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Unveiling the complexity of early childhood caries: *Candida albicans* and *Streptococcus mutans* cooperative strategies in carbohydrate metabolism and virulence

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ABSTRACT

Objective: To explore the mechanisms underlying the virulence changes in early childhood caries (ECC) caused by *Candida albicans (C. albicans)* and *Streptococcus mutans (S. mutans)*, with a focus on carbohydrate metabolism and environmental acidification.

Methods: A review of literature was conducted to understand the symbiotic relationship between *C. albicans* and *S. mutans*, and their role in the pathogenesis of ECC. The review also examined how their interactions influence carbohydrate metabolism and environmental acidification in the oral cavity.

Results: *C. albicans* and *S. mutans* play crucial roles in the onset and progression of ECC. *C. albicans* promotes the adhesion and accumulation of *S. mutans*, while *S. mutans* creates an environment favorable for the growth of *C. albicans*. Their interactions, especially through carbohydrate metabolism, strengthen their pathogenic potential. The review highlights the importance of understanding these mechanisms for the development of effective management and treatment protocols for ECC.

Conclusion: The symbiotic relationship between *C. albicans* and *S. mutans*, and their interactions through carbohydrate metabolism and environmental acidification, are key factors in the pathogenesis of ECC. A comprehensive understanding of these mechanisms is crucial for developing effective strategies to manage and treat ECC.

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Introduction

Early childhood caries (ECC) is a prevalent chronic disease among young children and represents a significant public health challenge across the globe. It affects approximately half of all preschool-aged children, displaying an epidemic spread with notable geographical disparities, particularly among impoverished communities [1,2]. ECC can lead to severe destruction of the dental crown in primary teeth, resulting in increased pain and infection, ultimately compromising the child's quality of life [3].

Dental caries is recognized as a multifactorial disease, driven by the fermentation of dietary carbohydrates, acid production through bacterial metabolism, and biofilm-mediated dysbiosis that culminates in the demineralization of tooth tissues [4–6]. Recent evidence indicates that the role of *Streptococcus mutans* (*S. mutans*) in ECC is paralleled by the involvement of *Candida albicans* (*C. albicans*), both of which have been frequently detected in the oral saliva and dental plaque of ECC-affected patients [7]. A study examining saliva and dental plaque from 30 preschool children across two different locations revealed that those with ECC exhibited higher quantities of *C. albicans* and *S. mutans* compared to their healthy counterparts, as determined by log DNA copy numbers and their proportion relative to total oral bacteria [8]. The tooth surface is increasingly acknowledged as a conducive environment for the colonization by *C. albicans* and the formation of pathogenic dental biofilms [9].

Filamentation is considered a key virulence attribute of *C. albicans*, wherein its ability to transition from yeast to hyphal is deemed critical for pathogenicity [10,11]. However, recent research by Xiang et al. [12] suggests that filamentation may not be as essential for *C. albicans'* pathogenicity within the oral niche as previously thought. Isolates from children with severe ECC (S-ECC) displayed broad phenotypic variations but consistently exhibited traits conducive to cariogenesis, such as high proteinase activity, acidogenicity, and acid tolerance. Remarkably, these isolates enhanced sucrose metabolism and biofilm acidogenicity, creating a highly acidic environment (pH < 5.5) and forming robust biofilms with *S. mutans*, irrespective of their filamentous state. These findings suggest that the morphology of *C. albicans* does not solely determine its adaptive

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strategies on the tooth surface. Clinical studies have corroborated the frequent detection of *C. albicans* in dental plaque from children with S-ECC, implicating its association with increased caries severity [13–15]. The virulence of *C. albicans* within the oral cavity is significantly influenced by its interactions with commensal bacteria [16].

S. mutans, an acidogenic bacterium capable of biofilm formation, is a well-established pathogen in the context of ECC. It thrives in the acidic microenvironments created within dental biofilms, which confers a selective advantage over other microbial community members [17]. Notable research has demonstrated that *C. albicans* engages in biochemical, metabolic, and physical interactions with *S. mutans*, leading to the establishment of highly cariogenic interkingdom biofilms [18–20]. Given that the presence of *C. albicans* can alter the oral microbiome composition even in early biofilm development stages, it is essential to consider its potential role within healthy oral ecosystems and its impact on bacterial populations [21].

