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Tyler G. Myers
University of Tennessee, Knoxville, tmyers14@vols.utk.edu

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Investigating corollaries between Streptococcus gallolyticus subsp. gallolyticus virulence and colorectal cancer

TYLER MYERS
Correspondence: tmyers14@vols.utk.edu

Streptococcus gallolyticus subsp. *gallolyticus* (*Sgg*) is a gram-positive, non-beta-hemolytic bacterium that belongs to the Lancefield group D of streptococcal pathogens. For the past several decades, associations between *Sgg* bacteremia and colorectal cancer (CRC) have been apparent, with gastroenterologists routinely recommending colonoscopies for positive cases of *Sgg* in either blood or stool samples. However, current research has not wholly established whether *Sgg* acts as a causative agent of CRC or if pre-existing cancers make the lumen of the colon more hospitable to its colonization. Regardless, *Sgg* has been implicated in the modulation of host inflammatory pathways and subsequent remodeling of gut microbial communities, both of which may constitute predispositions for the formation of colorectal neoplasias. This literature review seeks to define and characterize molecular mechanisms unique to *Sgg* that not only assist in colonizing environments where cancerous tissue abounds but also perpetuate cancerous phenotypes. In addition, this review seeks to not only understand how these mechanisms are internally regulated but also intends to probe host pathways influenced by *Sgg* colonization.

Introduction

Of the many forms of host-microbe interactions that occur within the human body, those associated with the formation and progression of malignant cancers are notoriously difficult to parse. Though microbes have been implicated in a variety of cancer forms, one association of particular interest falls around the connection between microbiota and CRC, a disease of growing epidemiological concern. Globally, CRC ranks as the third most prevalent neoplastic cancer, following closely behind lung and breast cancer in women and lung and prostate cancer in men (Siegel et al., 2021). Following lung cancer, CRC presents the second greatest cause of cancer mortality, with an estimated 9.4% of cancer deaths worldwide in 2020 having a CRC basis (Xi and Xhu, 2021). This burden is expected to increase an estimated 60% throughout the current decade, contributing to more than 2.2 million cases and 1.1 million deaths (Arnold et al., 2017). As countries continue to move through demographic transitions and face the challenge of providing care to increasingly aging populations, needs for identifying causes and delivering treatments against CRC will only grow increasingly important.

As with many cancers, the etiology of colorectal neoplasia remains poorly understood, though genetic predispositions and environmental factors appear to vary in their degree of influence across individuals. At the cellular level, aberrations in signal transduction are regularly associated with CRC neoplasm formation, including signaling through Wnt, TGF-β, PI3K, RTK-RAS, and p53 (The Cancer Genome Atlas Network, 2012). Of note, the Wnt pathway, known to regulate homeostatic patterns in intestinal epithelium replacement, has been found to be constitutively induced in more than 90% of CRC neoplasms. Moreover, amorphic mutations in the negative regulator of Wnt, APC, have been found in around 80% of non-hypermutated CRC tumors and around 50% of hypermutated CRC tumors (The Cancer Genome Atlas Network, 2012). However, discerning the intracellular mechanisms of CRC induction fail to account for phenomena outside the cells of the intestinal epithelium that may drive these mutations in the first place. Such discernment brings into frame the gut microbiome, an extracellular component of the intestinal environment routinely associated with CRC development in the clinical setting (Braten et al., 2017; Flemer et al., 2017; Gao et al., 2017; Lucas et al., 2017). Observational studies of this association in animal models have established that cancer-associated bacteria in the gut microbiome can assume roles in cancer progression (Couturier-Maillard et al., 2013). Moreover, through the expanded metabolic capacity of the gut microbiome and known exertions on the immune system surrounding the intestinal tract, studies have revealed how microbiota

may modulate the effects of chemotherapy (Pope et al., 2017). Removal of this microbiome with antibiotics, in turn, has been shown to reduce the risk of the emergence of colorectal neoplasia (Schwake and John, 2013). In investigating this complex network of microbiota and molecular effects with more scrutiny, some strains have been identified as cancer-mediating within the large intestine, including colibactin-producing *Escherichia coli*, toxigenic *Bacteroides fragilis*, *Fusobacterium* spp., and, of great note, *Streptococcus gallolyticus* subsp. *gallolyticus* (*Sgg*).

