Streptococcus Mutans Membrane Vesicles Enhance Candida albicans Pathogenicity and Carbohydrate Metabolism

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Streptococcus mutans and Candida albicans, as the most common bacterium and fungus in the oral cavity respectively, are considered microbiological risk markers of early childhood caries. S. mutans membrane vesicles (MVs) contain virulence proteins, which play roles in biofilm formation and disease progression. Our previous research found that S. mutans MVs harboring glucosyltransferases augment C. albicans biofilm formation by increasing exopolysaccharide production, but the specific impact of S. mutans MVs on C. albicans virulence and pathogenicity is still unknown. In the present study, we developed C. albicans biofilms on the surface of cover glass, hydroxyapatite discs and bovine dentin specimens. The results showed that C. albicans can better adhere to the tooth surface with the effect of S. mutans MVs. Meanwhile, we employed C. albicans biofilm-bovine dentin model to evaluate the influence of S. mutans MVs on C. albicans biofilm cariogenicity. In the S. mutans MV-treated group, the bovine dentin surface hardness loss was significantly increased and the surface morphology showed more dentin tubule exposure and broken dentin tubules. Subsequently, integrative proteomic and metabolomic approaches were used to identify the differentially expressed proteins and metabolites of C. albicans when cocultured with S. mutans MVs. The combination of proteomics and metabolomics analysis indicated that significantly regulated proteins and metabolites were involved in amino acid and carbohydrate metabolism. In summary, the results of the present study proved that S. mutans MVs increase bovine dentin demineralization provoked by C. albicans biofilms and enhance the protein and metabolite expression of C. albicans related to carbohydrate metabolism.

Keywords: Streptococcus mutans, Candida albicans, membrane vesicles, cross-kingdom, dental caries
INTRODUCTION

Streptococcus mutans, an important cariogenic bacterium in the oral cavity, is considered a microbiological risk marker of dental caries (Grier et al., 2021). Candida albicans, the most common fungus in the oral cavity, is both acidogenic and aciduric and in this regard has similar properties to S. mutans (Klineke et al., 2009; Pereira et al., 2018). A number of studies have reported that C. albicans is able to adhere to the surfaces of hydroxyapatite, dentin and cementum (Pereira et al., 2018; Thanh Nguyen et al., 2021). In addition, secreted aspartic proteases of C. albicans can degrade dentin collagen fibers and may play an important role in early childhood caries (ECC) (Li et al., 2014). Recent research found that C. albicans is frequently isolated in the oral mycobiome of early childhood caries and causes more severe dental caries (Falsetta et al., 2014; Cui et al., 2021; Alkhars et al., 2022). As the core resident of dental plaque biofilms, C. albicans interacts with various oral bacteria, such as Streptococcus gordonii and S. mutans (Bertolini and Dongari-Bagtzoglou, 2019). C. albicans may be a “keystone commensal” of plaque biofilms and can work synergistically with classic cariogenic bacteria (Pereira et al., 2018; Young et al., 2020).

The cross-kingdom interactions of S. mutans and C. albicans are considered to be associated with severe dental caries (Koo et al., 2018), which has become of increasing interest. S. mutans is involved in C. albicans virulence regulation in dental plaque biofilms. Mutanobactin and competence-stimulating peptide of S. mutans inhibit C. albicans hyphal formation (Joyer et al., 2010). However, S. mutans degrades sucrose into glucose and fructose, which provide for C. albicans catabolism in the sucrose environment (Falsetta et al., 2014). S. mutans antigen I/II and glucosyltransferase (GtfS) secreted by S. mutans promote C. albicans adhesion and biofilm formation (Ellepola et al., 2017; Yang et al., 2018). S. mutans GtfB can bind to mannan on the cell surface of C. albicans and promote coadhesion and symbiotic biofilm development (Koo et al., 2018).