This review diverges from previous discussions centered on *Candida-Streptococcus* biofilm interactions. Instead, we concentrate on the aspects of carbohydrate metabolism and environmental acidification, exploring the cooperative mechanisms employed by *C. albicans* and *S. mutans* that modulate their growth and virulence within the oral cavity. We aim to underscore the implications of these interactions on the oral health of children.

Alteration of oral microbiome composition in ECC through interactions between *C. albicans* and *S. mutans*, and the influence of external factors

In the oral cavity, environmental factors such as diet and oral hygiene play a pivotal role in the progression of ECC. *S. mutans* is a primary cariogenic bacterium that secretes glucosyltransferases (Gtfs), enzymes that catalyze the conversion of sucrose into extracellular glucans. These glucans form the major components of exopolysaccharides (EPSs), which facilitate *S. mutans* adhesion and promote microbial cohesion within the oral biofilm. Notably, glucosyltransferases B (GtfB), a key exoenzyme, binds to the mannan layer of the *Candida albicans* cell wall, enhancing extracellular matrix formation and fostering coexistence within biofilms [22]. The *C. albicans Checkpoint Kinase 1* (*CHK1*) gene as an essential component of two-component signal transduction system (TCS) plays a significant role in modulating the pathogenicity of *C. albicans* and its response to the host environment [23]. The *CHK1* gene in *C. albicans* is involved in various functions, including enhancing its virulence, upregulating the quorum sensing and enabling responses to stress [24]. Recent studies have highlighted the importance of *CHK1* gene during oral mucosal infections, its plays a crucial role in mediating cross-kingdom interactions, which can potentially increase the cariogenic potential of these microbiomes within the oral cavity [23,25,26].

The expression of Gtfs is regulated by the *VicK* gene, part of the VicRKX three-component system in *S. mutans*. Research conducted by Yelan Deng and colleagues [27] demonstrated that mutations in the *VicK* gene influenced EPS production and biofilm formation. This finding aligns with Yaqi Liu's observations that the *C. albicans CHK1* gene affects the VicRK pathway in *S. mutans*, enhancing the expression of *GtfB, GtfC*, and *GtfD* genes, and subsequently promoting biofilm and EPS production [26]. The VicRKX system comprises VicK, a Histidine protein kinase; VicR, a global response regulator; and VicX, a putative hydrolase. Beyond its capacity to produce EPS via Gtf upregulation, *S. mutans* collaborates with *C. albicans* in the oral environment, contributing to the initiation and progression of ECC.

Recent RNA-Seq study reveals that *C. albicans* can upregulate genes associated with carbohydrate transport and metabolism when co-cultured with *S. mutans* [19]. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway impact analysis indicated that both pyruvate and galactose metabolism pathways were enhanced in *S. mutans* during co-culture [19]. These findings suggest that *C. albicans* significantly influences the carbohydrate utilization patterns of *S. mutans*. Subsequent research has identified that *S. mutans* genes related to the phosphotransferase system (PTS), ABC sugar transporter system, and carbohydrate metabolism, along with glycogen biosynthesis, are also upregulated in the presence of *C. albicans* [18]. Proteomic analysis of dual-species biofilms has shown increased activity in carbohydrate metabolism, glucan biosynthesis and transferase activity, and peptidoglycan and cell wall biosynthesis in *S. mutans* [18]. These studies collectively highlight the possibility that *C. albicans* contributes to the acidification of mixed-species biofilms, in part through a GtfB-mediated mechanism, thereby enhancing the acidogenic and aciduric capabilities of *S. mutans*.

Gtfs and their role in ECC pathogenesis

Gtfs, including GtfB, GtfC, and GtfD, are critical enzymes in the pathogenesis of *S. mutans*-mediated ECC. These enzymes enable *S. mutans* to enhance biofilm formation and colonization by accumulating extracellular polysaccharides (EPSs) [28]. Although the Gtfs share structural similarities, each possesses unique functions. GtfB, for instance, primarily synthesizes insoluble glucans that facilitate microbial attachment and alter biofilm structure, playing an essential role in the interaction with other oral microbiota [29] (Figure 1).