In clinical contexts, streptococcal strains were first identified as infectious agents upon their initial discovery in wound infections and pus samples. Their denotation as opportunistic pathogens came later with their associated with chronic endocarditis, as determined through blood agar culturing (Schottmüller, 1903). The correlation between streptococcal-associated endocarditis and CRC was first reported in the early 1950s (McCoy and Mason, 1951), with reemerging interest on the topic appearing two decades later (Hoppes and Lemer, 1974). These subsequent studies noted that in clinical cases of endocarditis affiliated with *S. bovis*, the superseding Lancefield Group D taxonomic identifier, nearly two-thirds of individuals presented with concomitant gastrointestinal dysbiosis. Moreover, carriage rate in feces of *S. bovis* in patients with CRC was nearly five times higher than in otherwise healthy controls (Klein et al., 1977). The specific association between *S. gallolyticus* and CRC was not established until much later with the advent of biotype differentiation within Group D streptococcal strains. Those strains capable of synthesizing organic acids from mannitol, like *S. gallolyticus*, were separated from those that cannot (Schlegel et al., 2003). Moreover, in a unique fashion, *S. gallolyticus*, was found to be capable of decarboxylating gallate, an organic acid derived from the hydrolysis of tannins found in the diets of many of their avian and mammalian hosts in nature (Osawa and Sasaki, 2004). Following the incorporation of more robust systems of phylogenetic classification with multilocus sequence typing (MLST), Group D was again reclassified into seven major subspecies: *S. gallolyticus* subsp. *gallolyticus* (*Sgg*), *S. gallolyticus* subsp. *macedonicus* (*Sgm*), *S. gallolyticus* subsp. *pasteurianus* (*Sgp*), *S. infantarius* subsp. *infantarius* (*Sii*), *S. lutetiensis (Sl)*, *S. alactolyticus (Sa)* and *S. equinus (Se)* (Jans et al., 2015). In its newer classification, *Sgg* was perceived as a species rarely detected in gut communities, until the application of more sensitive polymerase chain reaction (PCR) protocols revealed the carriage rate of *Sgg* to be over 60% in healthy individuals (Dumke et al., 2017). When examining patients with CRC, quantitative PCR analyses have revealed that nearly 75% of colon neoplastic tissues were positive for *Sgg* colonization (Kumar et al., 2017). Additionally, risk for CRC has been demonstrated to be increased in individuals who have serum antibody response to *Sgg* pilus protein (Butt et al., 2019). However, it is important to note that concurrent studies utilizing alternative molecular approaches have indicated a smaller prevalence of *Sgg*, falling below 5% (Andres-Franch et al., 2017). These differing perspectives on *Sgg* association with CRC highlight the notion that the causality of Group D streptococcal pathogens in CRC remains unproven, leaving the bacterial-driver-passenger model as an important means of describing potential mechanisms of CRC formation and progression through a microbial context (Tjalsma et al., 2012).

This last note establishes the goal of this investigation: to compile and survey recent literature that explores the implications of *Sgg* colonization and virulence in mediating CRC.

Through this search, several major patterns have emerged in the physiology of *Sgg* which may confer traits differential from other Group D streptococcal strains and potentially vital in understanding the relationship between *Sgg* and CRC.

Results

Sgg displays enhanced affinity for CRC epithelia through pili repertoire.

