The genomes of Group B Streptococcus and the relationship to pathogenesis of disease

H. H. L. Ma and M. Ip

Department of Microbiology, Chinese University of Hong Kong, Shatin, Hong Kong

Group B Streptococcus (*Streptococcus agalactiae*) is an opportunistic pathogen which can be carried asymptomatically, and be transmitted from mother to child causing meningitis and pneumonia. Infrequently, severe conditions like toxic-shock syndrome can be presented in previously healthy adults. A list of virulence factors, such as CAMP factor, β-hemolysin / cytolysin, C5a peptidase, and other cell-wall-anchored proteins related to adhesion and invasion has been identified through combination of microbiological and genomics approaches. With publications of GBS genomes of different serotypes made available, studies on pathogenesis could be applied on a wider spectrum of analysis. Besides traditional virulence factors that directly involve in disease progression, virulence-related transcriptional regulators that are involved in relaying signals from environmental changes, can be included to give a more dynamic view to understanding pathogenesis of GBS disease. In this review, classical microbiological and genomic approaches on investigating virulence factors in existing studies are compared, and the correlation of bioinformatics approaches with virulence-related transcriptional regulators is discussed.

Keywords Group B streptococcus; *S. agalactiae*; genome; pathogenicity

1. Introduction

1.1 Characteristics, disease burden and epidimiology of Group B Streptococcus

Streptococcus agalactiae, or Group B Streptococcus (GBS) due to its positive reaction with Lancefield group B serum, is a facultatively anaerobic Gram-positive coccus which produces β-hemolysis on blood agar plates. It can be classified into ten serotypes (Ia, Ib, II to IX) by different antigenicity of the capsular polysaccharide. [1, 2]

GBS is an opportunistic pathogen. It can be carried asymptomatically in genital and lower gastrointestinal tracts of pregnant mothers, and transmitted to infants *in utero* or during delivery, such as through vaginal tract or by aspiration. GBS can cause severe diseases like meningitis, pneumonia and septicemia in both mothers and children. [3] GBS transmitted to newborns can cause disease as early as the first few hours after delivery, and this is classified as early-onset disease. The infant suffers from pneumonia, which can progress rapidly to bacteremia and septic shock. GBS can cause disease with onset at a later stage of life from a week to three months after delivery, and known as late-onset disease. GBS enters bloodstream and crosses the blood-brain barrier to infect the central nervous system, causing meningitis. [4]

GBS is a burden in adults, diseases especially in those with underlying diabetes, neurological impairment, breast cancer and cirrhosis, and presents with pneumonia, soft tissue, bone and joint infections. In rare cases, GBS infections can progress to necrotizing fasciitis and toxic shock syndrome. Association studies also found that adults with predisposing conditions like diabetes mellitis are more prone to soft tissue infections and toxic shock syndrome. [5, 6]

Epidemiology studies showed that most invasive isolates belong to serotypes Ia, III and V, while serotype III has been associated with neonatal invasive disease and meningitis. [7, 8] Serotype III can be further classifed into four subtypes according to sequence variation in polysaccharide genes. [9] Serotype III subtypes 1 (ST19) and 2 (ST17) have been associated with adult endocardits and neonatal meningitis respectively, while subtype 4 (ST11 and ST283) have been implicated in adult meningitis. [10, 11, 12]

1.2 A brief history of GBS pathogenesis studies

GBS has been identified from milk samples from cows before 1930s, and causes mastitis in cows. Interests into GBS pathogenesis began in the 1960s when increasing attention was drawn to neonatal meningitis caused by this organism. [13] By 1970s, a large number of publications were related to GBS infections in both cows and humans, on GBS colonization, immune responses, antibiotic treatment, and diagnoses. [14, 15, 16] Subsequent researches were directed to elucidating specific virulence factors such as the sialyated polysaccharide capsule, toxins and surface proteins since the 1980s. [17, 18, 19]

The study of GBS pathogenesis has progressed to a new era in this millennium. GBS genomes were published in 2002, first of a clinical isolate of serotype III strain, NEM316 [20] and followed by a serotype V strain, 2603V/R [21]. Since, three completed genomes (strains A909, NEM316, 2603V/R) and five draft genomes (strains 515, CJB111, COH1, H36B, 18RS21) have been made available from the NCBI (up till April 2010) [22]. Comparative genomics and advances to bioinformatics facilitated the search of target sequences and motifs among different streptococcal genomes

and led to identification of novel virulence factors, such as novel surface-anchored proteins [21], and even previously unknown pili-like structures in GBS [23].

This review is divided into three parts. The first part is a comparison on the approaches from the microbiological and genomics perspectives of studying GBS pathogenesis, followed by a second part on the correlation between results from classical and bioinformatics research of GBS. The final part is on how comparative studies on bacterial genomes and bioinformatics approaches would facilitate in the understanding of virulence and host-pathogen interactions of GBS.

2. Comparison on approaches taken in GBS pathogenesis studies

Table 1 shows a summary on the experimental approaches taken for elucidating important virulence factors known. The virulence factors are arranged in the order of ascending year of publication, tabulating their role in pathogenesis, functions and experimental proof for the listed properties. The role in pathogenesis is classified as '**A**dherence to host epithelial and endothelial surface', '**I**nvasion across host blood cells and epithelial layers' and '**R**esistance to innate immune clearance' for simplified characterization. The experimental proof from the publications are categorized into '**A**nimal infection model', '**B**iochemical assay', '**C**ell culture model' and '**S**equence-based approach' according to the research approaches taken to demonstrate the respective role in pathogenesis.

2.1 Microbiological approach on GBS pathogenesis study

Classical microbiology approach in studying pathogenesis begins by isolation of the bacterial pathogen and fulfilling the Koch's postulates to confirm its pathogenic potential. Often, the potential pathogen would be inoculated into an animal model to demonstrate comparable clinical disease as in humans. GBS was first identified as a pathogen in 1953 by Lancefield and Hace, from serological differentiation of pathogenic and non-pathogenic hemolytic streptococci [24]. By 1980s serotype III isolates were identified as a predominant serotype in neonatal meningitis when pathogenesis studies were more focused into studying more specific strains.

Virulence factors like CAMP factor, β-hemolysin / cytolysin were among the first factors discovered [25, 27]. Studies on these factors were probably related to the relative ease in the chemical methods in extraction of these proteins and detection of functional activities. These toxins are secreted products from GBS into the culture medium, and obtaining the supernatant of culture with simple purification approaches with injection into animal models for demonstration of their pathogenic effects. This was simpler than obtaining purified proteins from other parts of bacterial cell fractions. Capsular polysaccharides had also been a target of study due to its antigenicity, and to which experimental methods had been well-developed before 1980s [32, 33].

