydrolysis of XOS. In addition, glycolysis pathway and pentose phosphate pathway in *W. confusa* XU1 were enhanced when XOS were present, indicating that XOS were utilized more efficiently compared with other carbon sources. **[Conclusion]** This study reveals a proposed XOS utilization mechanism in *W. confusa* XU1.

Keywords: major facilitator superfamily (MFS) transporter, probiotics, transcriptome, *Weissella confusa*, xylo-oligosaccharides

Probiotics are beneficial microorganisms which are used for the purpose of improving general health conditions of hosts including human beings and farm animals<sup>[1-2]</sup>. Lactic acid bacteria *Weissella* together with *Bifidobacterium*, and *Lactobacillus* are employed as the most important probiotics for their long history of use in fermented foods with much positive effects on the health<sup>[3-4]</sup>. Previous studies have reported that some *Weissella* strains could be used as probiotics<sup>[5]</sup> and are important starters for fermented foods including kimchi and soy sauce<sup>[6]</sup>. The *Weissella* species are Gram-positive,

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non-endospore forming cells with rod-shaped or coccoid morphology<sup>[7–8]</sup>. *Weissella* species have been found in a variety of environments, including traditional fermented foods such as kimchi, and the gastrointestinal tracts of human and animals<sup>[9]</sup>, some of which have enzymatic activities that play important roles in food fermentation. For example,  $\beta$ -glucosidases of *Weissella confusa* 31 and *Weissella cibaria* 33 converts glycosides of foods into aglycones which show more bioactive than glycosides in human body<sup>[10]</sup>. Dextran and fructan synthesized by *Weissella* have the function of relieving gastrointestinal discomfort and increasing the absorption of trace elements<sup>[11]</sup>.

Xylo-oligosaccharides (XOS) are prebiotics and can not be hydrolyzed by enzymes or the low pH in human body. So XOS are not absorbed by human during transit through the small intestine. Based on special structure characteristics, XOS are easy to pass through the foregut and reach the colon, where they serve as fermentable substrates for the resident beneficial bacterium in the hindgut<sup>[12–13]</sup>. Therefore, XOS can increase the indigenous Bifidobacterium spp. biomass in gastrointestinal tract<sup>[14–15]</sup> and promote the growth of probiotics in human gut microbiota to make the bacteria stay within a healthy balance<sup>[16]</sup>. Moreover, studies have shown that XOS ingestion contributes to anti-oxidant, anti-bacterial, immune-modulatory and anti-diabetic activities<sup>[17-18]</sup>. XOS can be digested by prebiotics but the underlying molecular mechanism remains elusive. Recently, several researches reported that XOS could be utilized by *Bifidobacterium* and Pediococcus and the utilization pathways of XOS in these microbes were found out by transcriptome analysis<sup>[19-20]</sup>. XOS are the results of partial hydrolysis of xylan, which are the major components of most plant cell walls. The main classes of xylan are the glucuronoxylans, arabinoxylans and glucuronoarabinoxylans, which are main cell wall components of birchwood, wheat flour and corn bran, respectively<sup>[21]</sup>. The XOS used

in the current study have a degree of polymerization ranging from 2–7 and were hydrolyzed from corn glucuronoarabinoxylan. Because of the complexity of xylan structure, XOS used in the current study contains various side chains such as arabinose, galactose and xylose. Therefore, the degradation and utilization of XOS requires the synergistic activity of several glycoside hydrolases.

In the current study, *Weissella confusa* XU1 (*W. confusa* XU1) isolated from kimchi was found to be able to grow on the medium that XOS are the sole carbon and energy source. Similar to *Bifidobacterium* and *Pediococcus*, *Weissella* is able to utilize XOS. Transcriptome analysis was employed to identify *W. confusa* XU1 genes which are involved in XOS utilization. The molecular mechanism of XOS utilization in *W. confusa* XU1 may reveal novel pathways.

