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MINIREVIEW

Stress responses in *Streptococcus* species and their effects on the host

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Streptococci cause a variety of diseases, such as dental caries, pharyngitis, meningitis, pneumonia, bacteremia, endocarditis, erysipelas, and necrotizing fasciitis. The natural niche of this genus of bacteria ranges from the mouth and nasopharynx to the skin, indicating that the bacteria will inevitably be subjected to environmental changes during invasion into the host, where it is exposed to the host immune system. Thus, the *Streptococcus*-host interaction determines whether bacteria are cleared by the host's defenses or whether they survive after invasion to cause serious diseases. If this interaction was to be deciphered, it could aid in the development of novel preventive and therapeutic agents. *Streptococcus* species possess many virulent factors, such as peroxidases and heat-shock proteins (HSPs), which play key roles in protecting the bacteria from hostile host environments. This review will discuss insights into the mechanism(s) by which streptococci adapt to host environments. Additionally, we will address how streptococcal infections trigger host stress responses; however, the mechanism by which bacterial components modulate host stress responses remains largely unknown.

Keywords: *Streptococcus*, host environment, two-component system, heat-shock stress, oxidative stress, antibiotic-induced stress

Introduction

Streptococcus is a genus of Gram-positive bacteria that causes serious infectious diseases with high rates of morbidity and mortality. Many invasive streptococcal infections are considered threats to public health on a worldwide scale. For instance, pneumococcal disease causes over 800,000 deaths in infants annually (O'Brien *et al.*, 2009). Up until now, the taxonomy of *Streptococcus* included over 100 species. Phylogenic and whole genome sequencing analyses have indicated that *Streptococcus* has 4,514 genotypes (https://www.patricbrc.org), including *S. pneumoniae* (3,404 genotypes), *S. agalactiae* (300 genotypes), *S. pyogenes* (230 genotypes), *S. mutans* (165 genotypes), and others (415 genotypes). Based on their hemolytic activity, *Streptococcus* species can be divided into two major groups: β-hemolytic (e.g. *S. pyogenes*, *S. agalactiae*) and non-β-hemolytic (e.g. *S. pneumoniae*, *S. mutans*) (Facklam, 2002). However, streptococci can also be divided into six groups based on the similarity of their 16S rRNA gene sequence, and these groups are the pyogenic, mitis, salivarius, bovis, mutans, and anginosus groups (Fig. 1). The pyogenic (*S. pyogenes* and *S. agalactiae*) and mitis group (*S. pneumoniae*) are typically isolated from infected humans because these streptococci can cause several severe human diseases: *S. pyogenes* causes rheumatic fever, glomerulonephritis, and pharyngitis; *S. agalactiae* causes neonatal meningitis and sepsis; and *S. pneumoniae* is responsible for otitis media, bronchitis, sinusitis, meningitis, and pneumonia (Parks *et al.*, 2015). Moreover, *S. mutans* (of the mutans group) is a pathogen commonly associated with dental caries in humans. Thus, this review will focus on the stress responses of these four important *Streptococcus* species.

In the first stage of the infection, streptococci usually colonize mucosal organs, such as the nasopharynx and respiratory, gastrointestinal, and genitourinary tracts. After this colonization step, these bacteria spread to other organs. At these new sites, the streptococci may encounter hostile host environments, such as oxidative, thermal, and pH stress conditions as well as nutrient limitation, which may trigger a stress response in the bacteria. Therefore, *Streptococcus* species have evolved several mechanisms to overcome and survive this harsh milieu. Recently, several studies have shown that streptococcal two-component systems (TCSs) are major pathways for the regulation of stress-related genes in these bacteria. In this review, we will discuss the mechanism(s) by which TCSs contribute to stress tolerance and how streptococci can resist heat shock, oxidative stress, and other stresses during infection.

Regulation of stress responses by TCSs in *Streptococcus* species

In *Streptococcus* species, TCSs play important roles in regulating virulence gene expression and allow the bacteria to adapt to host-milieu-induced stress (McCluskey *et al.*, 2004; Santi *et al.*, 2009; Downey *et al.*, 2014). TCSs are comprised of membrane-bound sensory histidine kinases (HKs) and a cytosolic response regulator (RR). In these systems, the sensory HK is autophosphorylated by a variety of external stresses, such as thermal, oxidative, nutrient, pH, and osmotic stress, which is followed by transferring of the phosphate to the RR