S. mutans membrane vesicles (MVs) contain proteins, extracellular DNA (eDNA) and other biologically active substances that play roles in cell-to-cell communication, biofilm formation and disease progression (Cao and Lin, 2021). The S. mutans sfp, srtA and TnSmu2 genes are involved in the formation and secretion of MVs (Morales-Aparicio et al., 2020; Wen et al., 2021). It has been demonstrated that S. mutans MVs are involved in cell wall synthesis, bacterial adhesion and biofilm formation (Cao et al., 2020; Morales-Aparicio et al., 2020). Moreover, they can promote the biofilm formation of Streptococcus mitis, Streptococcus oralis, C. albicans and other microorganisms in the oral cavity (Senpuku et al., 2019; Wu et al., 2020). Our previous research found that S. mutans MVs harboring glucosyltransferases augment C. albicans biofilm formation by increasing exopolysaccharide production (Cao et al., 2020; Wu et al., 2020). However, the specific impact of S. mutans MVs on C. albicans virulence and pathogenicity is still unknown.

In the present study, we employed a C. albicans biofilm-bovine dentin model to evaluate the effect of S. mutans MVs on C. albicans biofilm pathogenicity. An integrative proteomic and metabolomic approach was used to identify the differentially expressed proteins and metabolites of C. albicans. The present study will identify the specific influence of S. mutans MVs on C. albicans cariogenic ability.

MATERIALS AND METHODS

Bacterial Strains And Culture Conditions

S. mutans UA159 (ATCC 700610) (Wu et al., 2020) and C. albicans SC5314 (ATCC MYA-2876) (Wu et al., 2020) were used in the present study. Brain heart infusion (BHI; Difco, Detroit, MI, United States) was employed to cultivate S. mutans, and Sabouraud’s dextrose broth (SDB, HKM, Guangzhou, China) was used to cultivate C. albicans. C. albicans biofilm development was achieved using-tryptone-yeast extract (TYE, OXOID, Hampshire, United Kingdom) medium supplemented with 1% sucrose. The method of cell culture was performed according to a previous study (Wu et al., 2020).

Preparation of S. mutans MVs

For the preparation of MVs from S. mutans, S. mutans was grown in 500 mL BHI broth at 37 °C for 16 h. The culture was centrifuged at 6,000 × g for 15 min at 4 °C and at 10,000 × g for 15 min at 4 °C to remove cells and cell debris. The supernatants were removed and filtered through 0.22 μm filters (Millipore, MMAS, United States). Then, the supernatants were concentrated by a 100 kDa Amicon ultrafiltration system (Millipore, MMAS, United States). S. mutans MVs were harvested by centrifugation at 100,000 × g for 70 min at 4 °C. The S. mutans MVs yield was quantified using a BCA assay (CWBO, Beijing, China) as we previously described (Wu et al., 2020).

Preparation of Bovine Dentin Specimens And Hydroxyapatite Discs

Bovine dentin specimens were prepared as we previously described (Wu et al., 2018). Briefly, dentin specimens (5 mm × 4 mm × 2 mm) were prepared from sound incisor bovine teeth that were freshly extracted. These teeth were devoid of stains, erosion and microcracks. Hydroxyapatite (HA) discs (Clarkinson Chromatography Products, South Williamsport, PA, USA) 9.7 mm in diameter and 1.5 mm thick were used in this study. Dentin surfaces and HA discs were ground and polished by silicon carbide sandpapers (600-, 800-, 1500- and 2000-grit) in order to avoid the influence of HA discs surface roughness in C. albicans biofilm formation (Wang et al., 2020b; Le et al., 2022). After polishing, the dentin specimens and HA discs were sonicated in distilled water for 10 min to remove residual debris. Before the start of the experiment, the dentin specimens were disinfected with 75% alcohol for 12 h and sterilized by ultraviolet light for 4 h, and the HA discs were autoclaved at 121 °C for 15 min.