### **1** Materials and Methods

# **1.1** Bacterial strains and bacterial growth curve assay

W. confusa XU1 previously isolated from kimchi bought from supermarket and was identified by 16S rRNA sequence analysis. Pre-grown W. confusa XU1 (1%, V/V) was inoculated to modified MRS medium (Oxoid peptone 10 g/L, XOS/glucose/ xylose 20 g/L, K<sub>2</sub>HPO<sub>4</sub> 2 g/L, CH<sub>3</sub>COONa·3H<sub>2</sub>O 5 g/L, triammonium citrate 2 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.2 g/L, MnSO<sub>4</sub>·4H<sub>2</sub>O 0.05 g/L, pH 6.2) containing XOS (LongLive Biotechnology), glucose (Sigma Aldrich) or xylose (Sigma Aldrich) as the only carbon source, respectively. Aliquots of cultures were sampled at regular intervals. All the cultures were incubated at 30 °C under anaerobic conditions. For anaerobic growth, media was incubated at 30 °C in a coy anaerobic chamber (10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>) for 16 hours before inoculation, and cultures were grown at 30 °C in flasks without shaking. The microbial growth was monitored by dry cell weight at every hour.

# **1.2 RNA isolation and library preparation for strand-specific transcriptome sequencing**

*W. confusa* XU1 cells for RNA isolation were harvested independently from triplicate cultures of each carbon source MRS medium at estimated early mid-exponential growth phase by centrifugation. Total RNA was extracted using Trizol and used for library preparation and Illumina sequencing. Sequencing and analysis of transcriptome of the samples were performed in Beijing Novogene Bioinformation Technology Co. Ltd (Beijing, China).

For each sample, mRNAs were purified using oligo (dT)-attached magnetic beads. The mRNA was fragmented and primed with random hexamers, and then submitted for the synthesis of the first-strand and second-strand cDNA. The cDNA fragments were processed for end repair and finally ligated to paired-end adaptors. The cDNA fragments with 150–200 bp size were selected and enriched by PCR amplification. The cDNA libraries were constructed and adopted the paired-end sequencing on an Illumina Hiseq-PE150.

#### 1.3 Data analysis

Clean data (clean reads) were obtained by removing reads containing adapter, reads containing ploy-N and low quality reads from raw data. Bowtie2-2.2.3 was used to build index of reference genome (*Weissella confusa* DSM 20196) and align clean reads to reference genome.

HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene.

Differential expression analysis of three conditions (three biological replicates per condition) was performed using the DESeq R package (1.18.0). The resulting *P*-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value<0.05 found by DESeq were assigned as differentially expressed.

Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with corrected *P*-value less than 0.05 were considered significantly enriched by differential expressed genes.

KOBAS software was used to test the statistical enrichment of differential expression genes in KEGG pathways.

#### 1.4 Data archiving

The raw transcriptome data generated during the current study are available in the National Center for Biotechnology Information Sequence Read Archive (SRA) under SRA accession number SRR9021087–SRR9021095.

### 2 Results

#### 2.1 Growth on XOS, xylose or glucose

The changes of *W. confusa* XU1 biomass with cultivation time of three different carbon sources were shown in Figure 1. Fermentation was completed within 12 hours. Unexpectedly, *W. confusa* XU1 grown on MRS media with XOS as the



Figure 1. Changes of *W. confusa* XU1 biomass with cultivation time of three different carbon sources. Mean values ( $\pm$ s.e.m.) of three replicates are shown. Experiments were repeated twice with similar results. XOS: xylo-oligosaccharides.

sole carbon source had the highest biomass in comparison with xylose or glucose as the sole carbon source. *W. confusa* XU1 was observed to grow best on XOS, followed by growth on the glucose. The biomass of *W. confusa* XU1 on XOS at 6 hour was about 1.51 and 2.63-fold greater than that on glucose and xylose, respectively.

