

Title	Cell wall polysaccharides of streptococci: a genetic and structural perspective
Authors	Kampff, Zoe;van Sinderen, Douwe;Mahony, Jennifer
Publication date	2023-01-01
Original Citation	Kampff, Z., Van Sinderen, D. and Mahony, J. (2023) 'Cell wall polysaccharides of streptococci: a genetic and structural perspective', Biotechnology Advances, 69, 108279 (11pp). doi: 10.1016/j.biotechadv.2023.108279
Type of publication	Article (peer-reviewed);journal-article
Link to publisher's version	10.1016/j.biotechadv.2023.108279
Rights	© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). - https://creativecommons.org/licenses/by/4.0/
Download date	2026-10-28 03:56:14
Item downloaded from	https://hdl.handle.net/10468/15249



Research review paper

Cell wall polysaccharides of streptococci: A genetic and structural perspective

Zoe Kampff, Douwe van Sinderen, Jennifer Mahony^{*}

School of Microbiology and APC Microbiome Ireland, University College Cork, Cork T12 YT20, Ireland

ARTICLE INFO

Keywords:

Lactic acid bacteria
Rhamnose-glucose polysaccharide
Exopolysaccharide
Serotype
Bacteriophage

ABSTRACT

The *Streptococcus* genus comprises both commensal and pathogenic species. Additionally, *Streptococcus thermophilus* is exploited in fermented foods and in probiotic preparations. The ecological and metabolic diversity of members of this genus is matched by the complex range of cell wall polysaccharides that they present on their cell surfaces. These glycopolymers facilitate their interactions and environmental adaptation. Here, current knowledge on the genetic and compositional diversity of streptococcal cell wall polysaccharides including rhamnose-glucose polysaccharides, exopolysaccharides and teichoic acids is discussed. Furthermore, the species-specific cell wall polysaccharide combinations and specifically highlighting the presence of rhamnose-glucose polysaccharides in certain species, which are replaced by teichoic acids in other species. This review highlights model pathogenic and non-pathogenic species for which there is considerable information regarding cell wall polysaccharide composition, structure and genetic information. These serve as foundations to predict and focus research efforts in other streptococcal species for which such data currently does not exist.

1. Introduction

The bacterial cell wall provides protection against the external environment as well as contributing to cell shape, integrity and strength. The Gram-positive cell wall contains a carbohydrate-rich layer of peptidoglycan that surrounds the cell membrane (Silhavy et al., 2010). The thick layer of peptidoglycan is a mesh-like structure composed of alternating β -1,4-linked *N*-acetylmuramic acid and *N*-acetylglucosamine strands covalently linked by penta-peptides (Vollmer et al., 2008). In addition to its primary functions in morphology and protection, it also acts as a scaffold for proteins and other glycopolymers including cell wall polysaccharides (CWPS) in Gram-positive bacteria (Garde et al., 2021). *Bacillus subtilis* and *Staphylococcus aureus* are among the best studied Gram-positive bacteria with respect to their cell walls and in which wall teichoic acids (WTAs) represent more than half of the total cell wall mass (Brown et al., 2013; Mistou et al., 2016). Unlike these model organisms however, not all Gram-positive bacteria synthesise WTA to the same degree or even produce and incorporate WTA structures into their cell wall (Mistou et al., 2016). Instead, some Gram-positive bacteria, including species of *Streptococcus* can produce peptidoglycan-anchored polysaccharides to the same extent e.g. more than 60% of the peptidoglycan dry weight of *Streptococcus agalactiae*

(Caliot et al., 2012). For streptococcal species, CWPS can encompass lipoteichoic acids (LTAs), rhamnose-glucose polysaccharides (RGPs) and exopolysaccharides (EPS) which decorate the exposed surface of the cell (Kawai et al., 2011; Weidenmaier and Peschel, 2008). The RGP and/or EPS may form part of the capsular polysaccharides produced by many streptococci, which may inhibit complement activity and phagocytosis and in so doing, is an important virulence factor in pathogenic streptococci (Hyams et al., 2010). Capsular and exopolysaccharides are also implicated in biofilm formation in pathogenic bacteria, therefore, these structures are essential to the virulence, establishment and maintenance of these organisms in their respective ecological niches (Balducci et al., 2023). *Streptococcus thermophilus* is a non-pathogenic streptococcal species that is associated with the dairy niche and many strains of this species are renowned for their ability to produce EPS although in many cases it presents more like a capsular polysaccharide being closely associated with the cell and with limited shedding into the surrounding medium. Therefore, it is timely to evaluate the state of the art of research pertaining to the cell wall polysaccharides of streptococcal species at the genetic and structural levels and to define their commonalities as well as species-specific cases that will guide future research in this field.

^{*} Corresponding author at: School of Microbiology & APC Microbiome Ireland, University College Cork, Western Road, Cork, Ireland.
E-mail address: j.mahony@ucc.ie (J. Mahony).

2. Historic classification of streptococci

Streptococcal diseases have affected human populations for centuries. Hippocrates described the disease “erysipelas” in the 4th century, writings of ancient scholars describe red tonsils and sore throats, and puerperal sepsis (or ‘childbed fever’). The latter of these was associated with high maternal mortality rates following childbirth across Europe and North America in the 18th and 19th century (Ferretti and Köhler, 2016). The first formal description of pathogenic streptococci was that of isolates from blood samples of women with puerperal fever by Louis Pasteur in 1879 (Ferretti and Köhler, 2016). *Streptococcus pyogenes* was named following its isolation from suppurative lesions while *Streptococcus erysipelas* was isolated from a patient with erysipelas. Early differentiation and nomenclature strategies were based on the origin/disease associated with the streptococcal strain. Subsequently, distinct haemolytic phenotypes were observed on blood agar plates and supported a method of distinguishing streptococcal species i.e., those with a clear zone surrounding a colony were named *Streptococcus haemolyticus*; and those presenting a green zone surrounding a colony were named *Streptococcus viridans*. On this basis, pathogenic streptococci are largely divided into these two phenotypic groups, i.e. the β -haemolytic pyogenic group or the α -haemolytic viridans group. Seminal work by Lancefield commencing in the 1920s facilitated the development of a new streptococcal classification system of β -haemolytic streptococci based on cell-surface antigen differences. The antigenic properties of group specific polysaccharides referred to as ‘C-substances’ initially enabled the serotype classification of Group A-E, these groupings expanded to include as many as 20 serotypes (Groups A - V) (Mistou et al., 2016). The typing system encompasses pathogenic streptococcal species linked to mild and severe infections in human and animal populations as well as non-pathogenic species that primarily occupy the human oral cavity.

In contrast to the pathogenic streptococcal species, one species of the *Streptococcus* genus is a major starter bacterial species used in dairy fermentation processes i.e. *S. thermophilus*. The earliest evidence of cheese making is documented as far back as 6000 years before the common era (BCE) in northern Europe (Salque et al., 2013), while yoghurt production is also documented in Indian Ayurvedic scripts, dating from the same time (Fisberg and Machado, 2015). In addition to its technological application in the food fermentation industry, *S. thermophilus* has also gained interest as a ‘probiotic’ with several purported human health promoting functionalities (Uriot et al., 2017).

While *S. thermophilus* is included in the heterogeneous group of lactic acid bacteria (LAB) and is related to *Lactococcus lactis*, it is phylogenetically closer to streptococcal species of the Viridans group. Members of the viridans group include streptococcal species that are not beta-haemolytic, cannot tolerate high pH or salt growth conditions and cannot grow at 10 °C. The salivarius group of viridans streptococci includes *S. thermophilus*, *Streptococcus salivarius* and *Streptococcus vestibularis* (Facklam, 2002). The group are closely related based on 16S rRNA gene sequence (Delorme et al., 2015). The taxonomic status of *S. thermophilus* has been evolved in recent years. Traditionally, it was grouped as part of the viridans group as a *S. salivarius* subspecies (Köhler, 2007). Based on DNA-DNA reassociation experiments under stringent conditions, *S. thermophilus* gained full species status (Facklam, 2002). It is also the only streptococcal species to be granted the ‘Generally Recognized As Safe’ (GRAS) status by the Food and drug administration (FDA) and the ‘Qualified Presumption of Safety’ (QPS) status by the European Food Safety Authority based on its long history of safe human consumption (EFSA) (Martinović et al., 2020). Although *S. salivarius* and *S. vestibularis* (viridans streptococci) are inhabitants of the human oral microbiome, several cases of meningitis, bacteraemia, and endocarditis have been associated with these organisms (Simsek et al., 2008; Wilson et al., 2012). The adaptation of *S. thermophilus* to the nutrient-rich milk substrate has been associated with genome decay and horizontal gene transfers (Bolotin et al., 2004; Hols et al., 2005). Consequently, a high proportion (approximately 10%) of the

S. thermophilus genome is constituted by pseudogenes (Bolotin et al., 2004) with their original functions being non-essential for growth in milk. As a starter culture, *S. thermophilus* rapidly converts lactose into lactic acid, causing a decrease in pH and subsequent coagulation of milk proteins. Additionally, many *S. thermophilus* strains confer flavour and textural properties to the final product (Cui et al., 2016; Iyer et al., 2010).

3. Role and significance of streptococcal cell wall glycans

As mentioned above, the three major glycans associated with the cell wall of streptococci are RGP, EPS and TAs. The RGP structures are particularly well characterised for many pathogenic and dairy streptococci due to their clinical and economic importance in their respective industries. RGPs of pathogenic streptococcal species have been associated with virulence, antimicrobial resistance, and immune evasion (Mistou et al., 2016). These glycopolymers may serve as functional replacements for WTAs in certain Gram-positive species (Fig. 1) (Mistou et al., 2016). Interestingly, RGPs are not universally present in all pathogenic streptococci. For example, *Streptococcus pneumoniae* instead incorporates WTAs and LTAs and the cell surface covered with a capsule, the structure of which has high strain-to-strain variability prompting methods for pneumococcal capsular serotyping (Jauneikaite et al., 2015; Vollmer et al., 2019).

Due to their association with animal and human diseases, the CWPS structure and composition of pathogenic streptococci have been studied extensively and particularly their RGPs (Sitkiewicz and Hryniewicz, 2010). Similarly, owing to their role in biofilm formation, EPS production in pathogenic streptococci has received significant research attention (Cugini et al., 2019; Klein et al., 2015; Li et al., 2022). Peptidoglycan anchored LTAs have been identified as virulence factors in *S. pneumoniae* (Heß et al., 2017) and more recently LTA synthesis of the closely related oral commensals *Streptococcus mitis* and *Streptococcus oralis* has been of interest due to their involvement in bacteremia and infective endocarditis (Kim et al., 2018; Wei et al., 2021). The recent resurgence of group A streptococcal disease in children in a number of European countries has focused research efforts towards the identification of virulence determinants (including CWPS) as well as vaccine development (Castro and Dorfmueller, 2021).

In the food industry, both RGP and EPS components of *S. thermophilus* have been implicated in (bacterio)phage-host interactions where they act as receptors for several phages (Romero et al., 2020). Phage infection of such starter cultures may lead to disrupted or failed dairy fermentations generating considerable food waste volumes and with economic consequences. However, from an applied perspective EPS produced by *S. thermophilus* is linked to desirable textures, mouth-feel and flavour of fermented dairy products and particularly so in the case of yoghurt-style products (Broadbent et al., 2003).

Comprehensive reviews of CWPS components of streptococci have predominantly focused on RGP analysis, highlighting their genetic diversity, biosynthesis, and physiological functions (Guérin et al., 2022; Lavelle and Mahony, 2021; Mistou et al., 2016). Among streptococcal species, the most thoroughly characterised with respect to CWPS composition and biosynthesis include *S. pyogenes*, *S. agalactiae*, *Streptococcus mutans*, *Streptococcus dysgalactiae*, and *S. thermophilus*. This review will discuss current knowledge of the diversity, biosynthesis, implications and/or applications of these cell wall glycans in both pathogenic and non-pathogenic streptococci with respect to their RGP and EPS production as well the LTA synthesis for certain species. Among these saccharides, RGP are undoubtedly the best studied cell wall polysaccharides and are, therefore, the primary focus of this review.