A growing body of evidence has suggested that the surface structures of *Sgg* confer a heightened sensitivity to epithelial tissues associated with colorectal neoplasms, being able to colonize these tissues at a rate not only higher than non-cancerous tissues but also at a rate that outcompetes native microbiota. Binding to epithelial and endothelial cells in the host is mediated by a suite containing three distinct pilus-encoding operons, the nature of which have been studied in strains TX20005 and UCN34 (Silanpaa et al., 2009; Rusniok et al., 2010). Two of these operons, *pil1* and *pil3*, appear to be highly conserved across *Sgg* strains following genome-wide analyses of several *Sgg* strains, while *pil2* is far more variable in nature and has received far less scrutiny (Silanpaa et al., 2009; Rusniok et al., 2010). As for *pil1* and *pil3*, a significant body of research has sought to compare the expression and function of their gene products, with several lines of evidence indicating that differential modes of virulence are conferred between the two pilus types. Principally, Pil1 has been demonstrated to assist *Sgg* in binding to the matrix protein collagen, particularly collagen types I and IV, in the context of attaching to heart valves and mediating the development of endocarditis in a rat model (Danne et al., 2011). This finding in strain UCN34 was coordinated through the detection of Pil1 in endocardial tissue through polyclonal antibodies and subsequent immunogold electron microscopy on tissue sections. Moreover, data have expanded Pil1's affinity to blood factor XII, potentially leading to activation, and prolongation, of the intrinsic coagulation pathway as observed through co-inoculation of UCN34 cells with human plasma and separation of cleaved factor XII through SDS-PAGE (Isenring et al., 2017). Pil1's association with type I and type IV collagen helps understand the distribution of *Sgg* infections throughout the body. While type I collagen is largely restricted to the heart, type IV collagen is more widespread, being found in the basal lamina underneath epithelial linings. As such, type IV collagen is the type most associated with mucosal and submucosal layers of the gastrointestinal tract. Interestingly, most colonic tumors, including colorectal neoplasms, produce significantly higher levels of type IV collagen compared to non-cancerous tissues (Skovberg et al., 2009). This finding may provide one gateway for increased prevalence of *Sgg* in colonic tumors compared to non-cancerous tissues.

Unlike Pil1, Pil3 does not appear to prioritize collagen as a substrate, instead displaying preference for mucins, long-chain glycoproteins frequently associated with the intestinal tract. Specifically, the Pil3 adhesin has been demonstrated to have high affinity for the gel-forming mucin subtypes MUC2 and MUC5AC (Skovbjerg et al., 2009; Martins et al., 2015, 2016). While MUC2 is a traditional component of the mucus lining of the intestinal tract, MUC5AC is moreso expressed in the respiratory tract, where it is associated with the mucociliary escalator. It is

important to note that several bodies of research have indicated the deviant expression of MUC5AC in various forms of colonic adenomas and carcinomas (Bartman et al., 1999; Sylvester et al., 2001; Bryant and Stow, 2004). Additionally, various mucinous subtypes of CRC appear to demonstrate elevated expression of MUC2, though other subtypes, interestingly, display decreased expression (Kasprzak et al., 2018). These findings may pinpoint the association of *Sgg* infection with forms of CRC and further elucidate the association between the two conditions.

One area of note is the apparent phase-variation observed in various clinical cases of *Sgg* involving pili expression. In an analysis of strain UCN34, samples demonstrating two subpopulations were revealed, in which roughly two-thirds of cells expressed a low number of pili on their cell surface and one-third of cells expressed a high number of pili (Danne et al., 2014). The mechanism behind this phase variation appears to be rooted in a form of transcriptional attenuation, in which the abundance of a leader peptide regulates high- and low-rate pilus transcription by destabilizing the stem-loop terminator upstream of the *pil1* operon. The *pil1* operon encodes three distinct genes: the adhesion molecule *pilA*, the primary pilin *pilB*, and the pilin polymerase *srtC*. Approximately 100 bp upstream of the ribosomal binding for *pilA* is an intergenic region encoding a leader peptide composed of 22 GCAGA repeats. Additionally, there exists a putative stem-loop terminator between the peptide and RBS. Following the deletion of *pil1* genes in *Sgg* (*Δpil1*), restoration of *pilA*, *pilB*, and *srtC* without this repeat promoter region resulted in homogenous expression of *pil1* within cultures. With this knowledge in mind, the researchers sought to compare the distribution of *pil1* expression across wildtype, *Δpil1*, fully rescued, and promoterless strains using flow cytometry. Results indicated that around 30% of wild-type and rescued cells possessed a high number of pili, 65% possessed a low number of pili, and 5% lacked pili. After isolating cells and sequencing this GCAGA repeat region, it was found that the addition or deletion of GCAGA repeats in the leader peptide did not alter the heterogeneity of UCN34, while the addition of frameshift mutations consistently produced a high-pili phenotype. Ultimately, frameshift mutants were frequently associated with having a high number of pili, and sequencing of a wild-type, high-pili promoter region revealed the encoding of a longer regulatory peptide than that observed in the low-pili phenotype, preventing the formation of the hairpin loop that reduces the rate of *pil1* translation (Fig. 1). The researchers noted that a very similar mechanism of phase variation was observed in the *pil3* operon, creating an entirely new layer of heterogeneity to *Sgg* populations.