### 2.2 RNA sequencing and assembly of the transcriptomes

To obtain a global view of W. confusa XU1 transcriptomes during different carbon sources utilization, RNA-seq was performed on W. confusa XU1 cultures containing XOS, xylose and glucose as the sole carbon source, and the gene expression profiles of each condition were compared. Nine cDNA libraries prepared from the pooled total RNA extracted from Weissella confusa XU1 grown under three different carbon conditions were paired-end sequenced on the Illumina Hiseq-PE150 platform. After filtering out repetitive, low-complexity, and low-quality reads, the clean reads were assembled according to the reference genome (Weissella confusa DSM 20196). The sequencing results for the nine libraries (designated Xylose\_1, Xylose\_2, Xylose 3, Glu 1, Glu 2, Glu 3, XOS 1, XOS 2 and XOS 3) are summarized in Table 1. For each library, all clean reads were mapped back to the Weissella confusa reference genome. The percentage of clean reads from each library that could be uniquely mapped ranged from 91.14% to 93.45%, thus providing good coverage of the transcript profiles.

#### **2.3 DEGs induced by different carbon sources**

Differentially expressed genes (DEGs) were

analyzed via pairwise comparisons of XOS vs glucose, XOS vs xylose and xylose vs glucose to investigate which genes involved in XOS utilization when *W. confusa* XU1 grew in presence of XOS, xylose, or glucose as the sole carbon source. Cluster analysis of DEGs among xylose, glucose and XOS was shown in Figure 2.

The expression model of DEGs in xylose showed the lowest and highest similarities with XOS and glucose treatment, respectively. In comparison of XOS vs glucose, most DEGs were related to metabolic pathways, biosynthesis of secondary metabolites and biosynthesis of antibiotics. In comparison of XOS vs xylose, most DEGs were related to metabolic pathways, biosynthesis of metabolites secondary and transporters. In comparison of xylose vs glucose, most DEGs were also related to metabolic pathways, biosynthesis of secondary metabolites and transporters.

The global gene expression profiles of three comparisons were shown in Figure 3. Lots of DEGs found in each comparison suggests that different carbon sources have significant effects on global gene expression. Compared to xylose and glucose treatment, several highly expressed genes (listed by *W. confusa* XU1 locus tag numbers) with highlight molecular functions in XOS treatment would be related to XOS utilization mechanisms.

# 2.4 MFS transporters involved in XOS and xylose uptake

Analysis of differentially up-regulated loci enabled the identification of several genes conferring the uptake of the XOS and xylose used in

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Table I	Statistics	of nine	libraries	reads	mannino
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Sample name	Xylose_1	Xylose_2	Xylose_3	Glu_1	Glu_2	Glu_3	XOS_1	XOS_2	XOS_3
Total reads	9800160	8798142	8558890	9789186	10406530	10107678	9970784	10524586	9914424
Total mapped	9407360	8447705	8241762	9508215	10107203	9791271	9526282	10062974	9473711
	(95.99%)	(96.02%)	(96.29%)	(97.13%)	(97.12%)	(96.87%)	(95.54%)	(95.61%)	(95.55%)
Uniquely	9041236	8119380	7910181	9131155	9724725	9383840	9087176	9606691	9039402
mapped	(92.26%)	(92.29%)	(92.42%)	(93.28%)	(93.45%)	(92.84%)	(91.14%)	(91.28%)	(91.17%)



Figure 2. Cluster analysis of DEGs in different treatments. Values of  $log_{10}$  (FPKM+1) were conducted normalized transformation before clustered. Red indicated high expressed genes; Blue indicated low expressed genes.

this study. As shown in Table 2, major facilitator superfamily (MFS) transporters were significantly up-regulated among three comparisons. Compared to xylose treatment, MFS transporters IV69\_RS08185 and IV69\_RS01700 were up-regulated 20.39-fold and 6.92-fold with *q*-value  $\leq 0.01$  under XOS treatment, respectively.