4. Rhamnose-glucose polysaccharides produced by streptococci

The polysaccharide components that are rich in L-rhamnose are described in literature as rhamnose-containing cell wall polysaccharides

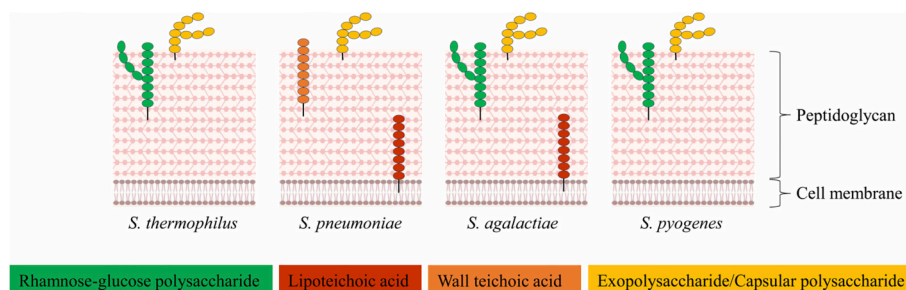


Fig. 1. Schematic representation of the major cell-wall polysaccharides in the cell wall of important streptococcal species. Rhamnose-glucose polysaccharides and exopolysaccharide/capsular polysaccharides are depicted by branched chains of circles highlighting their more complex structures compared to lipoteichoic and wall-teichoic acid structures. Exopolysaccharides and capsular polysaccharides have been grouped as there is a lack of clarity regarding the genetic and/or structural relationship between exopolysaccharides and capsular polysaccharides produced by streptococcal species.

(Lavelle and Mahony, 2021; Mistou et al., 2016), rhamnose-rich cell wall polysaccharides (Guérin et al., 2022), polyrhamnose polysaccharides, rhamnopolysaccharides (Rush et al., 2022), rhamnose polysaccharides (Edgar et al., 2019) and rhamnose-glucose polysaccharides (RGP) (Yamashita et al., 1998) among others. Despite the lack of a universal nomenclature, they are consistently described as complex heteropolysaccharides comprising a polyrhamnose chain (rhamnan backbone) accompanied by variations of oligosaccharide or polysaccharide chains (variable side chain) which can contain glucose, *N*-acetylglucosamine, galactose, *N*-acetyl galactosamine and phosphate (Mistou et al., 2016). Similar to WTA, the rhamnan backbone is proposed to be covalently attached to the peptidoglycan *N*-acetyl muramic acid residues. Both the rhamnan backbone and side chain components of pathogenic and dairy streptococci display strain-to-strain variability. Among streptococcal species, RGP structures share common features most notably the relatively conserved rhamnan backbone. However, the number of L-rhamnose residues and the glycosidic linkages may differ between species and even within species. In certain *S. thermophilus* strains and in *S. mutans* serotype c, e, and f strains, the repeating rhamnose unit also contains glucose (accounting for the RGP nomenclature) (Lavelle et al., 2022; Mistou et al., 2016) and group G streptococcal RGPs (*S. dysgalactiae* subsp. *equisimilis*) contain galactose in the rhamnan backbone (Zorzoli et al., 2019). The side chain components appear to account for much of the structural diversity among and within streptococcal species. This structural diversity has been the basis for pathogenic and non-pathogenic streptococcal typing systems (Szymczak et al., 2019).

The pioneering work by Lancefield on group-specific polysaccharides facilitated a link between human and animal diseases to group specific bacteria within the genus *Streptococcus* (groups A to V, excluding I and J) (Facklam, 2002; Köhler, 2007). The now 20 groups encompass key pathogenic species including *S. pyogenes*, responsible for a variety of diseases ranging from throat and skin infections to invasive infections including streptococcal toxic shock syndrome (Jespersen et al., 2020) and *S. agalactiae*, a causative agent of bovine mastitis and associated with skin and soft tissue infections in neonates, pregnant women, and immunocompromised individuals (Sitkiewicz and Hryniewicz, 2010; Ya'qoub et al., 2018). Group A carbohydrate (GAC) (*S. pyogenes*), and group C carbohydrate (GCC) (certain *S. dysgalactiae*) as well as *S. mutans* share a common disaccharide rhamnan backbone (repeating α -1,2-/ α -1,3-linked polyrhamnose backbone), with variation seen in their side chain components (Guérin et al., 2022). The gene cluster harbouring the biosynthetic machinery to form the final RGP structure (herein referred to as the *rgp* gene cluster) has been identified for many streptococcal species.

The leftward end of the *rgp* gene cluster, of GAC, GCC and *S. mutans* species exhibit high sequence identity and is proposed to encode the biosynthetic functions relating to the rhamnan backbone structure (Mistou et al., 2016). The decoration of the shared rhamnan backbone with specific side chain units produces structural diversity between

these groups and yields the discriminating epitopes for serotyping. The group specific antigen of Group B carbohydrates (GBC) (*S. agalactiae*) is unique as it contains a complex multiantenna branching with a final structure containing rhamnose, *N*-acetylglucosamine, glucitol, galactose, and phosphate (Wang et al., 2022).

Despite the many merits of the Lancefield typing system, it is important to highlight that it cannot distinguish streptococci at the species level. For example, *S. dysgalactiae* subsp. *equisimilis* can express Group A, C and G antigens (Mistou et al., 2016) and *S. mutans* strains can be classified into serotypes c, e, f or k based on their expressed polysaccharides (Nakano and Ooshima, 2009). Interestingly, *S. pneumoniae* cannot be classified into a Lancefield group. Instead of RGP structures described for other streptococcal species, the cell wall contains lipoteichoic acid (LTA) and wall teichoic acid (WTA) structures attached to the membrane or peptidoglycan, respectively (Vollmer et al., 2019). A predominant component of the pneumococcal teichoic acid is a ribitol teichoic acid termed the C-polysaccharide whose structure contains glucose, 2-acetamido-2,4,6- triideoxygalactose, galactosamine, ribitol phosphate, and phosphorylcholine covalently bound to the peptidoglycan layer. Each of these components have been identified as constituents of the capsular polysaccharide. Similar to RGP structures in the described pathogenic streptococci, the capsule structure is highly variable among pneumococcal species enabling the identification of at least 90 serotypes (Eletu et al., 2020) and the successful development of highly effective pneumococcal conjugate vaccines (Geno et al., 2015).

Undoubtedly, the varied nomenclature and typing systems that have been invoked over the past decades have impeded a universal understanding of the unifying features of distinct species in terms of cell wall polysaccharide composition. Furthermore, since strains of a given species may produce different group antigens (e.g. GAC or GCC), it is more useful to define the genetic regions associated with their biosynthesis and classify strains on this basis. This would allow a more generalised understanding of the conserved and variable elements of these moieties and allow a rapid classification of emerging isolates. Therefore, it may be useful to refine or reduce the nomenclature to RGP, L/WTA and/or EPS. Since RGP is a well-established naming scheme in place for several well studied species, it seems a natural choice to use this for studies pertaining to peptidoglycan-anchored cell wall polysaccharides. EPS should be only used as the nomenclature of genuine EPS phenotypes where the polysaccharide is shed into the surrounding medium and should be clearly distinguished from capsular polysaccharide-producing strains or species as there is a considerable lack of clarity on this point, particularly with respect to non-pathogenic streptococci.

4.1. Gene clusters associated with Rhamnose glucose polysaccharide (RGP) biosynthesis in streptococci

The genetic regions associated with RGP biosynthesis in streptococci were first identified in *S. mutans* through the characterization of four genes (*rmlA*, *rmlB*, *rmlC* and *rmlD*) whose products are indispensable for

dTDP-L-rhamnose production (Tsukioka et al., 1997). The *rml* genes in several streptococcal species including *S. mutans*, *S. pyogenes*, *S. agalactiae* and *S. thermophilus* are not all co-located. The *rmlA*, *B*, and *C* genes are typically clustered in the *rmlABC* locus and *rmlD* has been identified as part of a separate locus, termed the *rgp* locus. For *S. pneumoniae* the *rmlABCD* locus encodes the RmlA-D proteins that are required for dTDP-L-rhamnose synthesis (Bentley et al., 2006; Rockett et al., 2018). In addition to the *rmlD* gene, the *S. mutans* *rgp* loci also encodes six genes, *rgpA* through *rgpF*, essential for the synthesis of the rhamnan backbone (Yamashita et al., 1998) as well as several glycosyltransferase-encoding genes that were implicated in sidechain formation and an ABC membrane transport system encoded by *rgpC* and *rgpD*. Two additional genes (*rgpI* and *rgpH*) which encode proteins involved in the formation of the glucose side chain (RgpH) and branching frequency of side chain linkages (RgpI) have extended the boundary of the *rgp* locus downstream of *rgpF* in *S. mutans* (Shibata et al., 2003). Model pathways for the biosynthesis of the rhamnan backbone and subsequent addition of the variable side chain component for pathogenic streptococcal species have been proposed and will be outlined further below (Guérin et al., 2022; Mistou et al., 2016).

The biosynthesis of RGP of several streptococcal species shares common processes including initiation, elongation, translocation and modifications following cell wall anchoring (Mistou et al., 2016). The first enzymatic step in the synthesis of the rhamnan backbone involves the activation of the lipid carrier undecaprenyl phosphate by a UDP-GlcNAc:lipid phosphate transferase. In *S. mutans*, *rgpG*, a gene located outside of the *rgp* locus and performs the UDP-GlcNAc:Und-P-GlcNAc-1-P-transferase activity that is functionally equivalent to the TarO/TagO/WecA integral proteins of *B. subtilis*, *S. aureus* and *Escherichia coli*, respectively (Swoboda et al., 2010; Yamashita et al., 1998) is presumed to carry out the initiation step in rhamnan synthesis. Homologs of the phosphoglycosyltransferase RgpG, have also been identified (also located outside of the *rgp* locus) in *S. pyogenes* and *S. agalactiae* termed *gacO* and *gbcO*, respectively (Caliot et al., 2012; van Sorge et al., 2014). Deficiency of RgpG has been shown to affect cell division and biofilm formation in *S. mutans* (De et al., 2017). Following lipid carrier activation, biosynthesis of the complete RGP structure likely occurs through the addition of monosaccharides by specific glycosyltransferases contained within the *rgp* cluster. It is proposed that the step-wise additions are initiated by the rhamnosyltransferase RgpA, which transfers the first rhamnose residue from the dTDP-L-rhamnose precursor onto a *N*-acetylglucosamine-PP-undecaprenyl (GlcNAc-PP-Und) a lipid intermediate acceptor (Zorzoli et al., 2019). Subsequently, RgpB and RgpF are proposed to elongate the rhamnan chain through the addition of additional rhamnose moieties. RgpE is hypothesised to prevent further elongation of the rhamnan chain thus regulating rhamnan chain length in *L. lactis* (Theodorou et al., 2019). In *S. mutans* RgpE contributes to the formation of glucose side chains and is responsible for glucose side chain linkage (Kovacs et al., 2019). An *rgpE*⁻ mutant strain was capable of growing to the same maximal density (however with a reduced growth rate) as the parent strain and overall displayed little overall fitness defects, suggesting RgpE functions after the backbone has been assembled and alludes to a protective role of the rhamnan backbone in *S. mutans* (Kovacs et al., 2019). Similarly, the inactivation of *gacF* in *S. pyogenes* (which shares moderate sequence identity with *rgpE* of *S. mutans*) had no effect on cell viability, and the GAC produced by the *gacF* mutant strain displayed a wild-type antigenic profile. The ABC transport system encoded by *rgpC* and *rgpD*, subsequently transports the complete rhamnose-containing glycan across the membrane (Shibata et al., 2003). Although *S. mutans* can present four separate serotypes (based on the site-specific linkages of the glucose side chain) the rhamnan synthesis-associated genes *rgpA* through *rgpF* are conserved between strains reflective of their identical α -1,2/ α -1,3-linked rhamnan backbone structures (Shibata et al., 2003). The final step involving the rhamnan backbone is the covalent anchoring of the polymer to the peptidoglycan layer. This is most likely achieved by a transferase in the LytR-CpsA-Psr

(LCP) family (Stefanović et al., 2021).