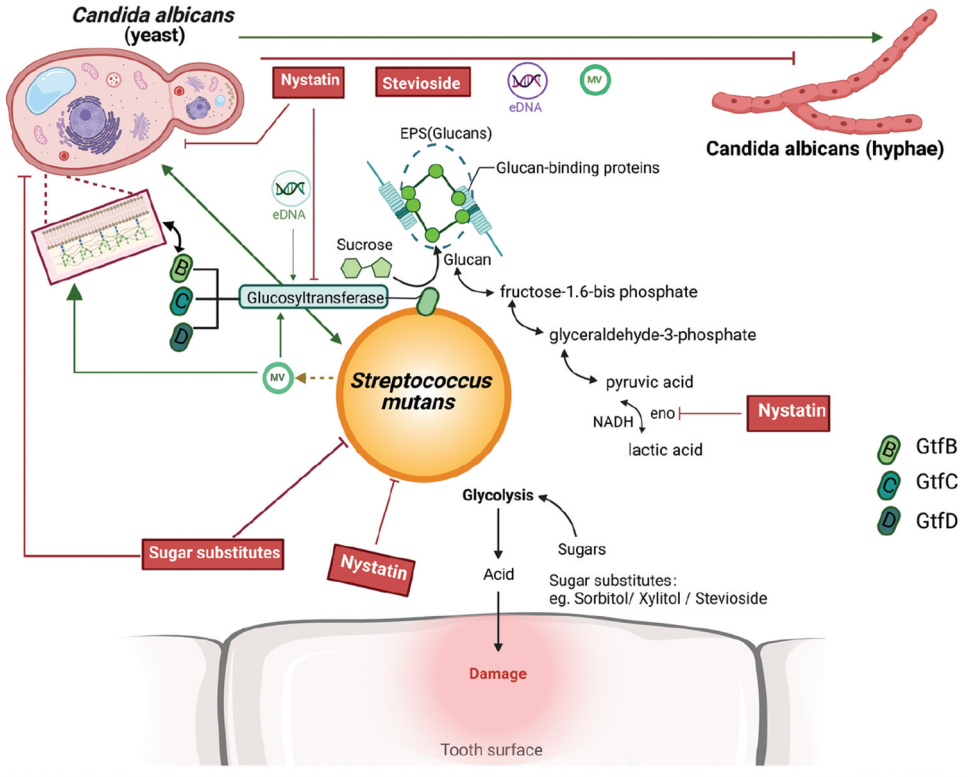

Figure 1. Alteration of the Virulence of *C. albicans* effecting with *S. mutans* in the development of ECC. *S. mutans* prompt both the growth and pathogenesis of *C. albicans* in the oral via secreted and cell surface molecules. *S. mutans* secrete Glucosyltransferase that can attach firmly to the *C. albicans* cell wall that are critical for pathogenesis and virulence. EPSs present on the cell surface of *S. mutans* mediate the sugar metabolism transforming sucrose to glucan to sustain the virulence of *S. mutans* in the oral, which enhances their capacity to cariogenic. (Created with BioRender.com).

In a dual-species biofilm system, *S. mutans* influences a wide array of genes and proteins involved in carbohydrate metabolism, sugar transport systems, glycolysis, pyruvate degradation, and the production of ethanol and acetate, as well as the tricarboxylic acid cycle and electron transport chain [18,30]. Notably, while *C. albicans* predominantly utilizes glucose and cannot efficiently metabolize sucrose, *S. mutans* can decompose ingested sucrose into glucans via the Gtf system. This activity supports the growth of *C. albicans* and enhances acid production in the oral cavity [18,28,31,32]. Furthermore, the exoenzyme GtfB from *S. mutans* binds to the mannan layer of the *C. albicans* cell wall, promoting the in situ production of extracellular α-glucans. This interaction likely affects *C. albicans* colonization, and the resultant EPSs provide additional bacterial binding sites for *S. mutans* [22,33,34]. The structure of the *C. albicans* cell wall is thus crucial not only for its growth and survival but also for mediating interactions with the extracellular environment and may play a pivotal role in ECC development.