C. albicans Biofilm Formation

C. albicans biofilms were cultured on the surfaces of cover glass, HA discs and dentin specimens to assess the effect of S. mutans MVs on C. albicans biofilm formation. C. albicans biofilms were

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developed as we previously described (Wu et al., 2020), with slight modification. First, all cover glass, HA discs and dentin specimens were preserved in sterile artificial saliva (ChangFeng Technology, Guangzhou, China) for 2 h at 37 °C. Then, artificial saliva was removed, and SDB containing C. albicans (~10^6 CFU/mL) was added to culture plates and incubated at 37 °C for 90 min with a shaking speed of 90 rpm under aerobic conditions. Immediately afterward, sterile PBS was employed to wash unattached cells, and 1% sucrose TYE medium containing 40 μg/mL S. mutans MVs was added to culture plates to develop C. albicans biofilms at 37 °C under aerobic conditions for 24-96 h. The 1% sucrose TYE culture medium containing S. mutans MVs was replaced daily. As the control group, C. albicans biofilms were cultured with 1% sucrose TYE medium without S. mutans MVs. Dentin specimens mixed with 1% sucrose TYE medium containing 40 μg/mL S. mutans MVs without C. albicans inoculation was the blank control group.

Analysis of C. albicans biofilms and dentin specimens by SEM.

Scanning electron microscopy (SEM) (Quanta 400F-FEI, Eindhoven, Netherlands) was employed to observe C. albicans biofilm and dentin specimen morphological characteristics (Wu et al., 2020). For C. albicans biofilms on cover glass, HA discs and dentin specimen analysis, the supernatants and unattached cells were removed by washing with sterile PBS three times. For dentin specimen surface observation, the attached C. albicans biofilm was removed by washing with deionized water. After fixation, dehydration and gold sputter-coating, the C. albicans biofilm was observed at 2,000 × magnification by SEM, and dentin specimens were observed at 4,000 × and 8,000 × magnification by SEM. The experiment was performed in 4 biological replicates.

### Measurement of Dentin Surface Microhardness and Roughness

C. albicans biofilms were developed on the dentin surface over 96 h, with culture medium replaced daily. Following biofilm development, the attached C. albicans biofilm was removed by washing with deionised water and dentin samples were stored in PBS at 4°C.

A Vikers microhardness tester (DuraScan-20, Struers, Germany) was employed to measure the surface microhardness of the dentin specimens. Three random points on each dentin specimen surface were subjected to measurement with a load of 0.2 HV for 15 s. The dentin surface hardness (SH) of each group (n = 8) was determined before and after C. albicans biofilm formation. Surface hardness loss (%SHL) is an indicator of demineralization, which was calculated as %SHL = (SH1 - SH2)/SH1 × 100%, with SH1 being the SH of the dentin specimen before C. albicans biofilm formation, and SH2 being the SH of the dentin specimen after C. albicans biofilm formation (Sampaio et al., 2019). SH1 and SH2 were measured at three different defined regions of the sample, and the mean values were calculated. Confocal laser scanning microscopy (CLSM) (LSM700-Carl, Zeiss, Germany) was used to detect the surface roughness of the dentin specimens. Three measurement areas of 300 μm × 300 μm on each dentin specimen surface were randomly selected to obtain three-dimensional topography images and analyze the average roughness (Ra). The Ra of each group (n = 8) was determined before and after C. albicans biofilm formation, and the increase in the surface roughness of the dentin was calculated. Ra was measured at three different defined regions of the sample and the mean values were calculated.

### Sample Preparation for Proteome and Metabolome Analysis

C. albicans biofilms were developed on cover glass for 24 h, with supernatants and planktonic cells discarded following incubation. Biofilm samples were collected in PBS and washed by centrifugation at 12, 000 rpm for 5 min at 4°C. All samples were stored at -80°C for no longer than one month.