Decoration of the RGP backbone with specific side chain moieties is accomplished through the activity of multiple glycosyltransferases encoded within the *rgp* locus. These side chain decorations underpin the Lancefield streptococcal classification system and most notably can distinguish between GAC, GCC and *S. mutans* who share a common rhamnan backbone structure. For *S. mutans*, glycosyltransferases RgpE and RgpI add α 1,2-linked glucose to rhamnan backbone (Yamashita et al., 1998), whereas *gacI* in *S. pyogenes* is proposed to transfer the characteristic β -linked GlcNAc side chain (van Sorge et al., 2014).

Detailed analysis of GAC synthesis and its assembly pathway in *S. pyogenes* has been established (Rush et al., 2017; van Sorge et al., 2014). With a similar architecture to that of *S. mutans* RGP synthesis, the gene cluster involved in GAC synthesis comprises twelve genes, *gacA-L*, with *gacO* typically located beyond the GAC gene cluster (van Sorge et al., 2014). As with *S. mutans*, the components of the GAC cluster include a *rmlD* homolog, *gacA*, rhamnosyltransferase-encoding genes *gacB*, *gacC* and *gacG* and an ABC-type transport system encoded by *gacD* and *gacE* (Rush et al., 2017). Recently a common initiation step for RGP synthesis (Group A, B, C and G antigen synthesis) was discovered through heterologous expression and complementation studies whereby the substitution of *S. pyogenes* *gacB* with the homologous gene from *S. agalactiae*, *S. equi* subsp. *zooepidemicus*, *S. dysgalactiae* subsp. *equisimilis*, or *S. mutans* complemented the GAC biosynthesis pathway (Zorzoli et al., 2019). Similarities in architecture and gene content between important pathogenic streptococci has immense importance for future studies in understanding pathogenesis and potential therapeutics.

While the early studies of CWPS biosynthesis were aimed at the development of clinical diagnostics and a basis to predict pathogenicity of streptococcal species, recent studies seek to apply the CWPS composition and structure information to improve drug targeting, vaccine design and phage therapy systems (Dale and Walker, 2020; Mangalea and Duerkop, 2020; van der Beek et al., 2019). Furthermore, there is a significant and growing volume of data pertaining to the diversity, composition, structure and biosynthesis of CWPSs of the dairy-associated species *S. thermophilus* (Lavelle et al., 2022; McDonnell et al., 2020; Parlungungan et al., 2022). The *rgp* gene cluster of *S. thermophilus* shares synteny with those of their pathogenic counterparts (Fig. 2). However, in contrast to *S. mutans*, the *rgpA-F* cluster of *S. thermophilus* associated with rhamnan backbone biosynthesis is co-located with genes associated with variable side chain production (Hols et al., 2005; Lavelle et al., 2022). A model for the biosynthesis of the RGP in *S. thermophilus* has been recently proposed (Lavelle et al., 2022). The RGP component is universally present in all analysed *S. thermophilus* strains and is closely associated with the cell wall. RGPs of *S. thermophilus* are characterised as structures containing a poly-rhamnose backbone with some strains also incorporating glucose in their backbone structure. The final RGP structure of *S. thermophilus* strains can also contain galactose, *N*-acetylglucosamine or *N*-acetylglactosamine as well as the previously noted rhamnose and/or glucose subunits primarily in the side chain component (Fig. 3A) (Lavelle et al., 2022; Szymczak et al., 2018).

S. thermophilus has historically been considered to lack genetic diversity due to its widespread application as an industrial starter culture (Delorme et al., 2017). However, the *rgp* gene cluster displays high variability between strains (Parlungungan et al., 2022; Romero et al., 2020; Szymczak et al., 2019). Similar to Lancefield classification or pneumococcal serotyping techniques, the genetic diversity of *rgp* clusters has been harnessed to devise multiplex PCR systems for their differentiation and classification (Kouwen et al., 2019; Lavelle et al., 2022). Hierarchical clustering analysis of the *rgp* gene clusters *S. thermophilus* strains genomes revealed considerable strain level diversity, enabling the identification of seven distinct groups of *S. thermophilus* *rgp* clusters termed Rgp1–7 (Lavelle et al., 2022; Romero et al., 2020; Szymczak et al., 2019). Detailed analysis of the rhamnan (backbone) and side chain (variable)-associated regions of the *rgp* locus revealed three

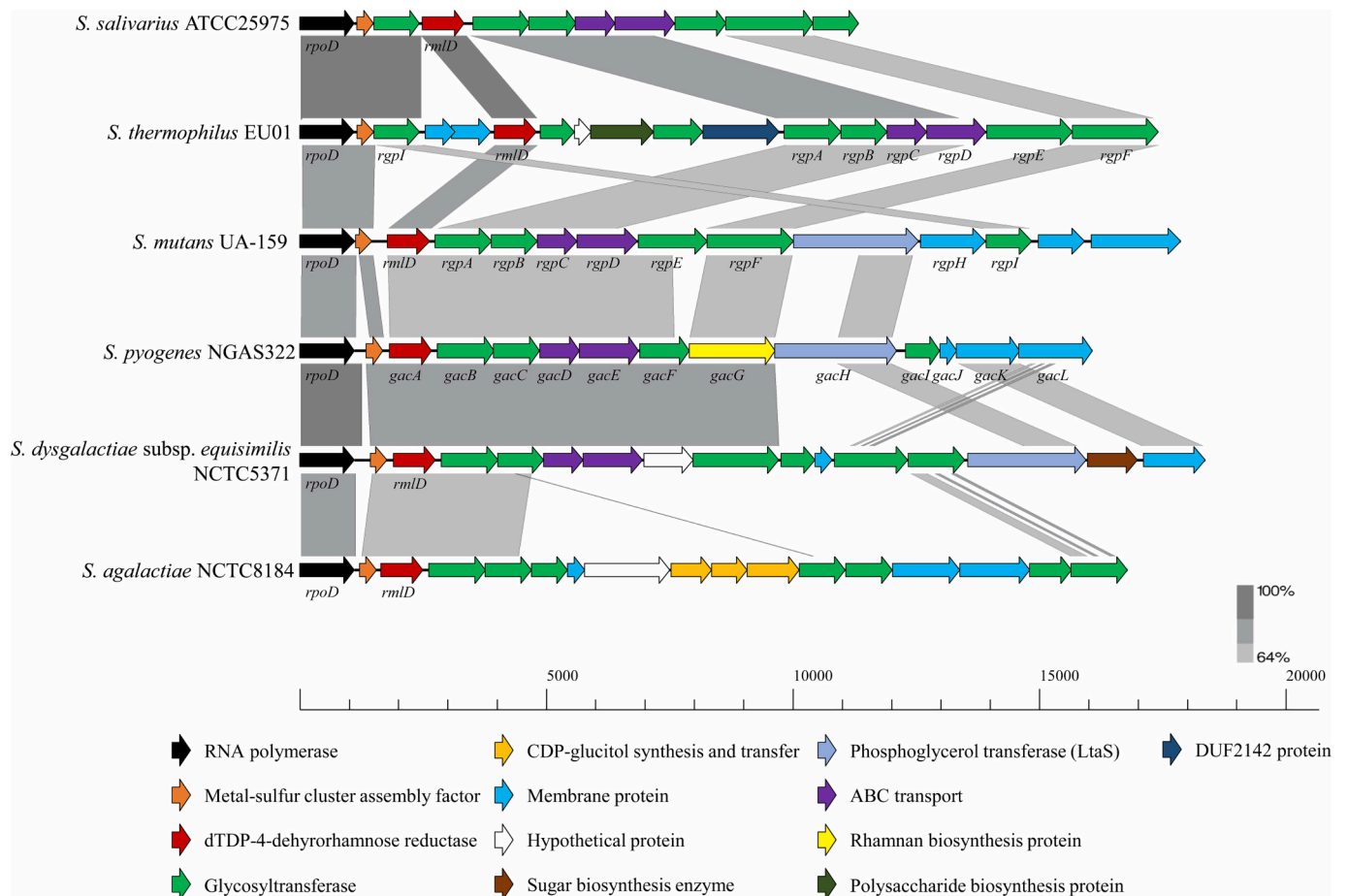


Fig. 2. Comparison of rhamnose-glucose polysaccharide (RGP) biosynthetic gene clusters of representative pathogenic and non-pathogenic streptococcal species. The 5' end of the *rgp* gene cluster is flanked by *rpoD* in the analysed species. The proposed functions of the gene products are indicated by colour-coded arrows with functions annotated under the scale bar. Regions of homology are joined by blocks in grayscale and generated using Easyfig (Sullivan et al., 2011). The scale bar is measured in base pairs. The gene clusters of the following strains are shown: *S. salivarius* ATCC 25975, *S. thermophilus* EU01, *S. mutans* UA-159, *S. pyogenes* NGAS322, *S. dysgalactiae* subsp. *equisimilis* NCTC5371, *S. agalactiae* NCTC8184.

distinct backbone genotypes (Bt) and five variable genotypes (Vt) (Lavelle et al., 2022). Theoretically 15 unique combinations of backbone and side chain may exist for *S. thermophilus* strains. To date however, just seven of the potential 15 combinations have been identified and five complete RGP structures have been elucidated, each representing a unique combination of backbone and side chain components and associated *rgp* genotype (Lavelle et al., 2022).

4.2. Biosynthesis of Rhamnose glucose polysaccharides

The *rgp* locus, encoding the biosynthetic machinery for both backbone and side chain synthesis, is a region of approximately 20–30 kb based on a recent study of 23 *S. thermophilus* strains (Parlindungan et al., 2022; Szymczak et al., 2018). While the size and gene content of the *rgp* locus appears to be strain-specific, the general organisation of the gene cluster is conserved among strains. In *S. thermophilus*, the *rgp* locus comprises a conserved set of genes associated with rhamnan backbone synthesis, including rhamnosyltransferases RgpA, RgpB, and RgpF as well as an ABC transporter/transport system (RgpC and RgpD), involved in exporting the side chain component to the outer surface of the cell membrane (Guérin et al., 2022; Hols et al., 2005). The *S. thermophilus* *rmlD* and *rgpA* through *rgpF* homologs share high identity with their *S. mutans* counterparts. Based on *rgp* gene cluster analysis of a total of 78 strains, the ABC transport system (RgpC and RgpD) is present in all *S. thermophilus* strains analysed (Parlindungan et al., 2022; Romero et al., 2020). As with pathogenic streptococci, the attachment of the

variable side chain is likely executed via a multicomponent glycosyltransferase system with a number of encoded glycosyltransferases present in *S. thermophilus* strains. The exact mechanisms by which the RGP backbone and side chain components are synthesised and their ultimate convergence to produce the final RGP structure remain to be experimentally proven for *S. thermophilus*.

dTDP-L-rhamnose is also essential for RGP synthesis in the non-pathogenic *S. thermophilus*. The biosynthesis of dTDP-L-rhamnose is reliant on the *rml* operon which contains *rmlA*, *B* and *C* and is located outside of the *rgp* locus of all *S. thermophilus* strains. *rmlD* however is located at the beginning of the *rgp* locus and shares high identity between strains and with pathogenic streptococci (Fig. 2). For *S. thermophilus*, the initiation of rhamnan backbone synthesis is mediated by an RgpG homolog (encoded by *stu163*) (Lavelle et al., 2022). The rhamnosyltransferase RgpA, B and F are proposed to be functional analogues of those described in pathogenic streptococcal species. RgpA transfers the first rhamnose residue, RgpB and RgpF are proposed to elongate the rhamnan backbone through the addition of individual monosaccharide units while RgpE is hypothesised to regulate rhamnan chain length. The ABC-transport system (encoded by *rgpC* and *rgpD*) transports the backbone polymer across the membrane following its intracellular synthesis. The incorporation of the backbone into the peptidoglycan layer is most likely performed by LCP family proteins acknowledging the similarities between *S. thermophilus* and *L. lactis* in terms *rgp* gene content and architecture (LcpA being the major LCP protein involved in rhamnan backbone attachment to peptidoglycan in