Interestingly, another two MFS transporters (IV69\_RS01480 and IV69\_RS01450) were both significantly up-regulated more than 270-fold and 5-fold in xylose *vs* glucose and XOS *vs* glucose comparisons, respectively. However, there was no significant difference of these two MFS transporters (IV69\_RS01480 and IV69\_RS01450) in XOS *vs* xylose comparison (Table 2). These results indicated that these two MFS transporters (IV69\_RS01480 and IV69\_RS01480) and IV69\_RS01480 and IV69\_RS01480 and IV69\_RS01480 and IV69\_RS01480 and IV69\_RS01480) were only related to xylose uptake.

#### 2.5 DEGs involved in XOS catabolism

Several genes related to XOS degradation were found in the comparison of XOS and xylose (Table 3). Under XOS treatment, the extracellular GH43 enzymes (IV69\_RS09575 and IV69\_RS02985) encoding  $\beta$ -xylosidase, and extracellular GH2 enzymes



Figure 3. Volcano plots of pairwise comparisons of the carbon-induced differential global transcriptome in *W. confusa* XU1. A: XOS *vs* xylose; B: Xylose *vs* glucose; C: XOS *vs* glucose.

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ruble 2. – Statistically significant up regulated genes involved in 1005 uplake								
Gene	Gene apportation	Log <sub>2</sub> FoldChange	-Log <sub>10</sub>	Log <sub>2</sub> FoldChange	$-Log_{10}$	Log <sub>2</sub> FoldChange	-Log <sub>10</sub>	
No <sup>a</sup>	Gene annotation	$(XOS vs Xylose)^b$	(q-value)	(Xylose vs Glu)	(q-value)	(XOS vs Glu)	(q-value)	
08185	MFS transporter	4.35	70.40	4.38	113.93	8.71	175.92	
01700	MFS transporter (Na+/xyloside symporter related)	2.79	118.17	4.23	96.26	7.02	q-value=0	
01450	Sugar porter family MFS transporter (D-xylose-proton symporter)	-0.30	1.16	2.64	34.66	2.33	53.98	
01480	Sugar porter family MFS transporter (D-xylose-proton symporter)	0.11	0.30	8.13	118.06	8.23	<i>q</i> -value=0	

Table 2. Statistically significant up-regulated genes involved in XOS uptake

<sup>a</sup>: Gene number referenced as IV69\_RS XXXXX with XXXXX being a five-digit number; <sup>b</sup>: Significance of fold change data in XOS *vs* xylose is judged by having a *q*-value on no more than 0.05 (except 01450 and 01480).

Gene		Sub-cellular	Log <sub>2</sub> FoldChange	-Log <sub>10</sub>	Log <sub>2</sub> FoldChange	-Log <sub>10</sub>	Log <sub>2</sub> FoldChange	-Log <sub>10</sub>
No <sup>a</sup>	Gene annotation	localization <sup>b</sup>	(XOS vs Xylose) <sup>c</sup>	(q-value)	(Xylose vs Glu)	(q-value)	(XOS vs Glu)	(q-value)
09575	β-xylosidase	Extracellular	1.06	21.25	7.55	<i>q</i> -value=0	8.59	q-value=
		space						0
02985	β-xylosidase	Extracellular	0.46	1.81	1.84	13.55	2.29	30.49
		space						
05580	β-galactosidase	Extracellular	1.13	23.38	4.37	165.44	5.50	<i>q</i> -value=
		space						0
05575	β-galactosidase	Extracellular	0.73	9.49	4.36	122.72	5.08	q-value=
		space						0

Table 3. Statistically significant up-regulated genes involved in XOS hydrolyzation

<sup>a</sup>: Gene number referenced as IV69\_RS XXXXX with XXXXX being a five-digit number; <sup>b</sup>: Sub-cellular localizations were predicted by "LocTree3" method<sup>[22]</sup>; <sup>c</sup>: Significance of fold change data in XOS *vs* xylose is judged by having a *q*-value on no more than 0.05.