By 1990s, a range of deletion mutants had been designed based on well-characterized clinically virulent strains, such as serotype Ia (A909), serotype III (NEM316) and serotype V (2603 V/R), to study each of the postulated virulence factors. For each virulence factor, a series of biochemical experimentation including binding affinities to components of extracellular matrix (e.g. fibrinogen, fibronectin, laminin, plasminogen-binding proteins, etc.) [34, 36, 37, 43, 44]; activities to digest these proteins and other products related to host immune responses (e.g. serine protease, hyluronate lyase, C5a peptidase, superoxide dismutase, glycolytic enzymes, etc.) [34, 35, 38, 39, 42, 55, 60] were performed, supplemented with data from cell culture assays and animal infection models.

2.2 Genomics approach on GBS pathogenesis study

Genomics approach relies greatly on the availability of sequenced genomes and shared databases. NCBI served as a common platform for sharing sequences of virulence factors, but the use is still limited for obtaining an overview of pathogenesis when published genome were lacking.

After 2002, when GBS genomes were published, comparative genomic studies emerged through similarities of target factors in published genomes. Potential virulence factors are identified from homology searches of known virulence factors from other species (such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*), or related genes belonging to the same gene family (e.g. serine protease, adhesin). Novel surface proteins can also be identified by conserved cell-wall anchoring motif searches among published GBS genomes, such as on C-terminal LG(X)PT motifs (e.g. novel bacterial invasin, pili proteins, serine-rich repeat domain protein) [41, 45, 48-54, 56, 57]. Much of these were predictions using bioinformatics and reduced the laboratory demonstration through DNA-hybridization and protein extraction experimental work.

Table 1 Summary on experimental approaches taken on virulence factor investigation in various GBS strains.

Virulence factor (gene)	Role in pathogenesis	GBS strain(s) (serotype)	Experimental approach (Results)	Ref.
CAMP factor (*cfb*)	**(I)** Forms pores on erythrocytes by binding to GPI-anchored surface proteins		**(A)** Infection model in mice and rabbit (lethal effect) **(C)** Incubation of sheep erythrocytes with recombinant CAMP (Pores of 12-15nm are formed on erythrocytes) **(C)** Incubation of sheep erythrocytes with bacteria which GPI-anchored proteins are cleaved (Binding of bacteria to erythrocytes decreases)	24-26
β-hemolysin / cytolysin (*cyl* operon)	**(I)** Forms pores and lyses epithelial and endothelial cells **(R)** Induces cytolysis and apoptosis in phagocyte and macrophage **(R)** Triggers release of interleukins from macrophage	A909 (Ia); COH1 (III); COH 31 (III); NCTC10/48 (V)	**(A)** Pulmonary disease model in neonatal rabbit (β-h/c deletion mutant of NCTC10/48 (V) confers to decreased mortality, development of bacteraemia and blood bacterial count than wild-type) **(A)** Sepsis model in mouse (β-h/c deletion mutants of NCTC10/48 (V) and A909 (Ia) have decreased mortality than wild-type) **(C)** Incubation of bacteria with lung epithelial cells and lung microvascular endothelial cells (High-hemolytic mutants derived from NCTC10/48 (V), A909 (Ia), COH1 (III) and COH 31 (III) by random mutagenesis demonstrated injury to human cells) **(C)** Phagocyte and macrophage killing assay (β-h/c deletion mutant of NCTC10/48 (V) and A909 (Ia) have decreased growth than wild-type)	27-31
Capsular polysaccharide (*cps* operon)	**(R)** Resistance to killing by macrophage and neutrophil	COH 31 (III)	**(A)** Infection model in mouse (Transposon-mutant of COH 31 (III) which become uncapsulated is less virulent) **(C)** Mouse macrophages and neutrophil killing assay (Transposon-mutant of COH 31 (III) and COH 31 (III) treated with sialidase are more susceptible than untreated strains and wild-type)	32-33
Hyaluronate lyase (*hylB*)	**(I)** Degrades hyaluronic acid which is an important ECM component in placental tissues	3520 (III)	**(B)** Incubation of hyaluronate lyase from 3520 (III) with hyaluronic acid *in vitro* (Hyaluronic acid is cleaved) **(S)** Amplification of nucleotide sequence of *hylB* by primers based on the enzyme's amino acid sequence (Nucleotide sequence of *hylB* was identified from 3520 (III))	34-35
Laminin-binding protein (*lmb*)	**(A)** Adheres to laminin in ECM **(A & I)** Mediates adherence and invasion to BMEC	O90R (ATCC12386) (Ia)	**(B)** Incubation with immobilized laminin (Disruption mutant of O90R (Ia) in *lmb*, a putative homologue of LraI streptococcal family, failed to adhere immobilized laminin) **(C)** Incubation with BMEC (Disruption mutant of O90R (Ia) in *lmb* has decreased invasion)	36-37
C5a peptidase (*scpB*)	**(A)** Binds to fibronectin in ECM **(R)** Cleaves the complement-derived complement-derived C5a	7360 (III); COH1 (III)	**(B)** Incubation with recombinant human C5a (Recombinant human C5a is cleaved in 7360 (III) culture) **(B)** Fibronectin- binding assay(Decreased in fibronectin -binding of *scpB* deletion mutant of COH1 (III)compared to wild-type)	38-39
Superoxide dismutase (*sodA*)	**(R)** Neutralizes superoxide anions produced by macrophages	NEM316 (III)	**(A)** Virulence model in model (*sodA* deletion mutant of NEM316 (III) has decreased virulence than wild-type) **(B)** Oxygen radical resistance assay (*sodA* deletion mutant of NEM316 (III) is more susceptible to oxidative damage than wild-type) **(C)** Incubation with mouse macrophage (*sodA* deletion mutant of NEM316 (III) is more susceptible to oxidative burst from mouse macrophage)	60