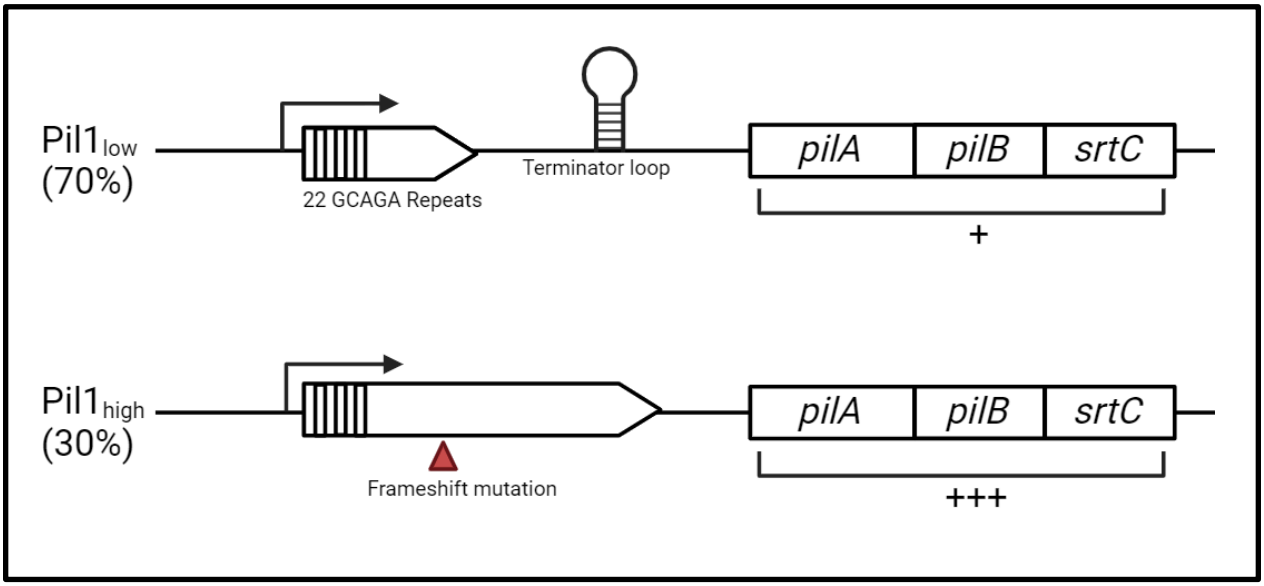

Figure 1: Phase variation within the *pil1* operon in *Sgg*. Note the elongated regulatory peptide in the promoter region of the high-pili schematic (bottom), with its extension blocking the formation of a terminator loop. This figure was generation in BioRender

Subsequently, Pil1 hyper-piliated bacteria were observed to be more susceptible to macrophage-mediated phagocytosis and fared poorly in human blood compared to the cells that expressed lower levels of pili (Danne et al., 2014). What this indicates about the endurance of *Sgg* in the colonic epithelium is unclear, though the "pruning" of high-*pil1*-expressing cells through phagocytosis may encourage a greater reliance on *pil3* gene products when attaching to host epithelia. This phenomenon may have already been validated through observations on a variety of Group D streptococcal strains, with *pil1* expression primarily noted in human blood-derived isolates from *Sgg*, *Sgp*, *Sii*, *Sl*, and *Sgm* (Vanrobaeys et al., 2000a; Sillanpää et al., 2008; Vollmer et al., 2010; Boleij et al., 2011b; Grimm et al., 2018). Similar observations involving the incidence of *pil3* across these strains may provide further insight into how the diverse suite of pili encoded by *Sgg* assists in colonization of host tissues, particularly those in the dynamic microenvironment of cancers.

Augmented metabolic capability and metabolite production outcompetes native microbiota.