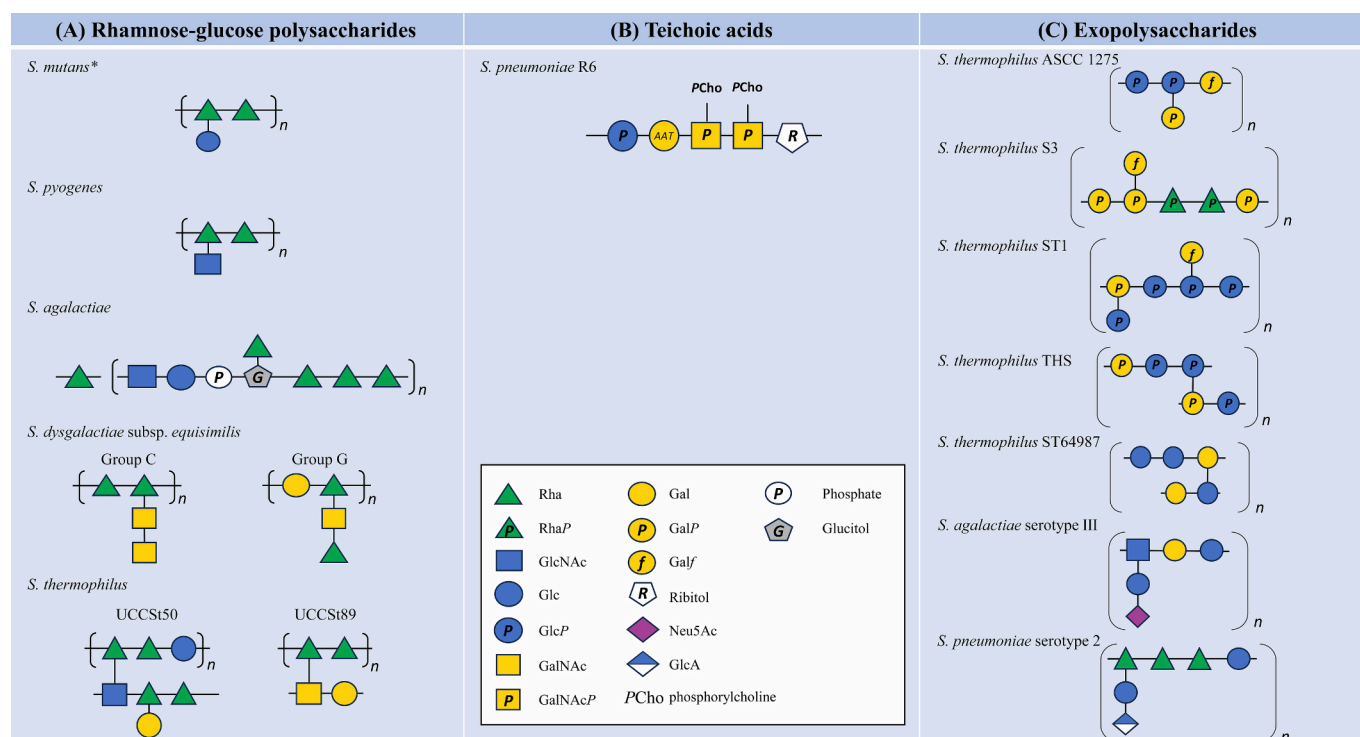


Fig. 3. Overview of the main cell-wall polysaccharide structures within different *Streptococcus* species. (A) Schematic representation of the rhamnose-glucose polysaccharide (RGPs) structures. *For *S. mutans* the glucose side chain decoration varies for each serotype (the glucose side chain be absent (serotype k) or be linked to the rhamnan backbone in a α -1,2 configuration (serotype c), β -1,2 configuration (serotype e), or a α -1,3 configuration (serotype f). In addition, GroP (glycerolphosphate) attachments have been identified on the serotype c carbohydrate (Zamakhaeva et al., 2021). GroP modifications have also been identified on the GlcNAc substituents in *S. pyogenes* (Edgar et al., 2019). (B) Schematic representation of the teichoic acid (TA) structure of *S. pneumoniae* R6 where ATTGal is 2-acet-amido-4-amino-2,4,6-trideoxygalactose. The repeating units can be attached to the glycolipid glucose-diacylglycerol (Glc-DAG) to yield membrane-anchored lipoteichoic acid (LTA) or to peptidoglycan via N-acetylmuramic acid (MurNAc) to produce wall teichoic acid (WTA) (Denapaité et al., 2012). (C) Schematic representation of the exopolysaccharide (EPS) structures produced by *S. thermophilus*, *S. pneumoniae* and *S. agalactiae* (Whitfield et al., 2020).

L. lactis) (Theodorou et al., 2019).

Biosynthesis of the side chain structure in the *S. thermophilus* is believed to be initiated by (ScbA) protein A, and its associated activator, ScbB (Lavelle et al., 2022). These proteins initiate side chain synthesis by the addition of a GlcNAc (or GalNAc in certain strains) moiety to the lipid carrier Und—P, representing functional homologs of *S. pyogenes* GacI/J (Rush et al., 2017). The elongation of the side chain is believed to be performed by glycosyltransferases in the *rgp* locus which are predicted to add monosaccharides sequentially to the lipid-linked GlcNAc foundation (Lavelle et al., 2022).

Translocation of the side chain across the cytoplasmic membrane typically requires proteins which ‘flip’ the saccharidic component following their intracellular synthesis often via a Wzy/Wzx-dependent pathway (Islam and Lam, 2014). The *S. thermophilus* ScbF of strain UCCSt50 shares structural similarity to an *E. coli* flippase and is hypothesised to be responsible for the translocation or ‘flipping’ of the saccharidic side chain across the cytoplasmic membrane (Lavelle et al., 2022). Interestingly, a putative Wzx-like flippase encoding gene is present for a majority of analysed *S. thermophilus* strains. The attachment of the side chain component to the rhamnan backbone is believed to be performed by a homolog of WpsJ, a GT-C fold glycosyltransferase discovered in the *L. lactis* strain MG1363 (Theodorou et al., 2019). Regulation of the side chain branching is likely the function of *rgpI* in *S. thermophilus* as it shares significant sequence identity to *rgpI* of *S. mutans*. Adding to the unique architecture, *rgpI* is the first gene in the *S. thermophilus* *rgp* cluster and one of distal genes in pathogenic streptococcal species (Fig. 2).

5. Teichoic acids produced by streptococci

The term TA has historically been used to describe all bacterial cell wall, membrane and capsular polymers which contain glycerolphosphate (GroP) or ribitolphosphate (RboP) (Percy and Gründling, 2014). Currently, the term encompasses two cell wall polymers in Gram-positive bacteria: wall teichoic acid (WTA) and lipoteichoic acid (LTA). LTAs are tethered to the plasma membrane and extend into the peptidoglycan layer, while WTAs are covalently attached to the *N*-acetyl muramic acid component of peptidoglycan (via a phosphodiester linkage) and extend through and beyond the cell wall (Brown et al., 2013; Swoboda et al., 2010). WTA glycopolymers are well studied in organisms such as *Bacillus* and *Staphylococcus* due to their crucial roles in bacterial physiology, cell division, antibiotic resistance and protection against host defence peptides and enzymes (Edgar et al., 2019). Interestingly, many streptococci lack the classically defined WTA and instead produce rhamnose-containing polysaccharides. Therefore, the idea that these glycopolymers act as functional homologs of TAs is one of note in the current literature (Caliot et al., 2012; Edgar et al., 2019; Kovacs et al., 2019; Mistou et al., 2016). Although early work reported the presence of glycerol and phosphate residues in a number of streptococcal species glycopolymers there remains a knowledge gap pertaining to TA synthesis in streptococci.

Recently, the presence of GroP in *S. pyogenes* and serotype c carbohydrate *S. mutans* was determined to be mediated by the product of *gacH* (or a *gacH* homolog) (Edgar et al., 2019). GacH, a membrane protein belonging to the alkaline phosphatase superfamily, catalyses GroP addition to the C6 hydroxyl group of GlcNAc substituents (Edgar et al., 2019). The GroP attachment present on ~25% of the GlcNAc side chains, converts the saccharide to polyanionic cell wall glycopolymer

decorations (Edgar et al., 2019). Similarly, GroP decorates the Glc side chain of the RGP in a *S. mutans* serotype c strain (Zamakhaeva et al., 2021). Based on phylogenetic analysis, GacH homologs are present in numerous streptococci, suggesting other species may also express glycopolymers with GroP additions on their side chain components (Edgar et al., 2019). Interestingly, GroP-modified side chains may have been largely overlooked due to acid-based methodologies for RGP extraction that may lead to GroP removal (Guérin et al., 2022).

The cell wall of *S. pneumoniae* is a complex structure that contains WTA and LTA surrounded by one of the 90 capsular polysaccharides produced by members of the species (Vollmer et al., 2019). *S. pneumoniae* is unique among streptococcal species in presenting identical WTA and LTA repeating units and only the anchoring location (peptidoglycan or membrane) defines their classification (Fig. 3B) (Denapaite et al., 2012; Heß et al., 2017; Vollmer et al., 2019). In most bacteria, these chain structures are different for the two polymers (Bui et al., 2012). The TA biosynthesis pathway has been proposed based on bioinformatic analysis of various pneumococcal strains (Denapaite et al., 2012). Genes associated with TA biosynthesis are located in three genomic loci, termed *lic1*, *lic2* and *lic3* (Fig. 4). These regions contain genes associated with choline uptake, CDP-choline and CDP-ribitol synthesis, teichoic acid flippase functions, and phosphotransferases (Vollmer et al., 2019). Of note TacF, a putative teichoic flippase, binds specifically to the choline-containing TA precursor chains before transferring the structure across the cytoplasmic membrane, providing evidence that all mature TAs are decorated with choline residues, essential for the choline-dependent growth of *S. pneumoniae* (Damjanovic et al., 2007). Subsequently, the mature TA chain is anchored to peptidoglycan or cell membrane to form WTA or LTA, respectively (Vollmer et al., 2019). Similar to RGP anchoring to peptidoglycan, members of the LCP family of proteins have been implicated in the cell wall maintenance and assembly of *S. pneumoniae* (Kawai et al., 2011). For LTA structures, the membrane protein TacL, transfers the TA precursor chains onto the glycolipid anchor (Heß et al., 2017). Similar LTA structures to those described for *S. pneumoniae* are described in *S. mitis* and *S. oralis* (Wei et al., 2021).

6. Exopolysaccharides of *Streptococcus thermophilus*

While pathogenic streptococci such as *S. mutans* are renowned for their biofilm forming activity, the descriptions of the associated polysaccharides, their composition, structure and genetics is surprisingly

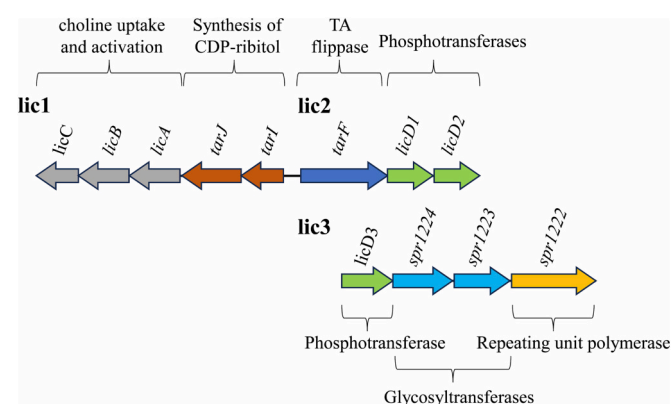


Fig. 4. Genetic organisation of the *lic* regions of *Streptococcus pneumoniae* R6 and associated functions of the gene products (Denapaite et al., 2012). The *lic1* operon contains genes associated with choline uptake, CDP-choline and CDP-ribitol synthesis. The adjacent *lic2* region encodes a putative teichoic acid (TA) flippase in addition to phosphotransferases. The *lic3* region is present in a disparate location to the *lic1* *lic2* gene clusters on the chromosome with proposed functions of gene products including a phosphotransferase, glycosyltransferase(s) and integral membrane protein(s).

limited. Biofilm formation may involve capsular polysaccharides or exopolysaccharides; however, this is not clearly delineated among pathogenic streptococci. It seems most likely that this property is invoked by capsular polysaccharides (CPS), which are described to be a widespread phenomenon among pathogenic streptococci (Skov Sørensen et al., 2016). The size (~16–36 kb) and organisation of the *cps* loci appears similar to those of *rgp* loci (Skov Sørensen et al., 2016) but remains to be experimentally established. Reports of EPS among pathogenic streptococci are rare and may, in fact, be CPS. The importance of TA and CPS in pathogenic species has dominated and likely overshadowed research of EPS in these species. In contrast, the EPS of *S. thermophilus* are thoroughly investigated with extensive reports of the genetic mechanisms underpinning their biosynthesis as well as their composition and structure. Here, the review presents an overview of the current knowledge of the genetics, composition and structure of the EPS of *S. thermophilus*.