Table 1 Continued

Virulence factor (gene)	Role in pathogenesis	GBS strain(s) (serotype)	Experimental approach (Results)	
Immunogenic bacterial adhesion protein (*bibA*)	**(A)** Adherence to human cervical and lung epithelial cells **(R)** Binds to C4-binding protein of the classical complement pathway	2603V/R (V)	**(A)** Infection model in mouse (*bibA* deletion mutant of 2603V/R (V) has reduced virulence than wild-type) **(A)** Incubation of recombinant BibA with human C4-binding protein (Recombinant BibA specifically binds to human C4-binding protein) **(A)** Incubation of recombinant BibA with human cervical and lung epithelial cells (Recombinant BibA binds to human cells) **(C)** Human blood survival and neutrophil resistance assay (*bibA* deletion mutant of 2603V/R (V) has severely reduced capacity of survival in human blood and resistance against neutrophils than wild-type) **(S)** Bioinformatics analysis to the eight published GBS genomes (A well-conserved, cell-wall located protein is identified) **(S)** Identification of BibA from published genomes withbioinformatics analysis (BibA is present in isolates from all serotypes)	41
Cell-surface protesase (*cspA*)	**(I)** Cleaves fibrinogen in ECM **(R)** Produces fibrin-like cleavage products that coat bacterial cell surface which interfere complement-mediated opsonophagocytic clearance	COH1 (III)	**(A)** Sepsis model in neonatal rat (COH1 (III) is ten times more virulent than its *cspA* deletion mutant) **(B)** Incubation with human fibrinogen (COH1 (III) cleaves human fibrinogen, while its *cspA* deletion mutant doesn't) **(S)** Identified from a transposon mutant with insertion in an ORF (*cspA*) of a surface-associated, cell-wall-anchored protein, with 55% similarity with putative extracellular protease in GAS **(S)** Southern blotting with *cspA* probe in clinical isolates (*cspA* is present in clinical isolates of all serotypes)	42
Fibrinogen-binding protein (*fbsA*, *fbsB*)	**(A)** Attaches to fibrinogen in ECM **(A & I)** Mediates adherence and invasion to BMEC	706 S2 (Ia); O176 (II); 6313 (III); SS1169 (V)	**(B)** Incubation with soluble fibrinogen (*fbsA* deletion mutants have decreased binding than wild-type) **(C)** Incubation with human epithelial and A549 cells (*fbsA* deletion mutants have decreased adherence to the human cells than wild-type) **(C)** Incubation with BMEC (Disruption mutant of 6313 (III) in fasA has decreased invasion to BMEC than wild-type)	34, 43-44
Leucine-rich –repeat protein (*lrrG*)	**(A)** Adherence to human epithelial cells		**(C)** Incubation of recombinant LrrG with human epithelial cells (Recombinant LrrG binds to the human cells in dose-dependent manner) **(S)** Identification of whole length nucleotide sequence of LrrG from all nine serotypes in GBS with by genome walking, (LrrG nucleotide sequence is present in all serotypes, with 81% similarity with homologue in GAS)	45
Penicillin-binding protein 1A (*ponA*)	**(R)** Resists AMPs and killing by alveolar macrophage and neutrophils	A909 (Ia)	**(A)** Aerosolized lung infection model in neonatal rat(*ponA* deletion mutant of A909 (Ia)has decreased survival than wild-type) **(B)** AMP killing assay with cathelicidins and defensins (*ponA* deletion mutant of A909 (Ia)is more susceptible to killing than wild-type) **(C)** macrophage and neutrophil killing assay (*ponA* deletion mutant of A909 (Ia)is more susceptible to killing than wild-type)	46-47

Table 1 Continued

Virulence factor (gene)	Role in pathogenesis	GBS strain(s) (serotype)	Experimental approach (Results)	
Pili-associated protein (*pilA*, *pilB*, *pilC*)	**(A & I)** PilB polymerizes pili backbone, which mediates adherence and invasion to BMEC **(R)** PilB resists killing by AMPs and associates with enhanced phagocytic resistance and systemic virulence **(A)** PilC contains two Ig-like domains required for binding to epithelial cells	COH1 (I); NEM316 (III), NCTC10/84(V); 2603V/R (V), JM9130013 (VIII)	**(A)** Infection model in mouse(*pilB* deletion mutant of NCTC10/84 (V) is less virulent than wild-type) **(B)** Immunogold microscopy and transmission electromicroscopy (With labeled-antibody raised from COH1 (I); JM9130013 (VIII)) and strains with *pilA* and *pilB* genes revealed pili-like structure) **(B)** Transmission electron microscope (Observation of pili-like structures) **(B)** AMP killing assay (cathelicidins) (*pilB* deletion mutant of NCTC10/84 (V)is more susceptible in than wild-type) **(C)** Incubation with BMEC(*pilA*, *pilB* deletion mutants of NCTC10/48 (V) showed decreased adherence than wild-type) **(C)** Macrophage killing, neutrophil killing and intracellular survival assays(*pilB* deletion mutant of NCTC10/84 (V)is more susceptible than wild-type) **(S)** Searching of LPXTG cell-wall anchoring motifs in published serotype V genomes(The genomes contain either one or both Pili pathogenicity islands composed of *pilA*, *pilB*, *pilC* (Pi-I, Pi-2a/b))	48-54
GADPH (*plr*)	**(I)** Degrades host ECM proteins by glycolysis **(A & I)** Binds to lysine residue of plasminogen, promotes invasion and systemic spread	NEM316 (III)	**(A)** Mouse model of infection (Virulence of NEM316 (III)is only shown with plasminogen and/or fibrinogen added) **(B)** Incubation with plasminogen and fibrinogen (NEM316 (III) bacterial cells binds plasminogen and degrades fibrinogen *in vitro*)	55
Serine-rich-repeat protein (*srr1*)	**(A)** Binds to human keratin 4 in saliva	NEM316 (III); 6313 (III)	**(B)** Incubation with human K4 protein(*srr1* deletion mutant of clinical isolate 6313 have decreased binding compared to wild-type) **(C)** Incubation with epithelial HEp-2 cell (*srr1* deletion mutant of clinical isolate 6313 have decreased binding compared to wild-type) **(S)** Searching for LPXTG cell-wall anchoring motifs in NEM316 (III) genome (ORF1529 is identified as *srr1*)	56-57

3 . Correlation between classical and bioinformatics researches on GBS

Classical pathogenesis research focuses on virulence factors that directly participate in disease progression. Availability of GBS genomes makes it possible to get a fuller view on transcriptional regulators that control the expression of virulence factors in response to environmental changes. Comparison of GBS genomes with other streptococcal genomes helps to identify transcriptional regulators homologous to those in other species, such as *Streptococcus pyogenes* (Group A streptococcus, GAS) and *Streptococcus pneumoniae*. [58, 59] The role of transcriptional regulators in pathogenesis is especially important for opportunistic pathogens like GBS, as differences in pH, temperature, ion concentrations, nutrient availability, etc. in carriage and invasive sites may alter the disease pathogenesis. Transcriptional regulators act as a switch to relay signals from environmental changes to virulence pathways [60, 61, 62, 83].

Studies of the roles of transcriptional regulators in pathogenesis combine classical microbiology and bioinformatics approaches. Target transcriptional regulators in GBS are identified by homology searches with those well-defined in other species, and deletion mutants can be constructed. It is predicted that there are 17-20 two-component systems (TCS), and more than a hundred signaling systems and transcriptional regulators in GBS [63, 64, 65]. Table 2 shows the relationship between important virulence factors (shown in Table 2) and transcriptional regulators.