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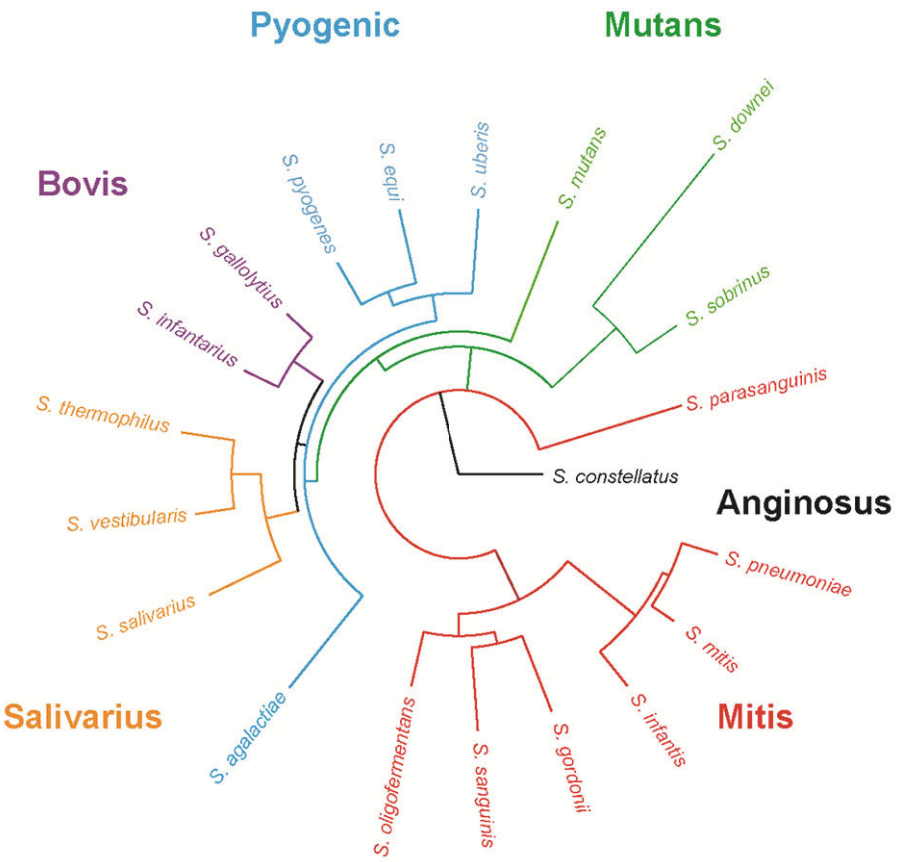

Fig. 1. Phylogenic tree of *Streptococcus* species based on the analysis of their 16S rRNA gene sequences. 16S rRNA gene sequences of selected *Streptococcus* species were aligned and analyzed with TreeDyn tools (www.treedyn.org/). The complete taxonomy of streptococci is shown at http://www.genome.jp.