### TMT Labeling Proteome Analyses

The TCA/acetone precipitation and SDT lysis method was employed to extract proteins. Protein quantitation was performed by a BCA assay (CWBio, Beijing, China), and separation detected by SDS-PAGE. The C. albicans protein samples were digested according to the manufacturer's instructions (Thermo Fisher Scientific). The subsequent procedures included peptide fractionation with reversed phase (RP) chromatography, mass spectrometry analysis, data analysis by Proteome Discoverer 2.2 software (Thermo Fisher Scientific), and bioinformatic analysis including Gene Ontology (GO) and KEGG Pathway annotations. Three biological replicates were prepared in each group. The final proteins that were deemed to be differentially expressed were screened by the following criteria: 1.2-fold changes (upregulation or downregulation) relative to the control group, and P value < 0.05.

### Metabolomic Analysis

The metabolites of collected samples were extracted with 50% methanol buffer, and pooled quality control (QC) samples were prepared at the same time. An ultra-performance liquid chromatography (UPLC) system (Sciex, UK) and an ACQUITY UPLC T3 column (Waters, UK) were used for sample chromatographic separation and reversed phase separation. Metabolites eluted from the column were detected by a high-resolution tandem mass spectrometer (TripleTOF5600plus, Sciex, UK). The mass spectrometry data were acquired in IDA mode. The quality of the acquired LC–MS data was analyzed by XCMS software. MetaX software was employed for metabolite identification, and quantification, and then the differential metabolites were screened. Metabolites were annotated through the open access databases, KEGG and HMDB. Six biological replicates were prepared in each group.

### Integrated Analysis of Metabolomics and Proteomics

The KEGG pathway database (http://www.genome.jp/kegg) and the Gene Ontology database (ftp://ftp.ncbi.nih.gov/gene/DATA/ gene2go.gz) were employed to analyze the significantly altered canonical pathways and molecular networks of differentially expressed metabolites and proteins. The final proteins and metabolites that were deemed to be differentially expressed were screened by the following criteria: proteins, 1.2-fold changes...
(upregulation or downregulation) relative to the control group, and 
$P$ value <0.05; metabolites, 2.0-fold changes (upregulation or 
downregulation) relative to the control group, and $P$ value <0.05.

**Statistical Analysis**
Statistical analysis was performed using SPSS 20.0. Significant
differences between two groups were analyzed by unpaired $t$-test 
and one-way ANOVA combined with a Student-Newman-Keuls 
(SNK) post hoc test. $P < 0.05$ was considered significant. Each 
assay was carried out as at least three biological replicates and 
three technical replicates.

**RESULTS**

**S. mutans MVs Promote C. albicans Biofilm Development**
The $S$. mutans MVs used for this study were isolated by 
ultracentrifugation and characterized as previously described 
(Wu et al., 2020). SEM images showed that $S$. mutans MVs 
enhanced $C$. albicans clustering and biofilm formation on the 
surfaces of cover glass (Figure 1A), hydroxyapatite discs 
(Figure 1B) and bovine dentin specimens (Figure 1C). In the 
$S$. mutans MV-treated group, there was biofilm extracellular 
matrix formation, and the biofilm structure was three-
dimensional (Figure 1).

**Effect of S. mutans MVs on C. albicans Biofilm Cariogenic Ability**
A $C$. albicans-bovine dentin biofilm model was employed to 
evaluate the effect of $S$. mutans MVs on $C$. albicans biofilm 
cariogenic ability. The dentin surface hardness loss of the $S$. 
mutans MV-treated group was 19.66%, which was significantly 
different from that of the control group (Figure 2A, $P < 0.05$). 
The increase in dentin surface roughness between the $S$. mutans 
MV-treated group and the control group was not significantly 
different (Figure 2B, $P > 0.05$). Compared to the control group 
and blank control group, the dentin surface morphology of the $S$. 

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**FIGURE 1** | The effect of $S$. mutans MVs on $C$. albicans biofilm formation. $C$. albicans biofilms were developed on (A) cover glasses, (B) hydroxyapatite discs, and (C) bovine dentin specimens. Scale bar, 5 µm.
mutans MV-treated group showed more dentin tubules exposure and broken dentin tubule (Figure 2C, Supplementary Figure 1).