For TCS, CovR/S is homologus to CsrR/S in GAS [66, 67, 68] and CiaR/H is homologus to CiaR/H in *S. pneumoniae* [69]. RgfC/A is identified by random mutagenesis of mutant with decreased fibrinogen binding [70], while CiaR/H is also identified from random mutagenesis mutants in *in vivo* survival assay [69]. For stand-alone regulators, RovS is in the same family as negative virulence regulator Rgg in GAS [71], RogB is in the same family as RofA-like regulators in GAS [72], while MtaR is a LysR-type transcriptional regulator [73, 74].

cDNA microarrays can be prepared from open reading frames predicted from GBS full genomes. Expression of virulence factors of wild-type strain versus deletion mutant are compared, then the regulon of targeted regulator can be identified. For example, CovR/S regulons are identified in 515 (Ia), NEM316 (III) and 2603V/R (V) by full genome microarrays with CovR/S deletion mutants of each strain [75]. The number of genes that are controlled by CovR/S differs in the three strains. A core regulon, that is genes regulated by CovR/S in all three strains, of 39 genes is identified. The core regulon includes classical virulence factors CAMP factor and *cyl* operon [75]. Other virulence factors regulated by CovR/S, the *cps* operon encoding polysaccharide capsule, C5a peptidase and fibrinogen-binding proteins, are controlled by CovR/S in a strain-dependent manner [66, 67, 68]. CovR/S did not control these factors in all strains characterized.

Strain-dependent phenomenon is also observed in stand-alone regulator RogB. RogB decreases *cps* operon expression in strain 6313 (III) [71], while homologue of RogB is not present in A909 (Ia) and COH1 (III) at all [63].

4. Comparative genomic studies on host-pathogen relationship and pathogenesis

Figure 1 summarizes the inter-relation between microbiology and genomic approaches taken on studying host-pathogen interaction. It shows that such studies are indeed the interaction of a population of pathogen with a population of host. So, obtaining information from more strains is better for a complete picture of the whole pathogenesis process. Besides looking at virulence factors and their interactions with virulence-related transcriptional regulators in GBS strains with full-genome published, mutations in transcriptional regulators themselves also shed light on pathogenesis study [84, 85].

One example is on streptococcal toxic-shock syndrome (STSS). Pathogenesis of toxic shock syndrome is well-characterized in GAS, with defined virulence factors such as pyrogenic exotoxins, and other enzymes streptokinases and streptolysins [86]. However, virulence factors in STSS caused by GBS remain unclear. One of the few available molecular hints in GBS STSS was published in 2009. Two variants of serotype Ib isolated from tissue site of infection from a patient with necrotizing fasciitis and STSS harbored a valine deletion in CovR, the response regulator of TCS CovR/S [87]. On the other hand, another hint comes from a report published in 2010 on GAS STSS isolates. Sequence analysis of TCS CsrR/S (homologue of CovR/S in GBS) and negative virulence regulator Rgg shows that mutations in these regulators are associated with invasiveness compared to non-invasive GAS isolates [85].

Table 2 Relationship of virulence factors with virulence-related transcriptional regulators in various GBS strains.

Virulence factor (gene)	Two-component system	Stand-alone transcriptional regulator	Others
Characterized in GBS strains (serotype)	**CiaR/H**: COH1 (III) [69] **CovR/S**: NEM316 (III) [66], 515 (Ia), 2603V/R (V) [67, 68] **DltR/S**: NEM316 (III) [76, 77] **RgfC/A**: O90R (ATCC 12386) [70]	**MtaR**: COH1 (III) [73, 74] **RogB (RofA-like family)**: 6313 (III) [72] **RovS**: 6313 (III) [71]	**Stk1 (signaling enzyme, phosphorylase):** A909 (Ia) [78, 80, 81], NCTC10/84 [81]
Inter-relation among regulators	**CovR/S**: **(X)** to RovS [66] **DltR/S**: **(+)** activates *dltA-D* in *dltRSA- BCD* operon [76] **RgfC/A**: **(-)** growth- phase dependent by RgfB, truncated in A909 (Ia), NEM316 (III) [63, 64]	**MtaR**: **(+)** to *metNPQ* and arginine biosynthesis **RogB**: Absent in A909 (Ia), COH1 (III) [63], **(+)** decreased expression in *rovS* mutant [71], **(X)** unchanged expression in *covRS* mutant[66], **RovS**: **(X)** unchanged expression in *covRS* mutant[66]	
CAMP factor (*cfb*)	**CovR/S**: **(+)** CovR binds to promoter [68, 78]	**RovS**: **(X)** [71]	**Stk1**: **(-)** decrease activation by CovR binding [78]
β-hemolysin /cytolysin (*cyl* operon)	**CovR/S**: **(-)** CovR binds to P*cylX* [66, 68]	**RovS**: **(+)** binds to P*cylX* [71]	**Stk1**: **(+)** relieves repression of CovR [78, 81]
Capsular polysaccharide (*cps* operon)	**CovR/S: (Strain-dependent)** **(+)** in NEM316 [66], (X) in A909 (Ia), 515 (Ia), 2603V/R (III) [68]	**RogB**: **(Strain-dependent)** **(-)** decreases expression of *cpsA* in 6313[72] (X) in A909 (Ia), COH1 (III) [63]	
C5a peptidase (*scpB*)	**CovR/S**: **(Strain-dependent)** **(-)** *scpB* increased expression in *covR* mutant in 515 (Ia), 2603V/R (V) [67], (X) in A909 (Ia) [68] **(-)** *scpB* increased expression in *rgfC* mutant [70]		Laminin-binding protein (*lmb)* is around 200bp upstream of *scpB*, should not be similarly regulated [82]
Superoxide dismutase (*sodA*)		**RovS**: **(+)** binds to promoter [71]	
Cell-surface protease (*cspA*)		**MtaR**: **(+)** [73, 74]	
Fibrinogen-binding protein (*fbsA*, *fbsB*)	**CovR/S**: **(-)** in NEM316 [66]	**MtaR**: **(-)** [73, 74] **RogB**: **(+)** growth-phase-dependent manner[72] **RovS**: **(-)** binds to promoter [71]	
Pili-associated protein (*pilA*, *pilB*, *pilC*)		**RogB**: **(+)** Not conserved in GBS strains NEM316, 2603V/R [79]	
Serine-rich-repeat protein (*srr1*)			Presence of a putative RofA-like transcriptional factor in Srr-1 operon [82]

Note:
(+) stands for increased expression, (-) stands for decreased expression, (X) stands for no effect on expression

Table 3 Information on published GBS genomes and the corresponding mutations in selected transcriptional regulators.