Another feature uniquely affiliated with *Sgg* lies within its remarkable metabolic capabilities, many of which help the bacterium to outcompete the native strains of the gut microbiome. For one, *Sgg* is predicted to be capable of synthesizing all twenty canonical amino acids as well as most vitamin coenzymes, thereby demonstrating little auxotrophy in the host environment (Rusniok et al., 2010). This availability to amino acids and coenzymes confers a heightened metabolic ability of *Sgg* when processing the abundant supply of carbohydrates present in the intestinal mucosa. Strains of *S. bovis* were previously identified to be one of the most efficient utilizers of liberated glucose in the gut microbiome, significantly outpacing nearly all other native constituents (Egert et al., 2007). In addition to glucose, *Sgg* is the only described taxon in the entire *Streptococcus* genus to metabolize malate, doing so using its own malolactic enzyme and malate transporter (Rusniok et al., 2010). Similarly, *Sgg* carries the gene *tanA*, giving it the unique propensity to degrade tannic acids that would otherwise be toxic to most constituents of the native microbiome (Papadimitriou et al., 2014). Other metabolizable compounds that may give *Sgg* a competitive advantage in the gut microbiome include bile salts, fructose 6-phosphate, 3-phosphoglycerate, and alanine (Boleji et al., 2012a). Considering the high rate of glycolysis observed in many cancers, including colorectal neoplasias, the ability to quickly harness glycolytic intermediates would provide immense help in colonizing the colon upon reaching the mucosal layer. Moreover, the ability to maintain its own full suite of pathways for amino acid synthesis while efficiently scavenging for metabolites in the intestinal lumen further demonstrates the metabolic versatility of this species.

The note on bile salt metabolism demonstrates an interesting point, as several gene products that may inhibit the growth of microbial competitors. One major antimicrobial found to be produced by *Sgg* has since been named gallocin, a two-peptide bacteriocin that specifically targets and kills phylogenetically closely related strains like commensal enterococci through membrane permeabilization (Proutière et al., 2023). Gallocin is encoded by the genes *gllA1* and *gllA2*, as recently named (Hill et al., 2020). In strain UCN34, production of gallocin was found to

significantly enhance colonization across the ileum and colon in mice already carrying intestinal tumors. These strategies for outcompeting native microbiota were not replicable in mice that lacked colonic tumors (Aymeric et al., 2018). Furthermore, the activity of this gallocin is strongly dependent on the secondary bile acids that would normally clear out many microorganisms including deoxycholic and lithocholic acids (Aymeric et al., 2017). The intracellular regulatory aspects of gallocin are rather complex, with stimulation of gallocin production occurring in a cell density-dependent manner through a two-component signaling pathway known as BlpH/BlpR (Proutière et al., 2021). By clearing out closely related native microbiota, *Sgg* can efficiently and effectively carve its own niche in the gut microbiome and presume former metabolic capacities through its repertoire of mechanisms for amino acid and vitamin synthesis.

To probe the mechanism of gallocin regulation in Sgg, researchers studied the previously sequenced genome of strain UCN34 within the gallocin locus and identified what was suspected to be a three-component regulatory system. This system is comprised of blpH, thought to encode a histidine kinase; blpR, thought to encode a response regulator protein; and a third gene that was hypothesized to encode an inducing peptide designated as gallocin-stimulating peptide (GSP). This GSP molecule had previously been described by another group as an analog of competence-stimulating peptide (CSP), noting its presence in the extracellular environment leading to activation of bacteriocin production in Sgg without the induction of competence (Harrington and Tal-Gan, 2018). This regulatory system is situated directly upstream of both gllA1 and gllA2, a gene encoding an immunity peptide against gallocin known as gip, and two genes encoding an ABC transporter, blpA and blpB. This ABC transporter was previously established to be associated with the secretion of gallocin into the extracellular environment (Harrington et al., 2020). To verify the function of the three-component regulatory system, in-frame deletions were generated in gsp, blpH, and blpR, and it was established that gallocin production was terminated in all three of these mutants. Moreover, supplementation of Δgsp cultures with synthetic GSP restored production of gallocin in a dose-dependent fashion. Gallocin production was monitored Streptococcus gallolyticus virulence and colorectal cancer 7 by inserting a GFP marker directly upstream of gllA2 within its promoter, with cells monitored for fluorescence over a growth curve.