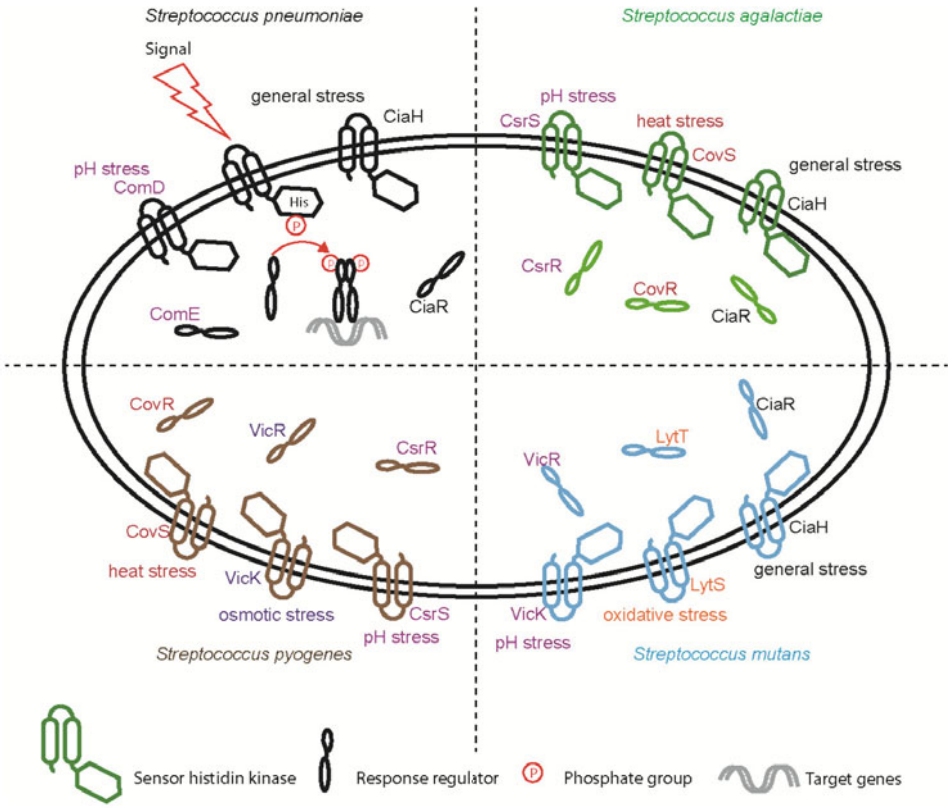

Fig. 2. Two-component systems (TCSs) in *Streptococcus* species' stress responses. Model illustrating the regulation of TCSs in *Streptococcus pneumoniae*, *S. agalactiae*, *S. pyogenes*, and *S. mutans*. In this model, sensory histidine kinases (HKs) sense stress signals, such as heat, oxidative, pH, and osmotic stress, selectively. HKs are autophosphorylated and transfer the phosphate to the cognate response regulators (RRs). As a result, RRs form heterodimers and bind to stress-related genes in order to turn on stress response signals.

Table 1. Summary of components and functions of major streptococcal two-component systems (TCSs) involved in stress responses

TCS(s)	*S. pneumoniae*	*S. agalactiae*	*S. pyogenes*	*S. mutans*
CiaR/H	Modulates HtrA and the acid tolerance response system to resist oxidative, acid, and heat-shock stress (Ibrahim *et al.*, 2004)	Present, but has other functions in resistance to host immune response (Quach *et al.*, 2009)	Involved in acid and oxidative stress responses (Tatsuno *et al.*, 2014)	Functions in the tolerance to stress (Ahn *et al.*, 2006)
ComD/E	Involved in protection of the bacteria against pH stress and heat shock (Cortes *et al.*, 2015)	Unknown	Unknown	Resistance to acid and oxidative stress (Ahn *et al.*, 2006)
CsrS/R	Unknown	The major acid response regulator (Cumley *et al.*, 2012)	Important in heat- and pH-induced stress (Dalton and Scott, 2004)	Involved in bacterial biofilm architecture and acid tolerance (Tsang *et al.*, 2005)
VicK/R	Involved in cell division and cell wall stress responses (Wayne *et al.*, 2012)	Unknown	Required for nutrient uptake, growth, and osmotic protection (Liu *et al.*, 2006)	Involved in osmotic, thermal, oxidative, and antibiotic stress responses, as well as the capacity to produce mutacin (Biswas *et al.*, 2008)

(Fig. 2). The phosphorylated RR modulates gene expression in response to stress signals. By regulating a set of stress-related genes, streptococci can overcome harsh environments and enhance their chances for survival (Ibrahim *et al.*, 2004; Suntharalingam *et al.*, 2009; Downey *et al.*, 2014).

Moreover, next genome sequencing analysis has indicated that the species *S. pyogenes* and *S. mutans* have 13 putative TCSs (Li *et al.*, 2008; Hertzén *et al.*, 2012), whereas *S. agalactiae* contain up to 21 TCSs (Vasilyeva *et al.*, 2015). Among the 13 pneumococcal TCSs, eight systems have been shown to play a key role in the regulation of virulence during *S. pneumoniae* infection (de Saizieu *et al.*, 2000). In this review, we will focus on the four most well-known and important TCSs in *Streptococcus* species, CiaR/H, ComD/E, CsrS/R (also known as CovS/R), and VicR/K.

CiaH/R TCS: In *S. pneumoniae*, the CiaH/R TCS has been shown to be involved in resistance to temperature and oxidative stress via the modulation of high-temperature requirement A (HtrA) serine protease expression (Ibrahim *et al.*, 2004). Furthermore, the CiaH/R system is required for the survival of *S. pneumoniae* under antibiotic stress conditions (Mascher *et al.*, 2006). Under pH stress conditions, CiaH/R systems protect *S. pneumoniae* from stress by utilizing the acid tolerance response (ATR) system (Cortes *et al.*, 2015). Interestingly, the CiaH/R system is also employed in stress tolerance in *S. mutans*. Furthermore, CiaH may cross-regulate gene expression not only via its cognate CiaR, but also through other response regulators (Ahn *et al.*, 2006).