Strain	2603V/R	A909	NEM316	18RS21	515	CJB111	COH1	H36B
NCBI genome project ID	330	326	334	15605	15606	15607	15609	15608
Sequencing status	Complete	Complete	Complete	Draft Assembly	Draft Assembly	Draft Assembly	Draft Assembly	Draft Assembly
Serotype	V	Ic (Ia)	III	III	Ia	V	III	Ib
Source	TIGR, clinical isolate	TIGR, clinical isolate from infant umbilicus culture	Instuti Pasteur, clinical isolate from a fatal case of septicaemia	TIGR, clinical isolate from infant umbilicus culture	TIGR, clinical isolate from infant umbilicus culture	TIGR, clinical isolate from infant umbilicus culture	TIGR, clinical isolate from infant with sepsis	TIGR, clinical isolate from infant umbilicus culture
CiaR	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
CiaH	A305T	Nil	Nil	A305T	Nil	Nil	Nil	Nil
CovR	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
CovS	Nil	Nil	V112A	273-501 a.a. missing	Nil	V343M	Nil	Nil
RgfC	Allele 2, Nil	Allele 1, Nil	Allele 2, 1-7 a.a. missing, I8M	Allele 2, Nil	Allele 2, Nil	Allele 1, T395M,R406Q	Allele 1, A148T, T157I, R159K, L179S, F182L, A200V, A321D	Allele 1, A321D
RgfA	Allele 1, Nil	Allele 2, 115-250 a.a. missing	Allele 1, Nil	Allele 1, Nil	Allele 1, Nil	Allele 2, 1-32 a.a. missing	Allele 2, 1-32 a.a. missing, E124D	No homologue
RgfB	N119E, L156F, MDF274-276LNL	Nil	1-5 a.a. missing, N119E, MDF274- 276LNL	N119E, L156F, MDF274-276LNL	N119E, MDF274-276LNL	1-5 a.a. missing, N119D	1-5 a.a. missing, K56T,N119D	Nil
MtaR	1-17 a.a. missing, S190N	F158L	Nil	Nil	Nil	P221T, V270I	Nil	S190N
RovS	Nil	Nil	Nil	No homologue	Nil	Y200C	Q186H	No homologue
RogB	Allele 1, 1-2 a.a. missing, M9L, RTFI 331-334EHLF, F330S, 335-509 a.a.missing, K372E	No homologue	Allele 1, 1-8 a.a. missing, Q145K, V147F, F164H, Q168R, M176L, H191Y, S229P, T232I, G316D, F328I, F330S, T338A, Q414K, L421S, K428Q, K456P, P460S, N479H, S481P, K489E, T506N, N511T	Allele 1, 1-8 a.a. missing	Allele 2	Allele 1, 1-8 a.a. missing	No homologue	Allele 1, M9L,Q145K, V147F, F164H, Q168R, M176L, H191Y, S229P, T232I, K372E
Stk1	Nil	Nil	T550A	1-22 a.a. missing	1-22 a.a. missing, K470R,T550A	1-22 a.a. missing	1-22 a.a. missing,T550A	Nil
Mga	Allele 1	Allele 1	Allele 1	Allele 1	Allele 2	Allele 2	Allele 1	Allele 1
CcpA	Nil	I163T	Nil	1-19 a.a. missing	1-19 a.a. missing	1-19 a.a. missing	1-19 a.a. missing	Nil

Table 3 shows mutations of selected transcriptional regulators in GBS strains with published genomes. The transcriptional regulators include those showed in Table 2, and homologues of major virulence-related regulators in GAS: multiple-gene global regulator, Mga [88] and catabolite control protein, CcpA [89].

For TCS, amino acid sequences of the response regulators of CovR/S (CovR) and CiaR/H (CiaR) are identical among all the eight published genomes, while only minor mutations are found in the histidine kinases (CovS, CiaH). These reflect that the two TCS may play essential roles in GBS. Another TCS RgfC/A and RgfB, the secreted peptide that acts on RgfC, are quite polymorphic, which may be a result of selection pressure from the environment [70], and this system may be one of the major components in sensing environmental changes.

For stand-alone regulators, Mga and RogB have two alleles among the eight GBS strains, though RogB is absent in A909 (Ia) and COH1 (III), its sequence is very polymorphic. Applying the same logic for mutations in transcriptional regulations in GAS STSS strains, clustering Mga and RogB sequences from isolates from patients with different clinical presentations may give very clearly separated clusters [84].

5. Conclusion and future perspectives

This review summarizes the approaches taken in studying virulence factors in GBS. Classical microbiological approach identifies virulence factors by biochemical assays, DNA-hybridization, molecular amplification, and with animal and cell-culture models; while recently developed genomics approach identifies virulence factors by searching for homologues in databases and published genomes. Besides virulence factors, virulence-related transcriptional regulators also play a role in pathogenesis by relaying signals from environmental change to virulence pathways. With full genomes of GBS, mutations in transcriptional regulators can be analysed and the predictions on the disease potential made, enabling further elucidation of the pathogenicity in human GBS disease.

As more GBS genomes become available, a more complete clustering of sequences of virulence factors and transcriptional regulators can be obtained. By then, a clearer relationship between mutations in transcriptional regulators and GBS disease may bring further advances to knowledge on GBS pathogenesis.