In expanding their search for gallocin-associated genes, the researchers also conducted whole-transcriptome analysis on each of the Δ*gsp,* Δ*blpH*, and Δ*blpR* mutants. This analysis flagged 24 genes downregulated when any of these three genes were nullified, with twenty of these genes confined within the gallocin locus as several were described above. Downregulated genes outside the locus included an additional putative ABC transporter and two hypothetical proteins without described function. Interestingly, one gene cluster was found to be upregulated in the absence of *gsp* specifically: *pil3*. It was posited that this upregulation may result from a mechanism of phase variation, like that of *pil1* as previously described, with interplay from GSP. Such results may therefore indicate that Pil3 is expressed in higher amounts at lower cell densities, with cells relying on other external structures besides Pil3 at higher cell concentrations (i.e. biofilms). Upon further examination of protein domains within each of the three components, it was observed that a putative protein encoded upstream of *blpR* possesses a DNA-binding domain similar in structure to *blpR* itself. In-frame deletion within this gene generated higher expression of gallocin, thereby revealing a

repressor named BlpS that counteracts the activity of the BlpR response regulator. In verifying these claims, the researchers found that overexpressing gallocin in a Δ*blpS* mutant generated cells that could far more effectively kill enterococcal bacteria in a gut microbiome-simulating competition assay. A depiction of the induction, inhibition, and secretion of gallocin, based on these findings, is provided in Figure 2.