Similar to RGP, EPS produced by *S. thermophilus* strains are heteropolysaccharides that primarily contain glucose, galactose, and rhamnose, and in some strains can contain fucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, ribose, and glucuronic acid as well as non-carbohydrate units such as glycerol, phosphate, pyruvyl, and acetyl groups (Faber et al., 2001; McDonnell et al., 2020; Nordmark et al., 2005; Padmanabhan and Shah, 2020a, 2020b; Säwén et al., 2010). The gene cluster that encodes the biosynthetic machinery for EPS production in *S. thermophilus*, termed the *eps* cluster exhibits considerable strain-to-strain variations in terms of size and gene content (Parlindungan et al., 2022). Furthermore, the locus termini appear to be highly conserved with variation primarily observed in the central regions of the cluster in which genes encoding glycosyltransferases are located (Alexandraki et al., 2019; Parlindungan et al., 2022). Hierarchical clustering analysis established the presence of six distinct groups of *S. thermophilus* *eps* genotypes designated as EPS A to F (Szymczak et al., 2019). Recent analysis of dairy streptococcal isolates identified a further four EPS genotypes (A to J) (Parlindungan et al., 2022). The genetic diversity of these clusters has yet to be linked to the compositional and structural diversity of these polysaccharides.

The *eps* gene cluster is approximately 15–30 kb in size and contains ~18 genes on average (Parlindungan et al., 2022; Szymczak et al., 2018). Despite the genetic diversity observed in the *eps* cluster of various *S. thermophilus* strains, the loci harbour genes that encode conserved functions associated with EPS chain length regulation, glycosyltransferase activity, polymerization and translocation (Fig. 5) (Cui et al., 2017; Parlindungan et al., 2022). The *eps* gene cluster contains three functional modules i.e. *epsAB* are located at the leftward end of the cluster and are involved in regulation of EPS production as well as *epsCD* whose products regulate EPS chain length (Cui et al., 2017). Two pairs of *epsC-epsD* genes were identified in the model *S. thermophilus* strain ASCC 1275, which are hypothesised to facilitate the strain producing two types of EPSs - a capsular EPS attached to the cell surface and a ropy EPS secreted into the extracellular environment (Fig. 5) (Wu et al., 2014). The second functional module is associated with the synthesis of repeating units of EPS (*epsE*, *epsF*, *epsG*, *epsH* and *epsI* and additional genes in some cases) and the third module is responsible for the polymerization of the repeating units and the export of the intracellularly synthesised EPS to the extracellular environment (*epsJ*, *epsK*, *epsL* and *epsM*) (Cui et al., 2017; Kong et al., 2022). The membrane-associated glycosyl-1-phosphate transferase (EpsE) catalyses the first step in the assembly of the EPS through the transfer of a sugar-1-phosphate to a lipid carrier located on the cytoplasmic surface of the membrane (Jolly and Stingle, 2001; Kong et al., 2022). Similar to RGP synthesis the transfer of the first unit triggers the addition of subsequent repeating units to the EPS (Cui et al., 2017).

The EPS produced by *S. thermophilus* strains is reported to be primarily composed of glucose, rhamnose and galactose (Pachekrepapal et al., 2017) while strain-specific combinations of galactose and rhamnose; galactose, glucose and *N*-acetylgalactosamine; galactose, glucose

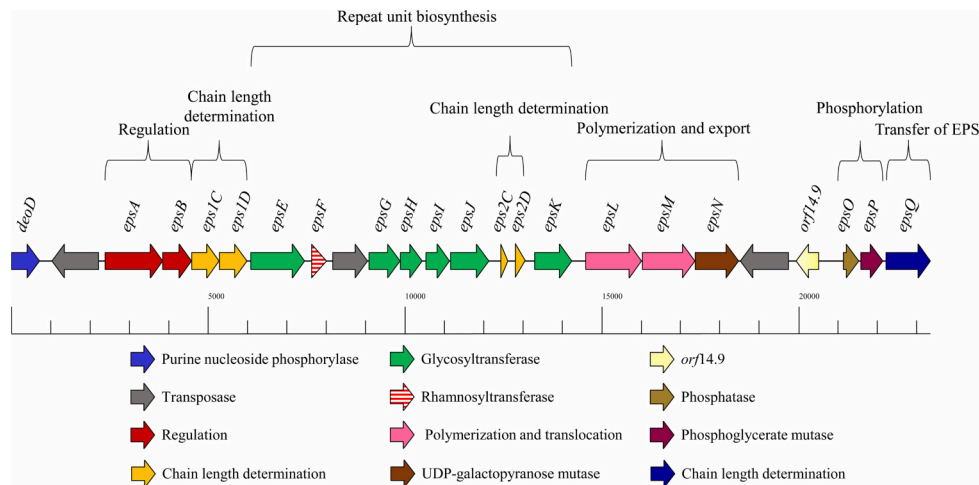


Fig. 5. Schematic depicting the architecture of the *eps* gene cluster in *S. thermophilus* ASCC 1275 as a representative of the species (Wu et al., 2014). Predicted functions are indicated by differently coloured arrows and highlight the main functions encoded by genes within the cluster including regulation, chain length determination, glycosyltransferases involved in repeat unit biosynthesis and polymerization and export.

and rhamnose; galactose, glucose, rhamnose and *N*-acetylgalactosamine have also been reported (Fig. 3C) (Faber et al., 2001; McDonnell et al., 2020; Nordmark et al., 2005; Padmanabhan and Shah, 2020a, 2020b; Sävén et al., 2010). The compositional diversity of these structures can be linked to the highly polymorphic nature of *S. thermophilus* *eps* cluster-encoded glycosyltransferases which commonly include galactosyltransferases, rhamnosyltransferases, glucosyltransferases, and *N*-acetylglucosamine transferases and less commonly include galactofuranose transferases, mannosyltransferases and fructosyltransferases (Cui et al., 2017). Polymerization-associated genes as well as export-associated genes are located at the 3' end of the *eps* cluster. The nomenclature associated with these functions is inconsistent and is entirely dependent on the number of genes preceding those associated with polymerization functions. For example, *epsJ*, *epsL* and *epsM* in one *S. thermophilus* strain are associated with polymerization and translocation of the EPS repeating units while *epsQ* is associated with the transfer of EPS between the membrane and peptidoglycan layer in another strain (Wu et al., 2014). Therefore, while there is an abundance of sequence and compositional data associated with EPS production in *S. thermophilus*, it is clear that there is significant diversity in both their genetic and chemical composition causing lack of clarity on some fundamental points including (i) the lack of a universal nomenclature for the *eps* associated genes and their encoded products; (ii) a lack of clarity regarding the nature of CPS and its genetic/structural relationship to either EPS or RGP and; (iii) the extent of chemical diversity of EPS in this species. To date the chemical composition of the EPS of a large number of strains of *S. thermophilus* have been elucidated while the chemical structure of only a handful have been completely resolved. This will likely represent an area of research growth in the coming years as it is imperative to understand the importance of these structures on microbiota- and immune-modulation in humans as well as the well-studied technological properties of fermented foods.

7. Implications of cell wall polysaccharide diversity in the food and healthcare industries

7.1. Starter cultures, probiotics, prebiotics and postbiotics

Bacteriophages, also known as phages, continue to be among the most significant and consistent threats to dairy fermentation processes (Romero et al., 2020). Disruption caused by phages in the dairy industry have significant technological and economic consequences while failed fermentations contribute to global food waste (Romero et al., 2020).

Therefore, food fermentation processes depend on reliable, technologically favourable, and robust starter cultures to reduce waste and increase the sustainability of production practices. Recent studies have highlighted the role of both RGP and EPS in strain-specific phage-host interactions and the development of multiplex PCR systems facilitate the rapid classification of dairy streptococcal strains based on their *rgp* genotype (Lavelle et al., 2022). Such classification may be applied to identifying strains that present distinct RGP structures on the cell surface and that reduce the risk of phage infection and loss of the fermentate. Such strategies could also be developed for the classification of *eps* genotypes for this species and further enhance the robustness of starter cultures. Currently, five genetically distinct phage groups of dairy streptococcal phages are known to exist and among these the Brus-sowviruses are known to recognise RGP receptors while EPS is the implicated receptor for 987 group phages. Limited information exists for the receptor of the remaining three phage groups although it is likely that one or both of these saccharides is required by these phages also. Owing to the pervasiveness of phages in the industrial setting, it is suggested that this pressure has underpinned the diversity of the *rgp* and *eps* loci. As the food industry adapts to growing demands for fermented products derived from plant-based alternatives to dairy, knowledge of *S. thermophilus* genetic composition and metabolic capabilities will underpin industry's ultimate success and ability to adapt to these, and other emerging, demands and challenges.

The metabolism of lactose by *S. thermophilus* has long been regarded as a defining probiotic trait of strains of this species. However, the survivability of *S. thermophilus* strains in the human gastrointestinal tract is the subject of debate and is likely a strain-specific attribute. Despite this, there are numerous possible benefits associated with *S. thermophilus* EPS including such as the limitation of pathogen colonisation/biofilm formation, immune system activation, anti-inflammatory and prebiotic activities (Angelin and Kavitha, 2020). Furthermore, even in the absence of viability, the EPS may impart such effects as a "post-biotic" further highlighting the potential of this species and its associated CWPS in contributing to human health. While the true benefits of *S. thermophilus* may emanate from its co-association with proto-cooperative organisms such as lactobacilli, the importance of *S. thermophilus* in the food and probiotics industries is undeniable.

Classical phage typing of pathogenic bacterial species was based on the unique interaction profiles of clinical isolates with phage panels known to infect strains of given serotype(s). These serotypes, being associated to cell wall polysaccharides, highlight the long-standing implications of cell wall polysaccharides including RGP, EPS and teichoic

acids. As the exact composition and/or structure of the majority of pathogenic streptococcal species is incomplete, it is not currently possible to define if there is an explicit link between cell wall polysaccharides that are involved in virulence and phage interactions or if these are distinct moieties; however, this is certainly an aspect that will become clear as an increasing number of cell wall polysaccharide structures and genome sequences of emerging strains become available.

7.2. Drug development and therapeutics

RGPs of pathogenic streptococcal species present promising opportunities in human therapeutic vaccine design, drug targeting and phage therapy approaches (Dale and Walker, 2020; Szafranski et al., 2017; van der Beek et al., 2019). The loss of virulence and suboptimal cell growth and cell morphology exhibited by a number of pathogenic streptococcal species following depletion of rhamnose, has highlighted the streptococcal L-rhamnose biosynthesis as a useful interesting drug target (van der Beek et al., 2019). Moreover, as dTDP-L-rhamnose is not produced or used by humans (Mistou et al., 2016), drugs targeting the L-rhamnose biosynthesis pathway would have limited undesirable side effects. A recent study identified compounds capable of inhibiting dTDP-L-rhamnose biosynthesis in streptococcal species (van der Beek et al., 2019). Further knowledge regarding this pathway in additional pathogenic species could optimize steps in drug design in the healthcare industry.