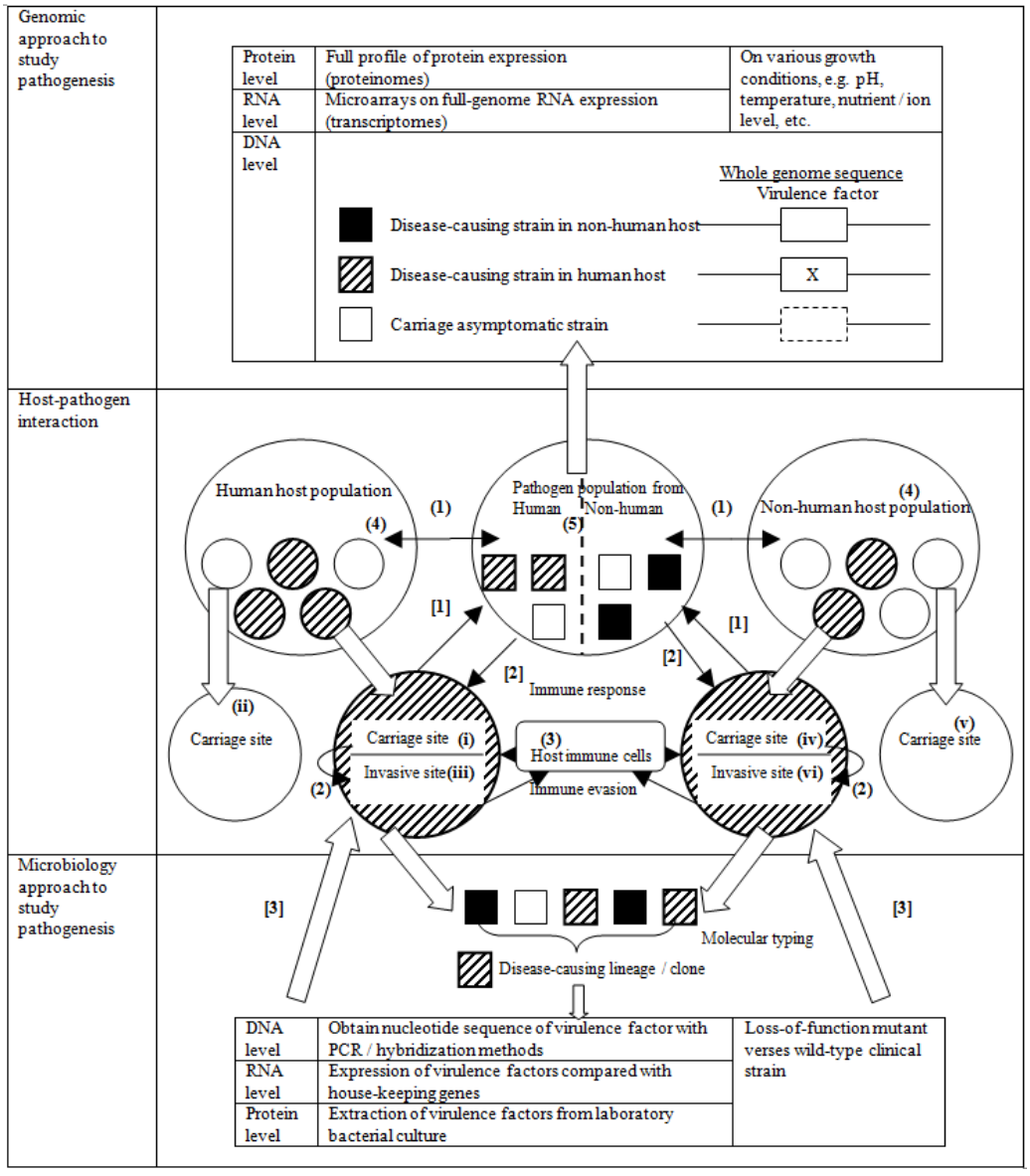

Fig.1 Schematic representation of host-pathogen relationship with possible approaches to study pathogenesis

Note:
(1) Transmission of pathogen to human and non-human hosts, (2) Transmission of pathogen among particular host's carriage and invasive sites, (3) Host immune response versus immune evasion from pathogen, (4) Individual factors among particular hosts that affect (1) to (3), (5) Strain-dependent factors among pathogens, [1] Isolation of pathogen from hosts, [2] Inoculation of pathogen back to host, [3] Molecular typing on disease-causing isolates to find potential clonal relationship, (i) Carriage site in diseased human host, (ii) Carriage site in asymptomatic human host, (iii) Invasive site in diseased human host, (iv) Carriage site in diseased non-human host, (ii) Carriage site in asymptomatic non-human host, (iii) Invasive site in diseased non-human host.

References

[1] Cieslewicz MJ, Chaffin D, Glusman G, et al. Structural and genetic diversity of Group B Streptococcus capsular polysaccharides. Infect Immun 2005 ; 73 :3096-3103.
[2] Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a proposed new *Streptococcus agalactiae* serotype. J Clin Micobiol 2007 ;45 :2929-2936.
[3] Mullaney DM. Grouop Strptococcal infections in newborns. J Obstet Gynecol Neonatal Nurs 2001 ; 30 :649-658.
[4] Cowgill K, Taylor TH Jr, Schuchat A, Schrag S. Report from CDC. Awareness of perinatal Group B Streptococcal infection among women of childrenbearing age in the United States, 1999 and 2002. J Womens Health (Larchmt) 2003 ; 12 :527-532.
[5] Sendi P, Johansson L, Norrby-Teglund A. Invasive Group B Streptococcal Disease in Non-pregnant Adults. A Review with Emphasis on Skin and Soft-tissue Infections. Infection 2008 ; 36 :100-111.
[6] Reich HL, Crawford GH, Pelle MT. Group B Streptococcal Toxic Shock-Like Syndrome. Arch Dermatol 2004 ; 140 :
[7] Tyrrell GL, Senzilet LD, Spika JS, et al. Invasive disease due to group B streptococcal infection in adults : results from a Canadian, population-based, acive laboratort surveillance study-1996. Sentinel Health Unit Surveillance System Site Coordinators. J Infect Dis 2000 ; 182 : 168-173.
[8] Harrison LH, Elliot JA, Dwyer DM, et al. Serotype distribution of invasive group B streptococcal isolates in Maryland : implications for vaccine formulation. Maryland Emerging Infections Program. J Infect Dis 1998 ; 177 :998-1002.
[9] Kong F, S Gowan, D Martin, G James, and GL Gilbert. 2002. Serotype identification of group B streptococci by PCR and sequencing. J Clin Microbiol 40;216-226.
[10] Jones N, Bohnsack JF, Takahashi S, et al. Multilocus sequence typing for group B streptococcus. J Clin Microbiol 2003 ; 41 :2530-2536.
[11] Wilder-Smith E, Chow KM, Kay R, Ip M, Tee N. Group B streptococcal meningitis in adults : recent increase in Southeast Asia. Aust N Z J Med 2000 ; 30 :462-465.
[12] Ip M, ESC Cheuk, MHY Tsui, F Kong, TN Leung, GL Gilbert. Identification of a Streptococcus agalactiae serotype III subtype 4 clone in association with adult invasive disease in Hong Kong. J Clin Microbiol 2006 ;44 :4252-4254.
[13] Jones HE, Howells CH. Neonatal meningitis due to Streptococcus agalactiae. Postgrad Med J. 1968 Jul;44(513):549-51.
[14] Klesius PH, Zimmerman RA, Mathews JH, Krushak DH. Cellular and humoral immune response to group B streptococci. J Pediatr. 1973 Dec;83(6):926-32.
[15] Paredes A, Wong P, Yow MD. Failure of penicillin to eradicate the carrier state of group B Streptococcus in infants. J Pediatr. 1976 Aug;89(2):191-3.
[16] Schauf V, Hlaing V. Group B streptococcal colonization in pregnancy. Obstet Gynecol. 1976 Jun;47(6):719-21.
[17] Wilkinson HW, Jones WL. Radioimmunoassay for measuring antibodies specific for group B streptococcal types Ia, Ib, Ic, II, and III. J Clin Microbiol. 1976 May;3(5):480-5.
[18] Gibson RL, Nizet V, Rubens CE. Group B streptococcal β-hemolysin promotes injury of lung microvascular endothelial cells. Pediatr Res. 1999;45:626–634.
[19] Spellerberg B, B Pohl, G Haase, S Martin, J Weber-Heynemann, R Lutticken. Identification of geneteric determinants for the hemolytic activity of Streptocccus agalactiae by ISS1 transposition. J Bacteriol 1999;181:3212-3219.
[20] Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of Streptococcus agalactiae, a pathogen causing invasive neonatal disease. Mol Microbiol. 2002 Sep;45(6):1499-513.
[21] Tettelin H, Masignani V, Cieslewicz MJ,et al.Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V Streptococcus agalactiae. Proc Natl Acad Sci U S A. 2002 Sep 17;99(19):12391-6. Epub 2002 Aug 28.
[22] http://www.ncbi.nlm.nih.gov/sites/entrez (Search "Genome" for "streptococcus[orgn] OR streptococcus agalactiae[orgn]"
[23] Lauer P, CD Rinaudo , M Soriani, I Margarit, D Maione, R Rosini, et al. Genome analysis reveals pili in Group B streptococcus. Science 2005;309:105.
[24] Lancefield RC, Hare R. The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci. J Exp Med 1935; 61:335-349.
[25] Lang S, Palmer M. Characterization of Streptococcus agalactiae CAMP factor as a pore-forming toxin. J Biol Chem. 2003 Oct 3;278(40):38167-73.
[26] Lang S, Xue J, Guo Z, Palmer M. Streptococcus agalactiae CAMP factor binds to GPI-anchored proteins. Med Microbiol Immunol. 2007 Mar;196(1):1-10.
[27] Nizet V, et al. Group B streptococcal β-hemolysin expression is associated with injury of lung epithelial cells. Infect Immun. 1996;64:3818–3826.
[28] Gibson RL, Nizet V, Rubens CE. Group B streptococcal β-hemolysin promotes injury of lung microvascular endothelial cells. Pediatr Res. 1999;45:626–634.
[29] Doran KS, et al. Group B streptococcal β-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8. J Infect Dis. 2002;185:196–203.