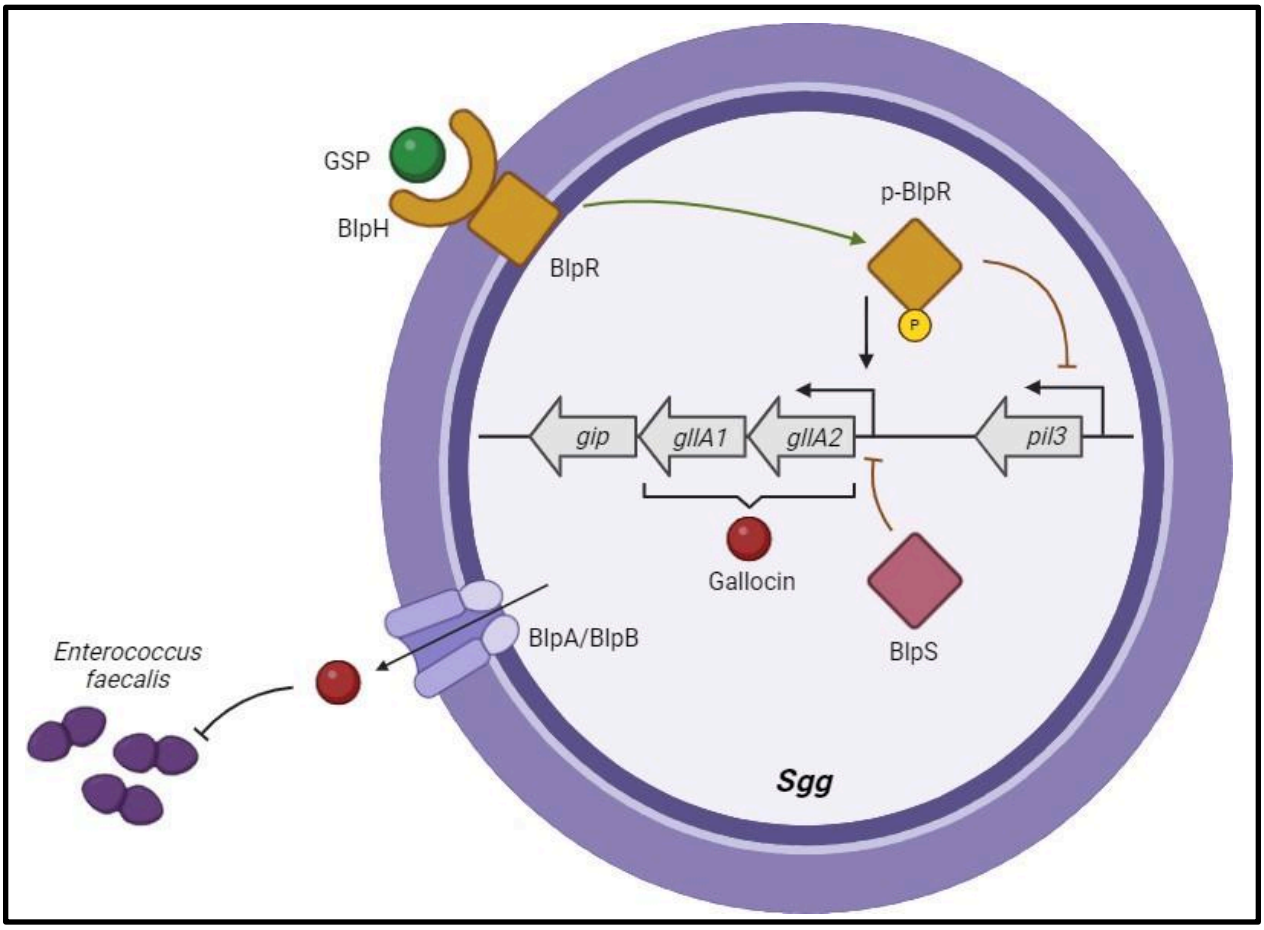

Figure 2. **A four-component system mediates gallocin production in *Sgg***. Note that, in the presence of both BlpR and BlpS, phosphorylated BlpR maintains higher affinity to the *gllA* promoter than BlpS. In the absence of a GSP signal, BlpS binding predominates. This figure was generated in BioRender.

One factor that may heavily contribute to the diversity of metabolic and toxin-producing systems in *Sgg* and enhance its ability to overtake native strains is the degree to which horizontal gene transfer has contributed to niche adaptation for Group D streptococcal pathogens. When examining the range of genomes within Group D, nearly all demonstrate a degree of horizontal gene transfer related to varying forms of carbohydrate metabolism, capsular polysaccharide formation, antimicrobial resistance, and tannic acid degradation, the last of which being unique to *Sgg* (Rusniok et al., 2010; Hinse et al., 2011b; Lin et al., 2011b; Jans et al., 2013; Papadimitriou et al., 2014; Kambarev and Caté, 2015; Grimm et al., 2017a; Kambarev et al., 2017). The source of the efficacy of these transfers appears to stem from competence operons and genes directing pseudopilus formation directly derived from *Sgg* and conferred horizontally to close relatives (Rusniok et al., 2010; Lin et al., 2011b; Jans et al., 2016). The impact of horizontal transfer has varied across members of the group; while some strains, such as *Sii*, *Sgm*, and *Sgp* encountered niche adaptation through gene loss, particularly in components of the biosynthesis of pantothenic acid and biotin, other strains like *Sgg* expanded their footing to occupy niches held by strains ousted by *Sgg* itself (Lin et al., 2011b; Papadimitriou et al., 2014). This adoption of a "generalist" mode further propels *Sgg* into a role well-suited for cancer tissue colonization and adaptation to a rapidly changing environment.

Immune evasion and modulation occur through capsule structure and surface proteins.

Once established within the epithelium of the colon, *Sgg* cells must continually ensure a range of measures to minimize detection from the host immune system. Interestingly, the capsule of *Sgg* has been configured in a manner that minimizes immune cell detection to a rather high degree. For one, the extracellular capsule of *Sgg* has been demonstrated to share many structural features with that of *Streptococcus pneumoniae* serotypes 16F and 23F (Li et al., 2019), a trait that likely contributes to a minimized pro-inflammatory response that would normally be mediated by cytokines such as interleukin-8 (Boleji et al., 2011). Within the polysaccharide-imbued capsule itself, particular residues like glucose and rhamnose, both of which are also found in *S. pneumoniae*, occur in differing iterations and patterns. The polysaccharides that comprise this capsule have varying identities, with different proteins assisting the formation of different manifestations of the capsule. When active, the glycosyltransferase GtfA generates insoluble α- 1,3-linked glycosidic polymers. On the other hand, the glycosyltransferase GtfB produces both α- 1,3-linked water-insoluble and α-1,6-linked glycosidic soluble polysaccharides (Lin et al., 2011b). Both arrangements have been demonstrated to play specific roles in the adhesion and colonization of host surfaces and the evasion of the host immune system (Nobbs et al., 2009; Isenring et al., 2018).