Cell wall compositions and their impact on ECC

The cell wall of *C. albicans* comprises an outer layer of mannan fibrils and an inner core of cross-linked chitin and β-glucan, covalently connected to mannoproteins modified by N-linked and/or O-linked mannosylation and phosphomannosylation. These modifications regulate the dynamic nature of the fungal wall and contribute to the complexity of mechanisms controlling its structure and viscoelastic properties [35,36]. The composition and architecture of the *C. albicans* cell wall are also pivotal in modulating the host immune response. In mixed-species biofilms, *S. mutans* can enhance *C. albicans* genes involved in hyphal formation and cell wall properties, resulting in the upregulation of genes related to filamentous growth and cell wall components such as mannans and glucans, thereby reinforcing *Candida* biology and virulence [18].

The architecture of the *C. albicans* cell wall is influenced by the available carbon source. Research by Elizabeth R. Ballou and colleagues [37] demonstrated that L-lactate, produced by host cells or bacteria within the microbiota, can trigger β-glucan masking in *C. albicans*. The level of β-glucan exposure is a critical pathogen-associated molecular pattern (PAMP), and their findings indicate that changes in carbon source can regulate the visibility of *C. albicans* to the immune system and influence its virulence. Moreover, the expression of enzymes that link mannans to β-glucan in the *C. albicans* cell wall is affected by different carbon sources. It has been

Table 1. The impact of various external factors in the dual-species interaction.

Factors	Dual-species Biofilms	*C. albicans*	*S. mutans*	Sugar Metabolism	References
Membrane Vesicles (MVs)	+	±	Not correlation	+	[38]
Extracellular DNA (eDNA)	-	±	+	Not mentioned	[39]
Streptococcus Parasanguinis	-	-	-	-	[40]
Lactobacillus Plantarum	-	-	-	Not mentioned	[41]
L. P ATCC 14917	-	-	±	-	[41]
L. P 108	-	-	-	Not mentioned	[42]
L. P CCFM8724	Not mentioned	-	-	Not mentioned	[43]
Stevioside	-	-	+	-	[44]
Nystatin	Not mentioned	-	+	-	[45]
Xylitol	Not mentioned	-	-	Not mentioned	[46]
Sorbitol	Not mentioned	-	-	Not mentioned	[46]

'+' means affected, '-' means not affected.

suggested that the outer wall mannans function as a protective shield over the β-glucan layer [24]. These alterations in β-glucan exposure appear to be dependent on the carbon source rather than pH levels. Specifically, *C. albicans* can mask β-glucan in its cell wall when utilizing lactic acid – a byproduct of *S. mutans* metabolism – as an energy source, which is highly acidogenic in the oral cavity. Collectively, these findings suggest that in the oral environment, the presence of *S. mutans* alters the carbon sources and thus reducing the exposure of β-glucan on the *C. albicans* cell wall, facilitating the binding of GtfB to mannans (Table 1).

Role of membrane vesicles (MVs) in ECC pathogenesis

MVs of *S. mutans* are bilayer membranous structures formed when a section of the cytoplasmic membrane protrudes and buds off, encapsulating an array of components such as proteins, nucleic acids, lipids, and metabolites. These MVs are essential for processes like cell wall synthesis, cell-to-cell communication, bacterial adhesion, and biofilm formation [47–49]. Notably, *S. mutans* MVs are implicated in caries development, aiding in both the formation of its own biofilms and the colonization of host sites, and also promoting the biofilm formation of *C. albicans* on tooth surfaces [38,50,51]. Specifically, *S. mutans* MVs have been shown to enhance demineralization of bovine dentin by *C. albicans* biofilms and to augment the expression of proteins and metabolites in *C. albicans* related to carbohydrate metabolism [52]. Furthermore, Gtfs harbored within *S. mutans* MVs can increase EPS production, thereby boosting *C. albicans* biofilm formation [38,48]. Even under different pH conditions, *S. mutans* continues to release MVs containing proteins associated with cariogenesis, contributing to the impairment of host cell function. Yina Cao's bicinchoninic acid (BCA) assay results revealed that *S. mutans* discharges more MVs under acidic conditions than neutral ones, suggesting that MVs production amplifies the virulence of *S. mutans* and enhances adherence to tooth surfaces, ultimately leading to caries development [48]. Additionally, MVs in *S. mutans* have been identified as vehicles for eDNA release. Investigation into a putative glycosyltransferase of *S. mutans*, *SMU_833*, which is thought to modulate biofilm matrix dynamics, revealed that an increase in eDNA was accompanied by elevated production of MVs. This suggests that *SMU_833* may influence biofilm matrix composition through MV-mediated modulation of eDNA release [32].