 $(IV69_RS05575 \text{ and } IV69_RS05580)$  encoding  $\beta$ -galactosidase contributed to the degradation of the XOS generated in the periplasm.

Several genes related to xylose catabolism were also found significantly up-regulated under XOS treatment in the comparison of XOS and xylose, such as xylose isomerase (IV69 RS08095 and IV69\_RS09560) and xylulose kinase (IV69 RS09555). What's more, nine genes involved in pentose phosphate pathway (map00030) and glycolysis pathway (map00010) were also up-regulated in XOS treatment (Table 4).

Compared to glucose treatment, genes (IV69\_RS08795, IV69\_RS08800, IV69\_RS00890 and IV69\_RS05190) related to phosphoenolpyruvate production (map00010) were up-regulated under

XOS treatment. Moreover, a gene encoded L-lactate dehydrogenase (IV69\_RS04535) was significantly down-regulated under XOS treatment.

### **3** Discussion

#### 3.1 Debranch and hydrolysis of XOS backbone

XOS are oligomers that consist of 2–10 xylose residues connected through  $\beta$ -(1-4)-linkages as the result of partial hydrolysis of xylan. So, the conserved  $\beta$ -1,4-xylose backbone of XOS may be decorated with various kinds of short side chains, including L-arabinose and L-galactose.

Before transported into cells, the xylan-derived XOS may be degraded by a suite of primarily exoacting enzymes, such as  $\beta$ -xylosidase (IV69 RS09575

	Table 4. DEGs involved in XOS catabolism							
Gene No <sup>a</sup>	Gene annotation	EC No.	$Log_2FoldChange$ (XOS <i>vs</i> Xylose) <sup>b</sup>	$-Log_{10}$ ( <i>q</i> -value)	Log <sub>2</sub> FoldChange (XOS vs Glu)	$-Log_{10}$ ( <i>q</i> -value)		
08095	Xylose isomerase	5.3.1.5	2.12	73.86	7.91	<i>q</i> -value=0		
09560	Xylose isomerase	5.3.1.5	2.31	95.53	10.54	<i>q</i> -value=0		
09555	Xylulokinase	2.7.1.17	2.34	95.81	9.35	<i>q</i> -value=0		
01395	Xylulose-5-phosphate phosphoketolase	4.1.2.9	0.31	2.01	0.49	7.04		
08795	Type I glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	0.77	11.37	1.03	29.54		
08800	Phosphoglycerate kinase	2.7.2.3	0.53	5.50	0.81	18.51		
00890	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	5.4.2.11	1.04	20.74	0.74	14.99		
05190	Pphosphopyruvate hydratase	4.2.1.11	0.49	4.90	0.41	5.16		
03240	Pyruvate kinase	2.7.1.40	0.34	2.42	-0.73	15.04		
05010	Glucose-6-phosphate isomerase	5.3.1.9	0.67	8.39	-0.03	0.08		
00475	Glucose-6-phosphate 1-dehydrogenase	1.1.1.363	0.38	2.76	-0.90	10.56		
02530	6-phosphogluconolactonase	3.1.1.31	0.98	18.06	2.69	170.22		
00035	6-phosphogluconate dehydrogenase (decarboxylating)	1.1.1.343	1.53	40.64	-2.36	139.10		
04535	L-lactate dehydrogenase	1.1.1.27	-0.19	0.75	-2.80	90.70		

<sup>a</sup>: Gene number referenced as IV69\_RS XXXXX with XXXXX being a five-digit number; <sup>b</sup>: Significance of fold change data in XOS *vs* xylose is judged by having a *q*-value on no more than 0.05 (except 04535).

and IV69 RS02985) and β-galactosidase (IV69 RS05580 and IV69 RS05575). Some β-galactosidase enzymes had been reported that could cleave  $\alpha$ -L-arabinosides,  $\beta$ -D-fucosides and  $\beta$ -D-glucosides<sup>[23]</sup>. And the side chains of XOS may be cleaved by β-galactosidase (IV69 RS05580 and IV69 RS05575) and the conserved  $\beta$ -1,4-xylose backbone of XOS was hydrolyzed by β-xylosidase (IV69 RS09575 and IV69 RS02985), after which XOS were converted to xylose and XOS with a low degree of polymerization outside cells.