**Effect of S. mutans MVs on C. albicans Based On Proteomic Analyses**

A total of 4345 proteins were identified in the S. mutans MV-treated and control groups by tandem mass tag (TMT) (Supplementary Table 1). For proteins of the S. mutans MV-treated group 1.2-fold changes (upregulation or downregulation) relative to the control group, and a P value <0.05 were deemed to be significant. The expression levels of 170 proteins were significantly changed as a result of C. albicans being treated with S. mutans MVs. The expression levels of 73 proteins were upregulated and 97 proteins were downregulated (Figure 3A). Principal component analysis (PCA) showed that the S. mutans MV-treated and control groups were clearly separated (Supplementary Figure 2). These results indicated that S. mutans MV significantly influenced the protein expression of C. albicans.

WoLF PSORT was employed to analyze the subcellular localization of proteins. The results showed that most of the significantly changed proteins were located in the nucleus and cytosol (Figure 3B). To identify the biological functions of significantly changed C. albicans proteins, GO enrichment analysis was performed (Figure 3C). The significantly changed proteins were mainly involved in biological processes such as oxidation–reduction processes and cellular components such as extracellular regions. KEGG analysis showed that the significantly changed proteins were mainly involved in galactose metabolism, which was similar to our previous findings that S. mutans MVs harboring Gtfs are involved in exopolysaccharide production in C. albicans biofilms (Wu et al., 2020). In addition, metabolic pathways, microbial metabolism in diverse environments and mineral absorption were also enriched (Figure 3D).

**Metabolomic Analysis of the Effect of S. mutans MVs on C. albicans**

In total, 15,379 metabolites were identified in the S. mutans MV-treated and control groups (Supplementary Table 2). Metabolites of the S. mutans MV-treated group with 2.0-fold changes (upregulation or downregulation) relative to the control group and a P value <0.05 were deemed to be significantly changed. The expression levels of 937 metabolites were

![Figure 2](https://example.com/image.png)

**FIGURE 2** | S. mutans MVs promote C. albicans biofilm formation to induce bovine dentin demineralization. (A) Dentine surface hardness loss (%SHL) (n = 8). (B) Dentine surface roughness increase (n = 8). (C) SEM images of dentin surfaces. Each field of view was magnified 4,000× and 8,000×. The red boxes indicate the magnified viewing area. The white arrows show the exposed dentin tubules. The data are presented as the means ± SD. *P < 0.05 vs control group.
upregulated, and those of 857 metabolites were downregulated (Supplementary Figure 3). Quantitative metabolomic data of C. albicans samples were used for hierarchical clustering (Figure 4A). To identify the biological functions of significantly changed C. albicans metabolites under S. mutans MV treatment, KEGG enrichment analysis was performed (Figure 4B). The significantly regulated metabolites were mainly involved in metabolic pathways, which was consistent with the proteomics results. In addition, biosynthesis of amino acids, alanine, aspartate and glutamate metabolism, and glutathione metabolism were significantly enriched in regulated metabolites. These results indicated complex metabolic regulation in S. mutans MV-treated C. albicans.

**Integrated Analysis of Proteome and Metabolome Data in C. albicans**

Proteomic and metabolomic data were integrated through the same KEGG pathway, and proteins and metabolites involved in significant changes in the same biological process were identified. Most of the proteins and metabolites were involved in metabolic pathways related to energy metabolism, carbohydrate metabolism, lipid metabolism, and amino acid metabolism. The results suggested that the interaction between host and pathogen plays a critical role in the regulation of metabolic pathways.
processes, including amino acid metabolism and carbohydrate metabolism, consistent with the proteomic and metabolomic results (Figure 5A). We further performed enrichment analysis of the KEGG pathways, and the top 10 KEGG pathways were identified (Figure 5B), which included metabolic pathways, biosynthesis of secondary metabolites, carbon metabolism, and biosynthesis of amino acids.