eDNA in ECC development

eDNA is a critical component of the dental plaque biofilm and the biofilm extracellular matrix of *S. mutans*. It serves multiple functions, including maintaining biofilm structural integrity, initiating adhesion to dental surfaces, acting as a nutrient source, and facilitating horizontal gene transfer [53]. In the development of caries, the release of eDNA via MVs and lysis has been proposed as a potential virulence factor for *S. mutans*. Notably, eDNA and glucans have been observed to colocalize within biofilms [50], with glucans providing a supportive matrix for eDNA, particularly in environments rich in sucrose [54]. The presence of carbohydrates may trigger the activation of catabolite control protein A (CcpA), which regulates the two-component system (TCS) LytST, leading to degradation of the bacterial cell wall and subsequent eDNA release [53]. Under acidic conditions, eDNA contributes to the formation of insoluble glucan-independent biofilms by *S. mutans* [55].

Recent study highlights the essential role of eDNA in maintaining the structural integrity of dual-species biofilms formed by *S. mutans* and *C. albicans*. Interestingly, the removal of eDNA significantly disrupts the formation and structure of these dual-species biofilms without affecting the growth of either organism [39]. eDNA is particularly influential during the initial attachment and developmental stages of biofilm formation, but less so in mature biofilms.

These findings underscore the potential of targeting eDNA to mitigate dental plaque formation on tooth surfaces and promote oral health.

The critical role of saliva in regulating *C. albicans* and *S. mutans* interactions

In the oral cavity, various factors could shape the microbiota, including internal conditions and exogenous dietary intake. These factors significantly influence the interactions between the cariogenic bacterium *S. mutans* and the opportunistic fungal pathogen *C. albicans*. Understanding the impact of these factors on such microbial niches may offer new insights into preserving the dynamic balance of the oral microbiome.

Saliva as a represent of internal factor plays a multifaceted role in oral health by mediating pH buffering, lubrication, tooth mineralization, and enhancing host defenses [56]. Among its components, mucin O-glycans, salivary glucose, and amylase are particularly noteworthy. Amylase, a major salivary constituent, is thought to contribute to the reduction of plaque acids produced by *S. mutans* that can dissolve dental enamel [57]. Mucins, which are large gel-forming polymers, constitute a significant part of the salivary mucus barrier, with MUC5B and MUC7 being the predominant mucins in the human oral cavity [58,59]. [60] Notably, mucin O-glycans have been shown to inhibit hyphal formation in *C. albicans* through the Nrg1 pathway and regulate microbial community behaviors [58].

Recent research by Caroline A. Werlang et al. [59], utilizing an ex vivo saliva model, identified that mucin glycans alone are sufficient to suppress both genetic transformation and biofilm formation in *S. mutans*. This finding suggests that mucin glycans can inhibit quorum-sensing-regulated phenotypes, thereby providing evidence for their significant role in modulating the oral microbiota and presenting them as potential therapeutic agents for maintaining oral health [61].

Mechanistic studies focusing on the impact of other microorganisms on the dual-species system comprising *C. albicans* and *S. mutans* could establish a more mature and stable framework for future research. Such studies would enhance our understanding of the interaction mechanisms between *C. albicans* and *S. mutans* in the oral cavity, especially for potential experiments involving targeted pathway analyses (Figure 2).

Insights from interactions with other oral environmental factors

The oral cavity harbors a diverse microbial community comprising fungi and bacteria, which contributes to the increased complexity and severity of oral diseases. Among the bacterial species, *Streptococci* are predominant in the oral cavity and saliva, encompassing groups such as mitis, sanguinis, anginosus,