Different from *Pediococcus*<sup>[20]</sup> and Bifidobacterim<sup>[24]</sup>, XOS were hydrolyzed into xylose and XOS with a low degree of polymerization outside cells with exo-acting enzymes.

### **3.2** More energy-efficient XOS transportation by MFS transporters

Several reports have shown that XOS are transported into cell membrane only by an ABC transporter sugar system in bacteria, such as *P. acidilactici* BCC-1<sup>[20]</sup> and *B. lactis* B1-04<sup>[24]</sup>. In

this study, MFS transporters were observed to participate in XOS transportation. Proton-motive force (PMF) was utilized by many MFS transporters to drive the transport process<sup>[25–26]</sup>. Two ATP molecules (equivalent to about 6 protons) were consumed by ABC transporters per transport cycle. While, for MFS transporters, one proton was consumed to transport one molecule of an electroneutral substrate. Thus, MFS transporters appear to be more energy-efficient in terms of the stoichiometric ratio of substrate to protons than ABC transporters<sup>[27]</sup>. What's more, products hydrolyzed outside cells may be easier to be transported into cells than XOS.

Taken together, these findings suggested that *W. confusa* XU1 may possess more efficient catabolic pathway for XOS utilization than other lactic acid bacteria for its more energy-efficient transportation of XOS.

#### 3.3 XOS catabolism in W. confusa XU1

After transported into cells, xylose may be

further translated to glyceraldehyde-3P with the action of xylose isomerase (IV69\_RS08095 and IV69\_RS09560), xylulose kinase (IV69\_RS09555) and xylulose-5-phosphate phosphoketolase (IV69\_RS01395).

Glyceraldehyde-3P was converted to pyruvate which then may participate in TCA cycle (map00020) metabolism pyruvate (map00620) and by up-regulated genes glycolysis of pathway (map00010) in XOS treatment (Table 4), including type I glyceraldehyde-3-phosphate dehydrogenase (IV69 RS08795), phosphoglycerate kinase (IV69 RS08800), 2,3-bisphosphoglycerate-dependent mutase (IV69 RS00890), phosphoglycerate phosphopyruvate hydratase (IV69 RS05190) and (IV69 RS03240). pyruvate kinase Glycolysis pathway was enhanced under XOS treatment in W. confusa XU1 and there may be more pyruvate to participate in TCA cycle.

Pentose phosphate pathway can be divided into oxidative branch and non-oxidative branch. In this study, oxidative pentose phosphate pathway was also enhanced. Alpha-D-glucose-6-phosphate in glycolysis (map00010) was converted to D-ribulose-5phosphate by related genes which were up-regulated in xylose XOS VS (Table 4), including glucose-6-phosphate isomerase (IV69 RS05010), glucose-6-phosphate 1-dehydrogenase (IV69 RS00475), 6-phosphogluconolactonase (IV69 RS02530), and 6-phosphogluconate dehydrogenase (IV69 RS00035).

The oxidative branch consists of three irreversible reactions, which result in NADPH and pentose phosphate production. NADPH is used as a reducing agent in many biosynthetic pathways and is also important for protection against oxidative damage<sup>[28]</sup>. Previous studies reported that xylose fermentation rate was controlled by pentose phosphate pathway<sup>[29]</sup>. A prime control point in xylose fermentation would be the oxidative pentose phosphate pathway, since this pathway is the principal NADPH-generating pathway in the cell<sup>[30–31]</sup>. And prior study has reported that xylose consumption

rate drastically decreases with a non-functional oxidative pentose phosphate pathway<sup>[32]</sup>.