**DISCUSSION**

*C. albicans* biofilm consists of different morphological types of cells (yeast, pseudohyphal, and hyphal) and extracellular polymeric substances. *C. albicans* biofilm production is an important virulence factor of *C. albicans* (Lohse et al., 2018). Our study showed that *S. mutans* MVs promote *C. albicans* biofilm formation on the surface of cover glass, hydroxyapatite discs and bovine dentin specimens. This indicates that *C. albicans* can better adhere to tooth surfaces with the effect of *S. mutans* MVs. Experimental studies have reported that *C. albicans* enhances the severity of dental caries because of its synergistic action with classical cariogenic bacteria (Falsetta et al., 2014; Pereira et al., 2018; Kim et al., 2020). Coinfection with *S. mutans* and *C. albicans* increases the cariogenic potential of dental plaque biofilms, leading to aggressive carious lesions *in vitro* and *in vivo* (Falsetta et al., 2014; Sampaio et al., 2019). *C. albicans* colonization on the tooth root surface can disrupt the balance of dental plaque microbial ecology and enhance the cariogenic ability of dental plaque, leading to the severity of tooth demineralization and carious lesions (Du et al., 2021). However, the cariogenic ability of *C. albicans* biofilms with the influence of *S. mutans* MVs has not yet been evaluated.

To further investigate the effect of *S. mutans* MVs on *C. albicans* biofilm cariogenic potential, a *C. albicans* biofilm-bovine dentin model was employed according to Sampaio et al.’s experimental method (Sampaio et al., 2019). *S. mutans* MVs have no significant effect on the pH of the *C. albicans* biofilm culture medium supernatants, which was reported by our previous study (Wu et al., 2020). Enamel demineralization is mainly due to the acid production of plaque biofilms (Wu et al., 2020).
Gftfs are critical virulence factors of S. mutans (Cao and Lin, 2021). Our previous study found that S. mutans MV-treated group had more dentin tubule exposure than that of the control group. In the control group, C. albicans biofilms alone did not decrease bovine dentin hardness, which was consistent with previous research on C. albicans biofilms on dentin demineralization (Sampaio et al., 2019). The increase in dentin surface roughness between the S. mutans MV-treated group and the control group was not significant different. This result may be due to the adhesion of C. albicans on the bovine dentin surface influencing the surface roughness; the detection of surface roughness by CLSM can be easily affected by the environment, which could have led to the lack of a significant difference between the two groups. SEM images showed that the bovine dentin surface morphology of the S. mutans MV-treated group had more dentin tubule exposure and broken dentin tubules, indicating the erosion of peritubular dentin and consistent with previous research reports (Poggio et al., 2013; Su et al., 2021). Altogether, these findings provide evidence that S. mutans MVs increase bovine dentin demineralization provoked by C. albicans biofilms.

Proteomics analysis showed that a total of 4345 proteins were identified in the S. mutans MV-treated and control groups, similar to the findings of previous research on C. albicans proteomics detection (Wang et al., 2020a). The significantly changed proteins were mainly involved in biological processes involving oxidation-reduction processes and cellular components, including extracellular regions. Meanwhile, significantly changed proteins were mainly related to galactose metabolism, and metabolic pathways and microbial metabolism were also enriched. Biological processes and metabolic pathways are essential for C. albicans biofilm formation (Munusamy et al., 2021). In the proteomics analysis of S. mutans-C. albicans mixed-species biofilms, C. albicans proteins involved in carbohydrate metabolism and cell wall components such as mannan and glucan were upregulated (Ellepola et al., 2019). Metabolomic analysis demonstrated enhanced expression of C. albicans metabolites related to metabolic pathways when cocultured with S. mutans MVs, including biosynthesis of amino acids, alanine, aspartate and glutamate metabolism, and glutathione metabolism, consistent with the proteomic results. The combination of proteomics and metabolomics analysis indicated that significantly regulated proteins and metabolites were involved in amino acid and carbohydrate metabolism. Taken together, S. mutans MVs enhance the expression of proteins and metabolites of C. albicans related to carbohydrate metabolism.