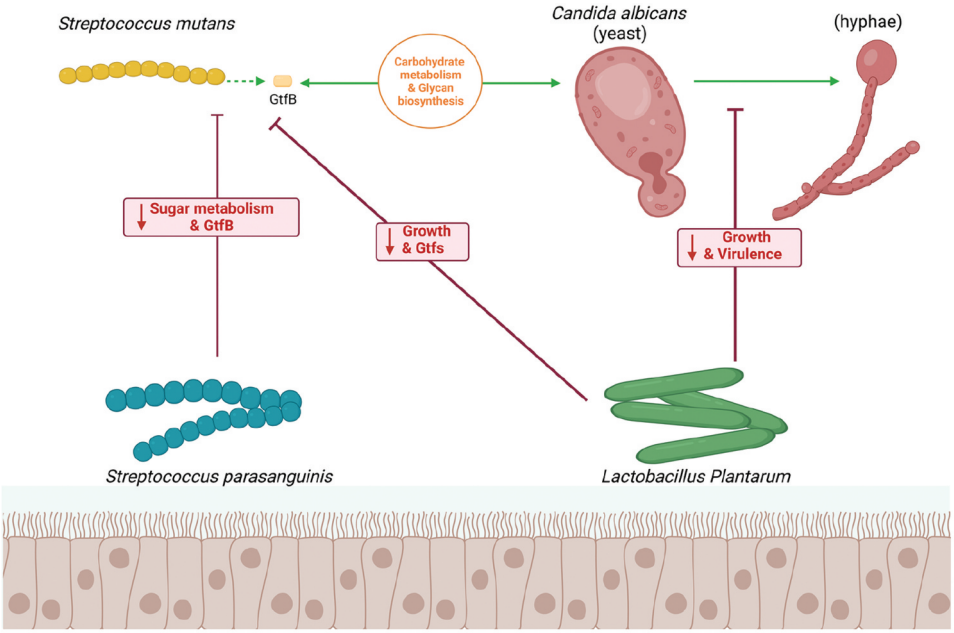

Figure 2. Model of the interplay between *C. albicans, S. mutans*, and *Streptococcus parasanguinis/Lactobacillus plantarum* in the human oral. *S. mutans* use GtfB to attach firmly to *C. albicans* to prompt each other's growth which could regulate the carbohydrate metabolism and glycan biosynthesis. *L. plantarum* reduce the growth of both *C. albicans* and *S. mutans;* however, *L. plantarum* has also been shown to suppress the *C. albicans* virulence through the hyphal morphogenesis an·d inhibit the expression of GtfB of *S. mutans*. Like *L. plantarum, S. parasanguinis* also disturb the sugar metabolism and reduce the expression of GtfB of *S. mutans*. (Created with BioRender.com).

salivarius, downei, and mutans groups [62]. Notably, common colonizers in the oral cavity include *Streptococcus parasanguinis (S. parasanguinis)*, *Streptococcus oralis (S. oralis)*, *Streptococcus gordonii (S. gordonii)*, *Streptococcus mitis (S. mitis)*, and *Streptococcus sanguinis (S. sanguinis)* [63]. Due to *S. mutans* and *C. albicans* association with the development of caries, mutans *Streptococci* – *Candida* interactions have raised attention. Mutans *Streptococci* including *S. mutans* and *Streptococcus sobrinus (S. sobrinus)* is among the most abundant genera present in caries incidence and prevalence, some studies have indicated that children harboring both *S. mutans* and *S. sobrinus* exhibit an elevated risk of caries development [64,65]. The association of *S. mutans* and *C. albicans* with caries development has drawn significant attention to *Streptococci-Candida* interactions. A recent study highlighted the essential cooperative strategy where the co-adherence of *C. albicans* to *Streptococci* is crucial for its robust colonization within the oral cavity [66,67].

S. parasanguinis

S. parasanguinis, a member of the mitis group *streptococci* like *S. mutans*, is commonly found on the tongue dorsum and within the oral cavity [63,68]. Unlike *S. mutans*, *S. parasanguinis* can antagonize oral pathogens by producing hydrogen peroxide (H_2O_2), which also exhibits antimicrobial activity against *S. mutans* [69,70]. Recent study demonstrates that *S. parasanguinis* significantly disrupts the biofilm synergy of *C. albicans* and *S. mutans*, even in the absence of H_2O_2 [40]. *S. parasanguinis* specifically hinders biofilm development by altering the sugar metabolism of *S. mutans* and inhibiting the activity of GtfB, a critical enzyme for *S. mutans* to bind to *C. albicans* mannan [40]. These findings suggest that *S. parasanguinis* could be exploited to disrupt the synergistic interaction between *C. albicans* and *S. mutans* in ECC, underscoring the pivotal roles of sugar metabolism and Gtf enzymes of cariogenic ability in the dual-species biofilm system.