[30] Hensler ME, et al. Virulence role of group B Streptococcus β-hemolysin/cytolysin in a neonatal rabbit model of early-onset pulmonary infection. J Infect Dis. 2005;191:1287–1291.
[31] Liu GY, et al. Sword and shield: linked group B streptococcal β-hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. Proc Natl Acad Sci U S A. 2004;101:14491–14496.[32] Skalka, B., and Smola, J. Lethal effect of CAMP-factor and UBERIS-factor--a new finding about diffusible exosubstances of streptococcus agalactiae and Streptococcus uberis. Zentralbl. Bakteriol. A 1981;249, 190–194.
[33] Marques MB, et al. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. Infect Immun. 1992;60:3986–3993.
[34] Heather C. Maisey, Kelly S. Doran, and Victor Nizet. Recent advances in understanding the molecular basis of group B Streptococcus virulence. Expert Rev Mol Med. 2008 September 22; 10: e27.
[35] Lin B, et al. Cloning and expression of the gene for group B streptococcal hyaluronate lyase. J Biol Chem. 1994;269:30113–30116.
[36] Spellerberg B, et al. Lmb, a protein with similarities to the LraI adhesin family, mediates attachment of Streptococcus agalactiae to human laminin. Infect Immun. 1999;67:871–878.
[37] Tenenbaum T, et al. Streptococcus agalactiae invasion of human brain microvascular endothelial cells is promoted by the laminin-binding protein Lmb. Microbes Infect. 2007;9:714–720.
[38] Bohnsack, J. F., K. W. Mollison, A. M. Buko, J. W. Ashworth, and H. R. Hill. 1991. Group B streptococci inactivate complement component C5a by enzymatic cleavage at the C-terminus. Biochem. J. 273:635-640.
[39] Beckmann C, et al. Identification of novel adhesins from Group B streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding. Infect Immun. 2002;70:2869–2876.
[40] Poyart C, et al. Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of Streptococcus agalactiae. Infect Immun. 2001;69:5098–5106.
[41] Santi I, et al. BibA: a novel immunogenic bacterial adhesin contributing to group B Streptococcus survival in human blood. Mol Microbiol. 2007;63:754–767.
[42] Harris TO, et al. A novel streptococcal surface protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen. J Clin Invest. 2003;111:61–70.
[43] Schubert A, et al. The fibrinogen receptor FbsA promotes adherence of Streptococcus agalactiae to human epithelial cells. Infect Immun. 2004;72:6197–6205.
[44] Tenenbaum T, et al. Adherence to and invasion of human brain microvascular endothelial cells are promoted by fibrinogen-binding protein FbsA of Streptococcus agalactiae. Infect Immun. 2005;73:4404–4409.
[45] Lang S, Xue J, Guo Z, Palmer M. Streptococcus agalactiae CAMP factor binds to GPI-anchored proteins. Med Microbiol Immunol. 2007 Mar;196(1):1-10.
[46] Hamilton A, et al. Penicillin-binding protein 1a promotes resistance of group B streptococcus to antimicrobial peptides. Infect Immun. 2006;74:6179–6187.
[47] Jones AL, et al. A streptococcal penicillin-binding protein is critical for resisting innate airway defenses in the neonatal lung. J Immunol. 2007;179:3196–3202.
[48] Lauer P, et al. Genome analysis reveals pili in Group B Streptococcus. Science. 2005;309:105.
[49] H. Tettelin et al., Proc. Natl. Acad. Sci. U.S.A. 99, 12391 (2002).
[50] Rosini R, et al. Identification of novel genomic islands coding for antigenic pilus-like structures in Streptococcus agalactiae. Mol Microbiol. 2006;61:126–141.
[51] Dramsi S, et al. Assembly and role of pili in group B streptococci. Mol Microbiol. 2006;60:1401–1413.
[52] Krishnan V, et al. An IgG-like domain in the minor pilin GBS52 of Streptococcus agalactiae mediates lung epithelial cell adhesion. Structure. 2007;15:893–903.
[53] Maisey HC, et al. Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells. J Bacteriol. 2007;189:1464–1467.
[54] Maisey HC, et al. A group B streptococcal pilus protein promotes phagocyte resistance and systemic virulence. FASEB J 2008; 22:1715-1724.
[55] Magalhaes V, et al. Interaction with human plasminogen system turns on proteolytic activity in Streptococcus agalactiae and enhances its virulence in a mouse model. Microbes Infect. 2007;9:1276–1284.
[56] Samen U, et al. The surface protein Srr-1 of Streptococcus agalactiae binds human keratin 4 and promotes adherence to epithelial HEp-2 cells. Infect Immun. 2007;75:5405–5414.
[57] Glaser, P., C. Rusniok, C. Buchrieser, F. Chevalier, L. Frangeul, T. Msadek, M. Zouine, E. Couve, L. Lalioui, C. Poyart, P. Trieu-Cuot, and F. Kunst. 2002. Genome sequence of Streptococcus agalactiae, a pathogen causing invasive neonatal disease. Mol. Microbiol. 45:1499-1513.
[58] Lamy MC, Zouine M, Fert J, et al. CovS/CovR of group B streptococcus: a two-component global regulatory system involved in virulence. Molecular Microbiology 2004;54:1250-1268.
[59] Mitchell TJ. The pathogenesis of streptococcal infections: from tooth decay to meningitis. Nature reviews microbiology 2003; 1:219-230.
[60] Mereghetti L, Sitkiewicz I, Green NM, Musser JM. Remodeling of *Streptococcus agalactiae* transcriptome in response to growth temperature. PloS One 2008; 3:e2785.
[61] Stikiewicz I, Green NM, Guo N, et al. Transcriptome adaptation of Group B *Streptococcus* to growth in human aminotic fluid. PloS One 2009; 4:e6114.
[62] Mereghetti L, Sitkiewicz I, Green NM, Musser JM. Extensive adaptive changes occur in the transcriptome of *Streptococcus agalactiae* (Group B *Streptococcus*) in response to incubation with human blood. PloS One 2009; 3:e3143.
[63] Tettelin H, Masignani V, Cieslewicz MJ, et al. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial 'pan-genome'. PNAS 2005; 102:13950-13955.
[64] Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. Mol Microbiol 2002; 45:1499-1513.
[65] Tettelin H, masignani V, Cieslewicz MJ, et al. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*. PNAS 2002; 99:12391-12396.