Glycolysis pathway and oxidative pentose phosphate pathway were both enhanced by XOS stimulation in *W. confusa* XU1, which may be one of the reasons for the maximum biomass accumulation under XOS treatment.

## **3.4** Different biomass production on XOS, xylose and glucose

In the current research, *W. confusa* XU1 with the growth capacity on XOS was reported (Figure 1). However, the findings of the current study are different from previous research<sup>[20]</sup>. XOS were more preferred by *W. confusa* XU1 than glucose and xylose.

According to DEGs analysis of XOS *vs* xylose, a lower expression of L-lactate dehydrogenase may also reduce the consumption of pyruvate to produce L-lactate and more pyruvate would participate in TCA cycle (Table 4), which may give an advantage to cell growth of *W. confusa* XU1.

A lower expression of L-lactate dehydrogenase, more energy-efficient transportation of XOS and enhancement of glycolysis pathway and oxidative pentose phosphate pathway, all these results illustrated that the maximum biomass production of *W. confusa* XU1 was observed when XOS were used as the solo carbon source. This character may be a results of growth competition happened in beneficial bacterium located hindgut of animal or human being, where XOS are more available than glucose or xylose.

Based on the results presented here, a possible pathway for XOS uptake and catabolism in *W*. *confusa* XU1 was proposed (Figure 4). XOS were first hydrolyzed by  $\beta$ -xylosidase and  $\beta$ -galactosidase outside cells to produce xylose and XOS with a low degree of polymerization. Then hydrolysis products were transported into cells by MFS transporters to participate in glycolysis pathway (map00010), pentose phosphate pathway (map00030) and TCA cycle (map00020), which could enhance sugar absorption and utilization.



Figure 4. Proposed pathways of XOS uptake and catabolism. XOS: Xylo-oligosaccharides; MFS: major facilitator superfamily; PEP: phosphoenolpyruvate; xpk: xylulose-5-phosphate phosphoketolase; TCA cycle: tricarboxylic acid cycle.

Weissella confusa is an important specific starter for food fermentation, which has potential in improving nutritional properties of foods. This study provides insights into the mechanism of utilization of XOS at molecular level in Weissella confusa, which may benefit to improve its fermentation process both *in vivo* and *in vitro*. Moreover, with energy-efficient transportation of XOS, W. confusa XU1 shows bright application prospects in food fermentation industry. XOS transportation pathway revealed here may be used as a referable tool to improve utilizing efficiency of xylan substrates for genetically engineered microorganism.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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# 基于转录组测序数据对 Weissella confusa XU1 中低聚木糖代谢 系统的分析

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摘要:【目的】近年来由于在发酵方面的良好特性,低聚木糖的益生作用越来越得到公众的关注。研究 发现相比于葡萄糖和木糖 Weissella confusa XU1 在以低聚木糖为唯一碳源时生长情况最好。本文将对 Weissella confusa XU1 中低聚木糖的代谢机制进行研究。【方法】本研究分别以葡萄糖、木糖和低聚木 糖作为唯一碳源对 Weissella confusa XU1 进行转录组测序并进行比较分析。【结果】通过转录组分析发 现以低聚木糖为唯一碳源的处理中部分编码 MFS 转运蛋白和糖基水解酶的基因转录水平显著上升, Weissella confusa XU1 中的糖酵解过程和磷酸戊糖途径也得到显著增强。【结论】本研究根据转录组数 据分析得出 Weissella confusa XU1 中的低聚木糖代谢机制。本研究首次在革兰氏阳性菌中发现 MFS 转 运蛋白参与到低聚木糖转运的过程,为提高微生物对木聚糖利用效率进行分子改造提供了改造方向,该 机制为低聚木糖代谢的研究和 Weissella 的工业化应用提供了新的思路。

关键词: MFS 转运蛋白, 益生菌, 转录组, Weissella confusa, 低聚木糖

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