In *S. mutans*, RgpG plays a crucial role in cell division which impacts biofilm formation, revealing a new potential target to develop anticaries vaccines or therapeutics (De et al., 2017). Similar strategies have been exploited in *S. aureus*, whereby the inhibition of the UDP-GlcNAc:lipid phosphate transferase, TarO, renders the bacterium avirulent (Sewell and Brown, 2014) and in *S. epidermidis* TagO deficiency was reported to cause major defects in biofilm development and formation (Holland et al., 2011).

CPSs of *S. pneumoniae* are immunoreactive components of the pathogen that have formed the basis of carbohydrate-based vaccines (Riu et al., 2022). Similarly, research into capsular polysaccharide conjugate vaccine development for *S. agalactiae* is underway, however no licensed vaccine is currently available. A *galI* mutant of *S. pyogenes* prevented GlcNAc side chain addition in *S. pyogenes* and was attenuated for virulence in two infection models (van Sorge et al., 2014). This represents additional opportunities to develop alternate vaccine antigens devoid of the presumptive infection-causing side chain components. As neither *S. pyogenes* nor *S. agalactiae* vaccines are currently available more research into understanding pathogenesis relating to CWPS is necessary for future vaccine designs.

There is growing interest in exploiting bacteriophages to combat bacterial infections due to the rise in nosocomial multidrug-resistant bacterial infections. Phages have evolved to recognise and adhere to bacterial cell surfaces with subsequent infection and replication within the cell. Targetted elimination of pathogenic streptococcal infections is gaining credence once again given the current antibiotic crisis. Endolysins, which are phage-encoded peptidoglycan hydrolases responsible for the degradation of peptidoglycan and subsequent cell lysis (Roach and Donovan, 2015) have become increasingly prevalent in the fight against streptococcal infections. An extensive review of the numerous streptococcal phages that target pathogenic species as well as the challenges that limit phages and endolysin application in the healthcare at present has recently been published (Wong et al., 2022).

Definition of the diversity and role of EPS and CPS in streptococcal species, especially in biofilm-forming species may lead to the development of novel antimicrobial agents by enhancing biofilm dispersion or by inhibiting the reproduction and metabolism of pathogens (Zhang et al., 2022). While biofilm formation and pathogenesis are well studied in *S. mutans*, it is less well defined and understood in other streptococcal species associated with the oral cavity and may represent an area of emerging interest in the coming years (Cugini et al., 2019).

8. Conclusions

Streptococci have evolved to diversify the cell surface glycans to adapt to a range of ecological niches. These glycans may be interchangeable depending on the species and the specific roles that they play in that host. Recent advances in the understanding of the genetic, compositional and structural diversity simultaneously increases awareness of the opportunities for tailored drug/vaccine development and sustainable food production systems. However, species- or niche-specific studies have also created a challenge as there is currently a lack of universal definition of the associated biosynthetic pathways and structures. While this may create a temporary impediment to progress in streptococcal glycobiology research in streptococci, the breadth of depth of activity that is currently underway gives cause for optimism in the context of therapeutics, diagnostics and the healthcare and food industries. To achieve this, it is imperative that the field unifies its efforts in linking the genetic diversity of the biosynthetic clusters to the composition and chemical structure of the final cell wall polysaccharide moieties. Secondly, it is essential that the field embarks on a systematic analysis of representatives of a breadth of streptococcal species to define the links between the various species and understand the biosynthetic processes that will have implications for the development of vaccines and therapeutics. Finally, it will be important to have a universal nomenclature to provide transparency in the field and a broader understanding of the common and unique aspects of the cell wall polysaccharides of streptococcal species that have profound impact on human health.

Funding statement

This publication has emanated from research conducted with the financial support of Science Foundation Ireland under grant numbers 20/FFP-P/8664 and 12/RC/2273-P2. For the purpose of open access, we have applied a CC BY public copyright license to any author accepted manuscript version arising from this submission.

CRediT authorship contribution statement

Zoe Kampff: Conceptualization, Writing – original draft, Visualization, Investigation. **Douwe van Sinderen:** Writing – review & editing, Supervision, Funding acquisition. **Jennifer Mahony:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

- Alexandraki, V., Kazou, M., Blom, J., Pot, B., Papadimitriou, K., Tsakalidou, E., 2019. Comparative genomics of *Streptococcus thermophilus* support important traits concerning the evolution, biology and technological properties of the species. *Front. Microbiol.* 10, 2916. <https://doi.org/10.3389/fmicb.2019.02916>.
- Angelin, J., Kavitha, M., 2020. Exopolysaccharides from probiotic bacteria and their health potential. *Int. J. Biol. Macromol.* 162, 853–865. <https://doi.org/10.1016/j.ijbiomac.2020.06.190>.
- Balducci, E., Papi, F., Capialbi, D.E., Del Bino, L., 2023. Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. *Int. J. Mol. Sci.* 24, 4030. <https://doi.org/10.3390/ijms24044030>.
- Bentley, S.D., Aanensen, D.M., Mavroidi, A., Saunders, D., Rabinowitsch, E., Collins, M., Donohoe, K., Harris, D., Murphy, L., Quail, M.A., Samuel, G., Skovsted, I.C., Kalltoft, M.S., Barrell, B., Reeves, P.R., Parkhill, J., Spratt, B.G., 2006. Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. *PLoS Genet.* 2, e31 <https://doi.org/10.1371/journal.pgen.0020031>.
- Bolotin, A., Quinquis, B., Renault, P., Sorokin, A., Ehrlich, S.D., Kulaukasas, S., Lapidus, A., Goltsman, E., Mazur, M., Pusch, G.D., Fonstein, M., Overbeek, R., Kyprides, N., Purnelle, B., Prozzi, D., Ngui, K., Masuy, D., Hancy, F., Burteau, S., Boutry, M., Delcour, J., Goffeau, A., Hols, P., 2004. Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. *Nat. Biotechnol.* 22, 1554. <https://doi.org/10.1038/nbt1034>.