Lactobacillus plantarum (L. plantarum)

L. plantarum has been shown to inhibit the growth and biofilm formation of the *C. albicans-S. mutans* system, similar to the effects observed with *S. parasanguinis* [41,42,71]. Studies have indicated that *L. plantarum* can modulate the gene expression of *S. mutans* and *C. albicans* involved in metabolic pathways. Notably, the *L. plantarum strain 108* reduces the attachment and biofilm formation of *S. mutans* in both initial colonization and preformed biofilms by downregulating the expression of the *Gtf* genes (*gtfB, gtfC, and gtfD*) and the *C. albicans* genes *HWP1, ALS1,* and *ALS3* [42] Additionally, strains *L. plantarum ATCC 8014* and *ATCC 14917* have been shown to downregulate genes involved in exopolysaccharide (EPS) formation, carbohydrate metabolism, and glycan biosynthesis and metabolism in the mixed-species biofilms [41]. Intriguingly, the inhibitory effect of *L. plantarum* was found to be more pronounced at higher sucrose concentrations (1%) compared to lower ones (0.1%) [41]. Using rat models, Qiuxiang Zhang's laboratory has reported a significant antagonistic relationship between *L. plantarum CCFM8724* and the mixed-species biofilms, with a potential for oral cavity colonization in both treatment and prevention contexts [43]. Collectively, these findings highlight the significant role of *L. plantarum* in modulating sugar metabolism and virulence gene expression, particularly affecting the levels of *S. mutans* and *C. albicans*.

The influence of external factors on oral microbiomes

Antifungal drugs

Antifungal drugs are well-known for targeting essential cell wall components such as β-glucans, mannans, and chitin, which play a crucial role in the interaction with GtfB of *S. mutans*. Nystatin, an antifungal agent with both fungicidal and fungistatic properties, is commonly used topically in dentistry [72]. This study shows that nystatin can alter the formation and characteristics of *C. albicans-S. mutans* dual-species biofilms in vitro, consistently reducing biofilm volume and microcolony size on substrate layers [45]. Notably, nystatin-treated biofilms exhibit distinctive halo-shaped microcolonies, and there is a downregulation in the core EPS coverage and expression of the *gtfD* and *atpD* genes of *S. mutans* [45]. Furthermore, investigations into the use of nystatin oral rinse have indicated its impact on oral carriage of *C. albicans* and *S. mutans*, suggesting that oral antifungal treatments may effectively influence *S. mutans* salivary carriage [73]. These findings point to antifungal treatments as a growing potential strategy for managing cariogenic microorganisms in oral environment. The influence of dietary factors will be discussed below.

Sugar substitutes

Sugar substitutes, representing exogenous dietary factors, play a significant role in shaping the oral microbiome and the development of ECC. The study revealed that different sugars could induce the alterations of the components and functions of microbiome biofilms [74]. The consumption of sugars is especially critical given its impact on the mixed-

species biofilm system in the oral cavity [75–77]. Sucrose, a fermentable sugar, serves as a substrate for acid and exopolysaccharide production by microorganisms and is widely recognized as the most cariogenic carbohydrate related to dental caries [78–80]. Sugar substitutes can indeed serve as an alternative to sucrose helping to reduce the risk of caries. In the recent study, Galacto-oligosaccharide (GOS), a low-calorie sweetener, has been found to suppress the hyphal formation of *C. albicans* and the acid resistance of the *C. albicans-S. mutans* interaction [81]. The finding suggests that sugar substitutes may have potential in inhibiting the growth and virulence of *C. albicans* and its cooperation with *S. mutans,* which could contribute to the prevention of caries.