[66] Lamy MC, Zouine M, Fert J, et al. CovS/CovR of Group B Streptococcus: a two-component global regulatory system involved in virulence. Mol Microbiology 2004; 54:1250-1268.
[67] Jiang SM, Cieslewicz MJ, Kasper MR. Regulatory of virulence by a two-component system in Group B Streptococcus. J Bacteriol 2005; 187:1150-1113.
[68] Jiang SM, Ishmael N, Hotopp JD, et al. Variation in the Group B Streptococcus CsrRS regulon and effects on pathogenicity. J Bacteriol 2008; 190:1956-1965.
[69] Quach D, van Sorge NM, Kristian SA, et al. The CiaR response reulator in Group B Streptococcus promotes intracellular survival and resistance to innate immune defenses. J Bacteriol 2008.
[70] Spellerberg B, Rozdzinski E, Martin S, et al. *rgf* encodes a novel two-component signal transduction system of *Streptococcus agalactiae*. Infect Immun 2002; 70:2434-2440.
[71] Samen UM, Eikmanns BJ, Reinscheid DJ. The transcriptional regulator RovS controls the attachment of *Streptococcus agalactiae* to human epithelial cells and the expression of virulence genes. Infect Immun 2006; 74:5626-5635.
[72] Gutekunst H, Eikmanns BJ, Reinscheid DJ. Analysis of RogB-controlled virulence mechanisms and gene repression in *Streptococcus agalactiae*. Infect Immun 2003; 71:5056-5064.
[73] Shelver D, Rajagopal L, Harris TO, et al. MtaR, a regulator of methionine transport, is critical for survival of Group B Streptococcus in vivo. J Bacteriol 2003; 185:6592-6599.
[74] Bryan JD, Liles R, Cvek U, et al. Global transcriptional profiling reveals *Streptococcus agalactiae* genes controlled by the MtaR transcription factor. BMC Genomics 2008; 9:607.
[75] Jiang SM, Ishmael N, Hotopp JD, et al. Variation in the Group B Streptococcus CsrRS regulon and effects on pathogenicity. Journal of Bacteriology 2008; 190:1956-1965.
[76] Poyart C, Lamy MC, Boumalia C, et al. Regulation of D-alanyl-lipotechoic acid biosynthesis in *Streptococcus agalactiae* involves a novel two-component regulatory system. J Bacteriol 2001; 183:6324-6334.
[77] Poyart C, Pellegrini E, Marceau M, et al. Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic cells. Mol Microbiol 2003; 49:1615-1625.
[78] Lin WJ, Walthers D, Connelly JE, et al. Threonine phosphorylation prevents promoter DNA binding of the Group B Streptococcus reponse regulator CovR. Mol Microbiol. 2009.
[79] Dramsi S, Caliot E, Bonne I, et al. Assembly and role of pili in Group B Streptococci. Mol Microbiol 2006; 60:1410-1413.
[80] Rajagopal L, Clancy A, Rubens CE. A eukaryotic type serine/threonine kinase and phosphate in *Streptococcus agalactiae* reversibly phosphorylate an inorganic pyrophosphate and affect growth, cell segregation, and virulence. J Biol Chem 2003; 278:14429-14441.
[81] Rajagopal L, Vo A, Silvestroni A, et al. Regulation of cytotoxin expression by converging eukaryotic-type and two-component signaling mechanisms in *Streptococcus agalactiae*. Mol Microbiol 2006; 62: 941-957.
[82] Rajagopal L. Understanding the regulation of Group B Streptococcal virulence factors. Future Microbiol 2009; 4:201-221.
[83] Santi I, Grifantini, Jiang SM, et al. CsrRS regulates group B streptococcus virulence gene expression in reponse to environmental pH: a new perspective on vaccine development. Journal of Bacteriology 2009; 191:5387-5397.
[84] Bessen DE, Manoharan A, Luo F, et al. Evolution of Transcription Regulatory Genes Is Linked to Niche Specialization in the Bacterial Pathogen *Streptococcus pyogenes*. J Bacteriol 2005; 187:4163-4172.
[85] Ikebe T, Ato M, Matsumura T, et al. Highly frequent mutations in negative regulators of multiple virulence genes in group A Streptococcal toxic shock syndrome isolates. PloS Pathogens 2010; 6:e1000832.
[86] Bisno AL, Stevens DL. Streptococcal infections of skin and soft tissues. N Engl J Med 1996; 334:240-245.
[87] Sendi P, Johansson L, Dahesh S, et al. Bacterial phenotype variants in group B Streptococcal toxic shock syndrome. Emerging Infectious Diseases 2009; 15:223-232.
[88] Mclver KS. Stand-alone response regulators controlling global virulence networks in *Streptococcus pyogenes*. Collin M, Schuch R (eds): Bacterial Sensing and Signaling. Contrib Microbiol. Basel, Karger, 2009, vol 16, pp103-119.
[89] Shelburne SA, Davenport MT, Beth DB, Musser JM. The role of complex carbohydrate catabolism in the pathogenesis of invasive streptococci. Trends in Microbiology 2008; 16:318-325.