- Broadbent, J.R., McMahon, D.J., Welker, D.L., Oberg, C.J., Moineau, S., 2003. Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a Review. *J. Dairy Sci.* 86, 407–423. [https://doi.org/10.3168/jds.S0022-0302\(03\)73619-4](https://doi.org/10.3168/jds.S0022-0302(03)73619-4).
- Brown, S., Santa Maria, J.P., Walker, S., 2013. Wall teichoic acids of gram-positive bacteria. *Annu. Rev. Microbiol.* 67, 313–336. <https://doi.org/10.1146/annurev-micro-092412-155620>.
- Bui, N.K., Eberhardt, A., Vollmer, D., Kern, T., Bougault, C., Tomasz, A., Simorre, J.-P., Vollmer, W., 2012. Isolation and analysis of cell wall components from *Streptococcus pneumoniae*. *Anal. Biochem.* 421, 657–666. <https://doi.org/10.1016/j.ab.2011.11.026>.
- Caliot, É., Dramsi, S., Chapot-Chartier, M.-P., Courtin, P., Kulakauskas, S., Péchoux, C., Trieu-Cuot, P., Mistou, M.-Y., 2012. Role of the group B antigen of *Streptococcus agalactiae*: a peptidoglycan-anchored polysaccharide involved in cell wall biogenesis. *PLoS Pathog.* 8, e1002756. <https://doi.org/10.1371/journal.ppat.1002756>.
- Castro, S.A., Dorfmueller, H.C., 2021. A brief review on group A *Streptococcus* pathogenesis and vaccine development. *R. Soc. Open Sci.* 8, 201991. <https://doi.org/10.1098/rsos.201991>.
- Cugini, C., Shanmugam, M., Landge, N., Ramasubbu, N., 2019. The role of exopolysaccharides in Oral biofilms. *J. Dent. Res.* 98, 739–745. <https://doi.org/10.1177/0022034519845001>.
- Cui, Y., Xu, T., Qu, X., Hu, T., Jiang, X., Zhao, C., 2016. New insights into various production characteristics of *Streptococcus thermophilus* strains. *Int. J. Mol. Sci.* 17, 1701. <https://doi.org/10.3390/ijms17101701>.
- Cui, Y., Jiang, X., Hao, M., Qu, X., Hu, T., 2017. New advances in exopolysaccharides production of *Streptococcus thermophilus*. *Arch. Microbiol.* 199, 799–809. <https://doi.org/10.1007/s00203-017-1366-1>.
- Dale, J.B., Walker, M.J., 2020. Update on group A streptococcal vaccine development. *Curr. Opin. Infect. Dis.* 33, 244–250. <https://doi.org/10.1097/QCO.0000000000000644>.
- Damjanovic, M., Kharat, A.S., Eberhardt, A., Tomasz, A., Vollmer, W., 2007. The essential *tacF* gene is responsible for the choline-dependent growth phenotype of *Streptococcus pneumoniae*. *J. Bacteriol.* 189, 7105–7111. <https://doi.org/10.1128/JB.00681-07>.
- De, A., Liao, S., Bitoun, J.P., Roth, R., Beatty, W.L., Wu, H., Wen, Z.T., 2017. Deficiency of RgpG causes major defects in cell division and biofilm formation, and deficiency of *LytR-CpsA-Psr* family proteins leads to accumulation of Cell Wall antigens in culture medium by *Streptococcus mutans*. *Appl. Environ. Microbiol.* 83. <https://doi.org/10.1128/AEM.00928-17> e00928–17.
- Delorme, C., Abraham, A.-L., Renault, P., Guédon, E., 2015. Genomics of *Streptococcus salivarius*, a major human commensal. *Infect. Genet. Evol.* 33, 381–392. <https://doi.org/10.1016/j.meegid.2014.10.001>.
- Delorme, C., Legravet, N., Jamet, E., Hoarau, C., Alexandre, B., El-Sharoud, W.M., Darwish, M.S., Renault, P., 2017. Study of *Streptococcus thermophilus* population on a world-wide and historical collection by a new MLST scheme. *Int. J. Food Microbiol.* 242, 70–81. <https://doi.org/10.1016/j.ijfoodmicro.2016.11.016>.
- Denapaite, D., Brückner, R., Hakenbeck, R., Vollmer, W., 2012. Biosynthesis of teichoic acids in *Streptococcus pneumoniae* and closely related species: lessons from genomes. *Microb. Drug Resist.* 18, 344–358. <https://doi.org/10.1089/mdr.2012.0026>.
- Edgar, R.J., van Hensbergen, V.P., Ruda, A., Turner, A.G., Deng, P., Le Breton, Y., El-Sayed, N.M., Belew, A.T., McIver, K.S., McEwan, A.G., Morris, A.J., Lambeau, G., Walker, M.J., Rush, J.S., Korotkov, K.V., Widmalm, G., van Sorge, N.M., Korotkova, N., 2019. Discovery of glycerol phosphate modification on streptococcal rhamnose polysaccharides. *Nat. Chem. Biol.* 15, 463–471. <https://doi.org/10.1038/s41589-019-0251-4>.
- Eletu, S.D., Sheppard, C.L., Rose, S., Smith, K., Andrews, N., Lim, W.S., Litt, D.J., Fry, N. K., 2020. Re-validation and update of an extended-specificity multiplex assay for detection of *Streptococcus pneumoniae* capsular serotype/serogroup-specific antigen and cell-wall polysaccharide in urine specimens. *Access Microbiol.* 2, acmi000094. <https://doi.org/10.1099/acmi.0.000094>.
- Faber, E.J., Van Den Haak, M.J., Kamerling, J.P., Vliegthart, J.F.G., 2001. Structure of the exopolysaccharide produced by *Streptococcus thermophilus* S3. *Carbohydr. Res.* 331, 173–182. [https://doi.org/10.1016/S0008-6215\(01\)00013-1](https://doi.org/10.1016/S0008-6215(01)00013-1).
- Facklam, R., 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin. Microbiol. Rev.* 15, 613–630. <https://doi.org/10.1128/CMR.15.4.613-630.2002>.
- Ferretti, J., Köhler, W., 2016. History of streptococcal research. In: Ferretti, J.J., Stevens, D.L., Fischetti, V.A. (Eds.), *Streptococcus Pyogenes: Basic Biology to Clinical Manifestations*. University of Oklahoma Health Sciences Center, Oklahoma City (OK).
- Fisberg, M., Machado, R., 2015. History of yogurt and current patterns of consumption. *Nutr. Rev.* 73 (Suppl. 1), 4–7. <https://doi.org/10.1093/nutr/nuv020>.
- Garde, S., Chodiseti, P.K., Reddy, M., 2021. Peptidoglycan: structure, synthesis, and regulation. *EcoSal Plus* 9. <https://doi.org/10.1128/ecosalplus.ESP-0010-2020>.
- Geno, K.A., Gilbert, G.L., Song, J.Y., Skovsted, I.C., Klugman, K.P., Jones, C., Konradsen, H.B., Nahm, M.H., 2015. Pneumococcal capsules and their types: past, present, and future. *Clin. Microbiol. Rev.* 28, 871–899. <https://doi.org/10.1128/CMR.00024-15>.
- Guérin, H., Kulakauskas, S., Chapot-Chartier, M.-P., 2022. Structural variations and roles of rhamnose-rich cell wall polysaccharides in gram-positive bacteria. *J. Biol. Chem.* 298, 102488. <https://doi.org/10.1016/j.jbc.2022.102488>.
- Heß, N., Waldow, F., Köhler, T.P., Rohde, M., Kreikemeyer, B., Gómez-Mejía, A., Hain, T., Schwudke, D., Vollmer, W., Hammerschmidt, S., Gisch, N., 2017. Lipoteichoic acid deficiency permits normal growth but impairs virulence of *Streptococcus pneumoniae*. *Nat. Commun.* 8, 2093. <https://doi.org/10.1038/s41467-017-01720-z>.
- Holland, L.M., Conlon, B., O'Gara, J.P., 2011. Mutation of tagO reveals an essential role for wall teichoic acids in *Staphylococcus epidermidis* biofilm development. *Microbiol. (Read.)* 157, 408–418. <https://doi.org/10.1099/mic.0.042234-0>.
- Hols, P., Hancy, F., Fontaine, L., Grossiord, B., Prozzi, D., Leblond-Bourget, N., Decaris, B., Bolotin, A., Delorme, C., Dusko Ehrlich, S., Guédon, E., Monnet, V., Renault, P., Kleerebezem, M., 2005. New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative genomics. *FEMS Microbiol. Rev.* 29, 435–463. <https://doi.org/10.1016/j.femsre.2005.04.008>.
- Hyams, C., Camberlein, E., Cohen, J.M., Bax, K., Brown, J.S., 2010. The *Streptococcus pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. *Infect. Immun.* 78, 704–715. <https://doi.org/10.1128/IAI.00881-09>.
- Islam, S.T., Lam, J.S., 2014. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Can. J. Microbiol.* 60, 697–716. <https://doi.org/10.1139/cjm-2014-0595>.
- Iyer, R., Tomar, S.K., Uma Maheswari, T., Singh, R., 2010. *Streptococcus thermophilus* strains: multifunctional lactic acid bacteria. *Int. Dairy J.* 20, 133–141. <https://doi.org/10.1016/j.idairyj.2009.10.005>.
- Jauneikaite, E., Tocheva, A.S., Jefferies, J.M.C., Gladstone, R.A., Faust, S.N., Christodoulides, M., Hibberd, M.L., Clarke, S.C., 2015. Current methods for capsular typing of *Streptococcus pneumoniae*. *J. Microbiol. Methods* 113, 41–49. <https://doi.org/10.1016/j.jmimet.2015.03.006>.
- Jespersen, M.G., Lacey, J.A., Tong, S.Y.C., Davies, M.R., 2020. Global genomic epidemiology of *Streptococcus pyogenes*. *Infect. Genet. Evol.* 86, 104609. <https://doi.org/10.1016/j.meegid.2020.104609>.
- Jolly, L., Stingle, F., 2001. Molecular organization and functionality of exopolysaccharide gene clusters in lactic acid bacteria. In: *International Dairy Journal, First International Symposium on Exopolysaccharides from Lactic Acid Bacteria: from Fundamentals to Applications*, 11, pp. 733–745. [https://doi.org/10.1016/S0958-6946\(01\)00117-0](https://doi.org/10.1016/S0958-6946(01)00117-0).
- Kawai, Y., Marles-Wright, J., Cleverley, R.M., Emmins, R., Ishikawa, S., Kuwano, M., Heinz, N., Bui, N.K., Hoyland, C.N., Ogasawara, N., Lewis, R.J., Vollmer, W., Daniel, R.A., Errington, J., 2011. A widespread family of bacterial cell wall assembly proteins. *EMBO J.* 30, 4931–4941. <https://doi.org/10.1038/emboj.2011.358>.
- Kim, S.L., Gordon, S.M., Shrestha, N.K., 2018. Distribution of streptococcal groups causing infective endocarditis: a descriptive study. *Diagn. Microbiol. Infect. Dis.* 91, 269–272. <https://doi.org/10.1016/j.diagmicrobio.2018.02.015>.
- Klein, M.L., Hwang, G., Santos, P.H.S., Campanella, O.H., Koo, H., 2015. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. *Front. Cell. Infect. Microbiol.* 5, 10. <https://doi.org/10.3389/fcimb.2015.00010>.
- Köhler, W., 2007. The present state of species within the genera *Streptococcus* and *Enterococcus*. *Int. J. Med. Microbiol.* 297, 133–150. <https://doi.org/10.1016/j.ijmm.2006.11.008>.
- Kong, L., Song, X., Xia, Y., Ai, L., Xiong, Z., 2022. Construction of a CRISPR/nCas9-assisted genome editing system for exopolysaccharide biosynthesis in *Streptococcus thermophilus*. *Food Res. Int.* 158, 111550. <https://doi.org/10.1016/j.foodres.2022.111550>.
- Kouwen RHME, van Sinderen, D., McDonnell B., Van Verloren TPE, Mahony, J., 2019. *Streptococcus thermophilus* starter cultures. *US patent* 20190367866.
- Kovacs, C.J., Faustoferr, R.C., Bischer, A.P., Quivey, R.G., 2019. *Streptococcus mutans* requires mature rhamnose-glucose polysaccharides for proper pathophysiology, morphogenesis and cellular division. *Mol. Microbiol.* 112, 944–959. <https://doi.org/10.1111/mmi.14330>.
- Lavelle, K., van Sinderen, D., Mahony, J., 2021. Cell wall polysaccharides of gram positive ovococoid bacteria and their role as bacteriophage receptors. *Comput. Struct. Biotechnol. J.* 19, 4018–4031. <https://doi.org/10.1016/j.csbj.2021.07.011>.
- Lavelle, K., Sadovskaya, I., Vinogradov, E., Kelleher, P., Lugli, G.A., Ventura, M., van Sinderen, D., Mahony, J., 2022. Bipartite rpg locus diversity in *Streptococcus thermophilus* corresponds to backbone and side chain differences of its Rhamnose-containing cell wall polysaccharide. *Appl. Environ. Microbiol.* 88, e0150422. <https://doi.org/10.1128/aem.01504-22>.
- Li, X., Liu, Y., Yang, X., Li, C., Song, Z., 2022. The oral microbiota: community composition, influencing factors, pathogenesis, and interventions. *Front. Microbiol.* 13, 895537. <https://doi.org/10.3389/fmicb.2022.895537>.
- Mangalea, M.R., Duerkop, B.A., 2020. Fitness trade-offs resulting from bacteriophage resistance potentiate synergistic antibacterial strategies. *Infect. Immun.* 88. <https://doi.org/10.1128/IAI.00926-19> e00926–19.
- Martinović, A., Cocuzzi, R., Arioli, S., Mora, D., 2020. *Streptococcus thermophilus*: to survive, or not to survive the gastrointestinal tract, that is the question! *Nutrients* 12, 2175. <https://doi.org/10.3390/nu12082175>.
- McDonnell, B., Hanemaaijer, L., Bottacini, F., Kelleher, P., Lavelle, K., Sadovskaya, I., Vinogradov, E., Loren, Ver, van Themaat, E., Kouwen, T., Mahony, J., van Sinderen, D., 2020. A cell wall-associated polysaccharide is required for bacteriophage adsorption to the *Streptococcus thermophilus* cell surface. *Mol. Microbiol.* 114, 31–45. <https://doi.org/10.1111/mmi.14494>.
- Mistou, M.-Y., Sutcliffe, I.C., van Sorge, N.M., 2016. Bacterial glycobiology: rhamnose-containing cell wall polysaccharides in gram-positive bacteria. *FEMS Microbiol. Rev.* 40, 464–479. <https://doi.org/10.1093/femsre/fuw006>.
- Nakano, K., Ooshima, T., 2009. Serotype classification of *Streptococcus mutans* and its detection outside the oral cavity. *Future Microbiol.* 4, 891–902. <https://doi.org/10.2217/fmb.09.64>.
- Nordmark, E.-L., Yang, Z., Huttunen, E., Widmalm, G., 2005. Structural studies of an exopolysaccharide produced by *Streptococcus thermophilus* THS. *Biomacromolecules* 6, 105–108. <https://doi.org/10.1021/bm0496514>.