Besides the arrangement of the capsule, the composition of lipoproteins in the membrane of *Sgg* mediates various host protein interactions of immunological significance. Based on genomic and protein domain analyses of strain UCN34, many of these proteins possess a serine-rich motif with a preceding cysteine residue (Rusniok et al., 2010). While the exact function of

these motifs is unknown, it has been suggested that their arrangement assists in mediating interactions with polysaccharides such as glycosaminoglycans in the intestinal lumen. Alongside these lipoproteins, another important surface protein identified in UCN34 mediating surface interactions is enolase. Enolase is an anchorless protein that was demonstrated to be involved in the crosslinking of *Sgg* with intestinal epithelial cells as mediated by the host protein cytokeratin 8 (Boleij et al., 2011a). While all intestinal epithelial cells, as well as epithelial cells in skin and other mucosal surfaces, constitutively express cytokeratin 8, colorectal neoplasms have been found to release the protein in elevated amounts (Boleij et al., 2011a). This enhanced crosslinking may provide *Sgg* yet another avenue for improved colonization of cancerous gut epithelial tissue.

Once colonized, several events involving immune regulation have been associated with *Sgg*. Reports have suggested that mRNA increases in genes encoding proinflammatory products like interleukin-1, interleukin-8, and COX-2 occur directly because of bacterial colonization following assessment of [mRNA] through reverse transcription PCR and association of cancerous tissue with *Sgg* infection through *in situ* hybridization of *Sgg*-derived *SodA* (Adbulamir et al., 2010). However, the specific mechanisms for these interactions other than cell-mediated response through toll-like receptor activation have been poorly described. Working from another angle, demonstrations in the *Sgg* strain TX20005 have revealed CRC development upon infection with direct observation of intestinal epithelial cell proliferation in a murine model (Kumar et al., 2017). This increased proliferation was found to be concordant with an upregulation of the signal protein β-catenin, associated with the Wnt pathway, as well as upregulation in several downstream targets like Myc and cyclin D. This same study found that, alongside increased epithelial cell proliferation, higher levels of dysplasia and an increased number of metastatic tumors were recorded in *Sgg*-infected mice compared to mice infected with a *Lactococcus lactis* control. While the bacterial-derived regulators of these pathways are still being researched, other research groups have posited that the delivery of any sort of mediator of increased CRC epithelial proliferation requires assembly of a specific type VII secretion system, identified as *Sgg*T7SS^{T05} (Taylor et al., 2021). This was demonstrated through the knockout of the system in strain TX20005 leading to decreased adherence and virulence in HT29 colorectal adenocarcinoma cells. As the nature of these effector proteins becomes more well understood, the pieces currently in place for understanding the role of *Sgg* first avoiding immune detection and then contributing the local immune activation will undoubtedly become clearer.

Discussion

Despite the examination of the lines of evidence presented above, the answer to whether *Sgg* infection precedes cancer formation in CRC patients or if CRC development creates hospitable environments for *Sgg* colonization remains somewhat dubious. It remains certain that the turbulent landscape of the intestinal epithelia generates several avenues for *Sgg* colonization, particularly through increased production type IV collagen, MUC5AC, and cytokeratin 8.

Considering that not all cancers will present these binding sites in a consistent manner, developing an understanding of *Sgg*'s ability to bind any of these structures may provide key details as to which patients may be particularly susceptible to infections that would further complicate cancer diagnoses and treatment regimens. Concurrently, observing *Sgg* entails an array of components intended for altering microbiome structure and points evidence in the direction of *Sgg* acting as a direct perpetrator of CRC formation through gut dysbiosis. Attempting to examine the association between *Sgg* and CRC through the lens of unidirectional causation is likely misleading. The culmination of evidence compiled through this investigation suggests that, while *Sgg* carries propensities for carcinogenesis in specific scenarios, the bacterium certainly also takes advantage of sites containing already-formed tumors, exercising those same molecular mechanisms to drive cancer progression.

One major area of study that would appear to greatly benefit from continued study of the interactions between *Sgg* and CRC is the degree to which effector proteins inspire immunological changes within host cells through alterations in cell signaling pathways. Isolation of the Wnt signaling pathway and its modulation by the presence of *Sgg* suggests that some gene product or metabolite from the bacterium may generate these host alterations in a manner that is currently undescribed. Additionally, more attention should be paid to the mechanisms of phase variation in Pil3 proteins. Their association with mucin subtypes specific to colorectal neoplasms provides important insight into their enhanced binding to cancer tissue. Considering the longstanding implications of *Sgg* and other Group D streptococcal pathogens with endocarditis, enhancing the study of pathogenicity from these microorganisms in the intestinal tract to this same level of rigor will likely parse the diverse ranges of molecular pathways in which this bacterium already has association.

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