S. mutans MVs carry various virulence proteins (Senpuku et al., 2019; Cao and Lin, 2021). Our previous study found that S. mutans MVs contain metabolic enzymes such as Gftfs, Gbps and DedX, which are related to carbohydrate metabolism (Cao et al., 2020). Gftfs are critical virulence factors of S. mutans that participate in sucrose metabolism and mediate sucrose-dependent adhesion, increasing the colonization of oral microorganisms (Loesche, 1986; Zhang et al., 2021). Gftfs also play a functional role in the cross-kingdom interactions of S. mutans and C. albicans. In the sucrose environment, S. mutans secretes Gftfs and breaks down sucrose into glucose and fructose, and C. albicans can utilize monosaccharides efficiently (Ellepola et al., 2017). S. mutans GtB binds to the cell surface of C. albicans and promotes C. albicans cell accumulation (Ellepola et al., 2017; Koo et al., 2018). Our previous study also demonstrated that S. mutans MVs harboring Gftfs promote exopolysaccharide production in C. albicans biofilms, and C. albicans genes correlated with mannan and glucan synthesis were upregulated by the effect of Gftfs in S. mutans MVs (Wu et al., 2020). Therefore, the reason why S. mutans MVs enhance C. albicans carbohydrate metabolism may be due to the effect of metabolic enzymes such as Gftfs carried by S. mutans MVs.

Carbohydrate metabolism can influence C. albicans pathogenicity (Vieira et al., 2010; En et al., 2014). The competitive consumption of glucose by C. albicans and macrophagocytes disrupts the glucose homeostasis of the host, which results in rapid macrophagocyte death (Tucey et al., 2018). C. albicans can escape from macrophage attack with the recovery of glycolysis ability (Ries et al., 2018). Moreover, recent research found that the carbohydrate metabolism of C. albicans biofilms is correlated with dental caries. S. mutans and C. albicans are considered microbiological risk markers of early childhood caries (Cui et al., 2021; Grier et al., 2021; Alkhars et al., 2022). Research found that C. albicans genes and proteins related to sugar transportation, pyruvate breakdown and the glyoxylate cycle were upregulated in S. mutans-C. albicans mixed-species biofilms (Ellepola et al., 2019). The transcriptomic analysis of dental plaque from the tooth root surface found that C. albicans genes associated with metabolic activity and glucose transportation were significantly enhanced in the dental caries group compared to the caries-free group (Ev et al., 2020). Our results also demonstrate that S. mutans MVs promote C. albicans carbohydrate metabolism and dentin demineralization provoked by C. albicans biofilms. Therefore, C. albicans promotes the occurrence and development of dental caries may be through the regulation of carbohydrate metabolism.

In summary, our present study showed that S. mutans MVs promoted C. albicans biofilm formation on the surfaces of cover glass, hydroxyapatite discs and bovine dentin specimens and increased bovine dentin demineralization provoked by C. albicans biofilms. Meanwhile, S. mutans MVs increased the protein and metabolite expression of C. albicans related to carbohydrate metabolism. Altogether, these results increase our understanding of S. mutans MVs on C. albicans virulence and pathogenicity. However, the effect of S. mutans MVs on C. albicans in dental plaque biofilms in vitro and in vivo needs to be confirmed, and the mechanism by which carbohydrate metabolism influences C. albicans cariogenic ability is complex and remains unclear. Further studies are needed to close the gap in knowledge of the contribution of S. mutans MVs to C. albicans virulence and pathogenicity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. The proteomic data have been deposited into the iProX database.
database https://www.iprox.cn/page/DSV021.html;?url=16521827363451p password: cN3M), and metabolic data is uploaded to the metabolights database (MTBLS5048 www.ebi.ac.uk/metabolights/MTBLS5048).

**AUTHOR CONTRIBUTIONS**

RW and HL designed the research. RW executed the experiments and analyzed the data. GC and YC provided technical and theoretical support. RW, WZ and HL co-wrote and revised the manuscript. All authors read and approved the submitted versions.

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**SUPPLEMENTARY MATERIAL**

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