- Pachekrepapol, U., Lucey, J.A., Gong, Y., Naran, R., Azadi, P., 2017. Characterization of the chemical structures and physical properties of exopolysaccharides produced by various *Streptococcus thermophilus* strains. *J. Dairy Sci.* 100, 3424–3435. <https://doi.org/10.3168/jds.2016.12125>.
- Padmanabhan, A., Shah, N.P., 2020a. Structural characterization of exopolysaccharide from *Streptococcus thermophilus* ASCC 1275. *J. Dairy Sci.* 103, 6830–6842. <https://doi.org/10.3168/jds.2019-17439>.
- Padmanabhan, A., Shah, N.P., 2020b. Structural characterization of exopolysaccharide from *Streptococcus thermophilus* ASCC 1275. *J. Dairy Sci.* 103, 6830–6842. <https://doi.org/10.3168/jds.2019-17439>.
- Parlindungan, E., McDonnell, B., Lugli, G.A., Ventura, M., van Sinderen, D., Mahony, J., 2022. Dairy streptococcal cell wall and exopolysaccharide genome diversity. *Microb. Genom.* 8, 000803 <https://doi.org/10.1099/mgen.0.000803>.
- Percy, M.G., Gründling, A., 2014. Lipoteichoic acid synthesis and function in gram-positive bacteria. *Annu. Rev. Microbiol.* 68, 81–100. <https://doi.org/10.1146/annurev-micro-091213-112949>.
- Riu, F., Ruda, A., Ibbá, R., Sestito, S., Lupinu, I., Piras, S., Widmalm, G., Carta, A., 2022. Antibiotics and carbohydrate-containing drugs targeting bacterial cell envelopes: an overview. *Pharmaceuticals (Basel)* 15, 942. <https://doi.org/10.3390/ph15080942>.
- Roach, D.R., Donovan, D.M., 2015. Antimicrobial bacteriophage-derived proteins and therapeutic applications. *Bacteriophage* 5, e1062590. <https://doi.org/10.1080/21597081.2015.1062590>.
- Rockett, R.J., Oftadeh, S., Bachmann, N.L., Timms, V.J., Kong, F., Gilbert, G.L., Sintchenko, V., 2018. Genome-wide analysis of *Streptococcus pneumoniae* serogroup 19 in the decade after the introduction of pneumococcal conjugate vaccines in Australia. *Sci. Rep.* 8, 16969. <https://doi.org/10.1038/s41598-018-35270-1>.
- Romero, D.A., Magill, D., Millen, A., Horvath, P., Fremaux, C., 2020. Dairy lactococcal and streptococcal phage-host interactions: an industrial perspective in an evolving phage landscape. *FEMS Microbiol. Rev.* 44, 909–932. <https://doi.org/10.1093/femsre/fuaa048>.
- Rush, J.S., Edgar, R.J., Deng, P., Chen, J., Zhu, H., van Sorge, N.M., Morris, A.J., Korotkov, K.V., Korotkova, N., 2017. The molecular mechanism of N-acetylglucosamine side-chain attachment to the Lancefield group A carbohydrate in *Streptococcus pyogenes*. *J. Biol. Chem.* 292, 19441–19457. <https://doi.org/10.1074/jbc.M117.815910>.
- Rush, J.S., Parajuli, P., Ruda, A., Li, J., Pohane, A.A., Zamakhaeva, S., Rahman, M.M., Chang, J.C., Gogos, A., Kenner, C.W., Lambeau, G., Federle, M.J., Korotkov, K.V., Widmalm, G., Korotkova, N., 2022. PpID is a de-N-acetylase of the cell wall linkage unit of streptococcal rhamnopolysaccharides. *Nat. Commun.* 13, 590. <https://doi.org/10.1038/s41467-022-28257-0>.
- Salque, M., Bogucki, P.I., Pyzel, J., Sobkowiak-Tabaka, I., Grygiel, R., Szmyt, M., Evershed, R.P., 2013. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature* 493, 522–525. <https://doi.org/10.1038/nature11698>.
- Sawén, E., Huttunen, E., Zhang, X., Yang, Z., Widmalm, G., 2010. Structural analysis of the exopolysaccharide produced by *Streptococcus thermophilus* ST1 solely by NMR spectroscopy. *J. Biomol. NMR* 47, 125–134. <https://doi.org/10.1007/s10858-010-9413-0>.
- Sewell, E.W.C., Brown, E.D., 2014. Taking aim at wall teichoic acid synthesis: new biology and new leads for antibiotics. *J. Antibiot. (Tokyo)* 67, 43–51. <https://doi.org/10.1038/ja.2013.100>.
- Shibata, Y., Ozaki, K., Seki, M., Kawato, T., Tanaka, H., Nakano, Y., Yamashita, Y., 2003. Analysis of loci required for determination of serotype antigenicity in *Streptococcus mutans* and its clinical utilization. *J. Clin. Microbiol.* 41, 4107–4112. <https://doi.org/10.1128/JCM.41.9.4107-4112.2003>.
- Silhavy, T.J., Kahne, D., Walker, S., 2010. The bacterial cell envelope. *Cold Spring Harb. Perspect. Biol.* 2, a000414. <https://doi.org/10.1101/cshperspect.a000414>.
- Simsek, A.D., Sezer, S., Ozdemir, N.F., Mehmet, H., 2008. Streptococcus vestibularis bacteremia following dental extraction in a patient on long-term hemodialysis: a case report. *NDT Plus* 1, 276–277. <https://doi.org/10.1093/ndtplus/sfn071>.
- Sitkiewicz, I., Hryniewicz, W., 2010. Pyogenic streptococci—danger of re-emerging pathogens. *Pol. J. Microbiol.* 59, 219–226.
- Skov Sørensen, U.B., Yao, K., Yang, Y., Tettelin, H., Kilian, M., 2016. Capsular polysaccharide expression in commensal *Streptococcus species*: genetic and antigenic similarities to *Streptococcus pneumoniae*. *mBio* 7. <https://doi.org/10.1128/mBio.01844-16> e01844–16.
- Stefanović, C., Hager, F.F., Schäffer, C., 2021. LytR-CpsA-Psr glycopolymer transferases: essential bricks in gram-positive bacterial cell wall assembly. *Int. J. Mol. Sci.* 22, 908. <https://doi.org/10.3390/ijms22020908>.
- Sullivan, M.J., Petty, N.K., Beatson, S.A., 2011. Easyfig: a genome comparison visualizer. *Bioinformatics* 27, 1009–1010. <https://doi.org/10.1093/bioinformatics/btr039>.
- Swoboda, J.G., Campbell, J., Meredith, T.C., Walker, S., 2010. Wall teichoic acid function, biosynthesis, and inhibition. *ChemBiochem* 11, 35–45. <https://doi.org/10.1002/cbic.200900557>.
- Szafrański, S.P., Winkel, A., Stiesch, M., 2017. The use of bacteriophages to biocontrol oral biofilms. *J. Biotechnol.* 250, 29–44. <https://doi.org/10.1016/j.jbiotec.2017.01.002>.
- Szymczak, P., Filipe, S.R., Covas, G., Vogensen, F.K., Neves, A.R., Janzen, T., 2018. Cell wall glycans mediate recognition of the dairy bacterium *Streptococcus thermophilus* by bacteriophages. *Appl. Environ. Microbiol.* 84 <https://doi.org/10.1128/AEM.01847-18> e01847–18.
- Szymczak, P., Rau, M.H., Monteiro, J.M., Pinho, M.G., Filipe, S.R., Vogensen, F.K., Zeidan, A.A., Janzen, T., 2019. A comparative genomics approach for identifying host-range determinants in *Streptococcus thermophilus* bacteriophages. *Sci. Rep.* 9, 7991. <https://doi.org/10.1038/s41598-019-44481-z>.
- Theodorou, I., Courtin, P., Palussière, S., Kulakauskas, S., Bidnenko, E., Péchoux, C., Fenaille, F., Penno, C., Mahony, J., van Sinderen, D., Chapot-Chartier, M.-P., 2019. A dual-chain assembly pathway generates the high structural diversity of cell-wall polysaccharides in *Lactococcus lactis*. *J. Biol. Chem.* 294, 17612–17625. <https://doi.org/10.1074/jbc.RA119.009957>.
- Tsukioka, Y., Yamashita, Y., Oho, T., Nakano, Y., Koga, T., 1997. Biological function of the dTDP-rhamnose synthesis pathway in *Streptococcus mutans*. *J. Bacteriol.* 179, 1126–1134.
- Uriot, O., Denis, S., Junjua, M., Roussel, Y., Dary-Mourou, A., Blanquet-Diot, S., 2017. *Streptococcus thermophilus*: from yogurt starter to a new promising probiotic candidate? *J. Funct. Foods* 37, 74–89. <https://doi.org/10.1016/j.jff.2017.07.038>.
- van der Beek, S.L., Zorzoli, A., Çanak, E., Chapman, R.N., Lucas, K., Meyer, B.H., Evangelopoulos, D., de Carvalho, L.P.S., Boons, G.-J., Dorfmüller, H.C., van Sorge, N.M., 2019. Streptococcal dTDP-L-rhamnose biosynthesis enzymes: functional characterization and lead compound identification. *Mol. Microbiol.* 111, 951–964. <https://doi.org/10.1111/mmi.14197>.
- van Sorge, N.M., Cole, J.N., Kuipers, K., Henningham, A., Aziz, R.K., Kasirer-Friede, A., Lin, L., Berends, E.T.M., Davies, M.R., Dougan, G., Zhang, F., Dahesh, S., Shaw, L., Gin, J., Cunningham, M., Merriman, J.A., Hütter, J., Lepenies, B., Rooijakkers, S.H.M., Malley, R., Walker, M.J., Shattil, S.J., Schlievert, P.M., Choudhury, B., Nizet, V., 2014. The classical lancefield antigen of group A *Streptococcus* is a virulence determinant with implications for vaccine design. *Cell Host Microbe* 15, 729–740. <https://doi.org/10.1016/j.chom.2014.05.009>.
- Vollmer, W., Blanot, D., de Pedro, M.A., 2008. Peptidoglycan structure and architecture. *FEMS Microbiol. Rev.* 32, 149–167. <https://doi.org/10.1111/j.1574-6976.2007.00094.x>.
- Vollmer, W., Massidda, O., Tomasz, A., 2019. The Cell Wall of *Streptococcus pneumoniae*. *Microbiol. Spectr.* 7 <https://doi.org/10.1128/microbiolspec.GPP3-0018-2018>.
- Wang, Z., Enotarji, J., Buffi, G., Pezzicoli, A., Gstötnner, C.J., Nicolardi, S., Balducci, E., Fabbri, M., Romano, M.R., van der Marel, G.A., Del Bino, L., Adamo, R., Codée, J. D.C., 2022. Chemical synthesis and immunological evaluation of fragments of the multiantennary group-specific polysaccharide of group B *Streptococcus*. *JACS Au* 2, 1724–1735. <https://doi.org/10.1021/jacsau.2c00302>.
- Wei, Y., Joyce, L.R., Wall, A.M., Guan, Z., Palmer, K.L., 2021. *Streptococcus pneumoniae*, S. Mitis, and S. Oralis produce a Phosphatidylglycerol-dependent, Itas-independent Glycerophosphate-linked glycolipid. *mSphere* 6. <https://doi.org/10.1128/mSphere.01099-20> e01099–20.
- Weidenmaier, C., Peschel, A., 2008. Teichoic acids and related cell-wall glycopolymers in gram-positive physiology and host interactions. *Nat. Rev. Microbiol.* 6, 276–287. <https://doi.org/10.1038/nrmicro1861>.
- Whitfield, C., Wear, S.S., Sande, C., 2020. Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu. Rev. Microbiol.* 74, 521–543. <https://doi.org/10.1146/annurev-micro-011420-075607>.
- Wilson, M., Martin, R., Walk, S.T., Young, C., Grossman, S., McKean, E.L., Aronoff, D.M., 2012. Clinical and laboratory features of *Streptococcus salivarius* meningitis: a case report and literature review. *Clin. Med. Res.* 10, 15–25. <https://doi.org/10.3121/cmr.2011.1001>.
- Wong, K.Y., Megat Mazhar Khair, M.H., Song, A.A.-L., Masarudin, M.J., Chong, C.M., In, L.L.A., Teo, M.Y.M., 2022. Endolysins against streptococci as an antibiotic alternative. *Front. Microbiol.* 13, 935145. <https://doi.org/10.3389/fmicb.2022.935145>.
- Wu, Q., Tun, H.M., Leung, F.C.-C., Shah, N.P., 2014. Genomic insights into high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275. *Sci. Rep.* 4, 4974. <https://doi.org/10.1038/srep04974>.
- Yamashita, Y., Tsukioka, Y., Tomihisa, K., Nakano, Y., Koga, T., 1998. Genes involved in cell wall localization and side chain formation of rhamnose-glucose polysaccharide in *Streptococcus mutans*. *J. Bacteriol.* 180, 5803–5807. <https://doi.org/10.1128/jb.180.21.5803-5807.1998>.
- Ya'qoub, L., Rehan, L., Parikh, S., Enriquez, J., 2018. *Streptococcus Agalactiae* infective endocarditis in a healthy middle-aged man: uncommon but life-threatening. *Cureus* 10, e2632. <https://doi.org/10.7759/cureus.2632>.
- Zamakhaeva, S., Chaton, C.T., Rush, J.S., Ajay Castro, S., Kenner, C.W., Yarawsky, A.E., Herr, A.B., van Sorge, N.M., Dorfmüller, H.C., Frolenkov, G.I., Korotkov, K.V., Korotkova, N., 2021. Modification of cell wall polysaccharide guides cell division in *Streptococcus mutans*. *Nat. Chem. Biol.* 17, 878–887. <https://doi.org/10.1038/s41589-021-00803-9>.
- Zhang, B., Zhao, M., Tian, J., Lei, L., Huang, R., 2022. Novel antimicrobial agents targeting the *Streptococcus mutans* biofilms discovery through computer technology. *Front. Cell. Infect. Microbiol.* 12, 1065235. <https://doi.org/10.3389/fcimb.2022.1065235>.
- Zorzoli, A., Meyer, B.H., Adair, E., Torgov, V.I., Veselovsky, V.V., Danilov, L.L., Uhrin, D., Dorfmüller, H.C., 2019. Group A, B, C, and G *Streptococcus* Lancefield antigen biosynthesis is initiated by a conserved α-d-GlcNAc-β-1,4-l-rhamnosyltransferase. *J. Biol. Chem.* 294, 15237–15256. <https://doi.org/10.1074/jbc.RA119.009894>.