In the quest for sugar substitutes that could prevent for caries, one research has indicated that stevioside significantly inhibits growth and biofilm formation in mixed-species cultures, suggesting its potential as an alternative to sucrose [44]. Interestingly, the addition of 1% sucrose to these cultures counteracted the inhibitory effects of stevioside, highlighting the importance of reducing sucrose intake for dental caries management [44,82]. Among various commercially available sugar substitutes, stevioside, along with other levorotatory carbohydrates such as xylitol and sorbitol, has demonstrated similar inhibitory effects on the mixed-species biofilm [83]. These sugar substitutes appear to be effective carbon sources in disrupting the synergy between *C. albicans* and *S. mutans*. The impact of stevioside and other sugar substitutes on sugar metabolism pathways and transcriptomic profiles is complex, pointing to multifaceted mechanisms underlying their effects on oral health [46].

Fluoride (F) as a caries protective agent

F is recognized for its role as a protective agent against caries, due to its ability to stabilize the dynamics of remineralization and demineralization in the oral environment [84,85]. This study indicates that children with access to fluoridated water and supplements exhibit a reduced risk of ECC [86]. The incorporation of F into oral care regimens may enhance enamel resistance to infection by diminishing the attachment of cariogenic bacteria and glycogen accumulation, thus offering a safe protective measure for children [87,88].

Research has demonstrated that F significantly suppresses the expression of genes involved in the transport and modification of mono- and oligosaccharides, as well as enzymes associated with glucose metabolism [89]. Although the impact of F on enolase gene expression was not found to be significant in one study, it is hypothesized that F's mediation of sugar metabolism, resulting in reduced acid production, may be the primary mechanism of action. Notably, the efficacy of F treatments in inhibiting the growth of *S. mutans* biofilms is closely linked to the stage of biofilm formation and the F concentration, with treatments being more effective during the early stages of biofilm development than in mature biofilms [90]. These findings suggest that F could potentially serve as an inhibitor of dual-species biofilms formed by *S. mutans* and *C. albicans*.

Supporting this notion, Thayse Yumi Hosida et al. [91] proposed that in mixed-species biofilms, F could mitigate the drop in pH and thereby reduce cariogenicity. Specifically, sodium F combined with hexametaphosphate (HMP) led to higher pH levels both before and after sucrose exposure [61,90]. Additionally, F has been shown to interfere with the acidogenicity of *S. mutans* by modulating gene expression related to glycosyltransferases and glycolytic pathways, leading to an increase in pH [90]. Another study reported that dual-species biofilms treated with calcium glycerophosphate and 500 ppm F exhibited a significant pH increase, resulting in the highest pH values and concentrations of F and calcium within the biofilm biomass, regardless of sucrose exposure [92].

Collectively, these findings highlight the significance of F in combating cariogenic infections.

Conclusion and future perspectives

The interaction between *C. albicans* and *S. mutans* is of paramount importance, as both are prevalent oral commensals that pose challenges in pediatric oral healthcare. While the cariogenic potential of *S. mutans* is relatively well-understood, significant knowledge gaps remain regarding the biological capabilities of mixed-species biofilms to cause ECC. Addressing these gaps is urgent, particularly as both *C. albicans* and *S. mutans* exhibit increasing virulence with more complex carbohydrate consumption. Moreover, examining physical interactions alone is insufficient, given the imminent threat of antibiotic resistance faced by both species. The distinct carbohydrate metabolism that largely regulates their interplay provides insights into their persistent attachment and virulence. Therefore, understanding the role of sugar metabolism in facilitating these processes offers opportunities for intervention before further invasion and provides broader perspectives on microbial interactions within various niches.

To date, numerous approaches have been developed to target the cross-kingdom biofilm interaction in oral disease. Gradually, the interaction between *S. mutans* and *C. albicans* has emerged as a significantly potential treatment target for ECC. Among the cooperative strategies reviewed here,

targeting Gtfs may offer the best choice to inhibit the production of EPSs and impede the co-adhesion between the two species. When dealing with children with poor compliance, rather than strictly restricting carbohydrate intake by eliminating sugar and other foods, a more feasible approach involves replacing sucrose with sugar substitutes and applying F under careful evaluation to delay the development of ECC and potentially achieve preventive effects.

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Authors' contributions

Pingping Jin: Conceptualization, Investigation, Data curation, Writing – original draft, Visualization. Lu Wang: Conceptualization, Investigation, Data curation, Writing – original draft, Visualization. Daozhen Chen: Data curation, Writing – review & editing. Yu Chen: Conceptualization, Writing – review & editing, Supervision, Project administration.

Data availability statement

I have shared my data in my review.

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