ComD/E TCS: The ComD/E TCS has been shown to be involved in protecting *S. pneumoniae* from pH stress via modulation of the ATR system (Cortes *et al.*, 2015). ComE is a known regulator of *S. pneumoniae Clp* genes, which encode members of the Clp family of ATP-dependent proteases (Chastanet *et al.*, 2001). Clp proteins are a family of heat-shock proteins involved in heat-shock tolerance and other types of stress resistance. In *S. mutans*, *comD/E* deletion mutations have been shown to result in a phenotype sensitive to acid stress, with deficiencies in biofilm formation and competence development (Ahn *et al.*, 2006).

CsrS/R or CovS/R TCS: In *S. agalactiae*, to adapt to pH stress, the CsrR response regulator of the CsrS/R system upregulates the expression of 317 genes and downregulates the expression of 61 genes (Santi *et al.*, 2009). Moreover, the CovS/R system has been shown to be an important TCS for resistance to pH stress and for the enhanced intracellular survival of *S. agalactiae* in macrophages (Cumley *et al.*, 2012). However, in *S. pyogenes*, the CovS/R system is required for resistance to heat- and pH-induced stress (Dalton and Scott, 2004).

VicK/R TCS: VicK/R and GcrR regulate the expression of ATR-related genes, such as *atpE/A*, *ffh*, *radA*, and *gcrR*, upon exposure to pH stress conditions (Downey *et al.*, 2014). In addition, the VicK/R system plays a crucial role in cell wall metabolism and nutrient uptake (Liu *et al.*, 2006). Interestingly, in *S. mutans*, VicK has been shown to transphosphorylate not only its cognate VicR, but also other RRs, such as GcrR (Downey *et al.*, 2014).

Other streptococcal TCSs: Under oxidative stress, pneumococcal TCS04 acts as a negative regulator of the pneumococcal surface antigen A (*psaA*) gene, which plays an important role in oxidative-stress resistance (McCluskey *et al.*, 2004). One study found that an *rr04* (a regulator of TCS04 in pneumococcus) mutant displayed an oxidation-susceptible phenotype which was related to the modulation of *psaA* expression (McCluskey *et al.*, 2004). During oxidative stress, the expression of the LytST system is enhanced in *S. mutans*; this system regulates potential oxidative stress genes, such as *yghU* (encoding an antioxidant enzyme), *tpx* (encoding thiol peroxidase), and *recJ*, a single-stranded DNA exonuclease that triggers the DNA repair system in response to oxidative stress (Ahn *et al.*, 2012). In addition, *S. mutans* has been shown to respond to cell envelop stress via the LiaFSR system, which upregulates the expression of several genes, including those involved in membrane protein synthesis, envelope chaperone/proteases, peptidoglycan biosynthesis, and transcriptional regulators (Suntharalingam *et al.*, 2009).

Role of streptococcal heat-shock proteins (HSPs) in heat-shock stress

Colonization and invasion are two key steps in streptococcal pathogenesis. Streptococci, especially *S. pneumoniae*, infect hosts via nasopharynx colonization. After the evasion of host mucosal defenses, *S. pneumoniae* invades other organs and body fluids, such as the ear, lung, blood, and brain, where it causes several serious diseases like otitis media, pneumonia, bacteremia, and meningitis, respectively. Once *S. pneumoniae* penetrates the mucosal nasopharynx (maintained at a temperature of 30°C or 34°C in humans and mice, respec-

tively) and enters the blood and/or brain (37°C, or higher during fever), it encounters heat stress conditions and can undergo heat shock (Kwon *et al.*, 2003). To overcome heat shock, *S. pneumoniae* synthesizes a set of highly conserved HSPs, which are also utilized against other stresses, including exposure to ethanol, antibiotics, and heavy metals, to increase the viability of the bacteria (Kwon *et al.*, 2003). Generally, HSPs are highly conserved proteins in both prokaryotes and eukaryotes and are molecular chaperones that refold stress-induced denatured proteins back into their native structure or mediate the proteolysis of damaged proteins, thus protecting the bacteria from stress (Park *et al.*, 2015). HSPs form a large family of proteins that are categorized based on their molecular weight and include hsp10, hsp40, hsp60, hsp70, hsp90, and hsp100. In *Streptococcus* species, several HSPs have been identified as virulence factors, such as DnaJ (hsp40) (Cui *et al.*, 2011), GroEL (hsp60) (Khan *et al.*, 2009), DnaK (hsp70), and caseinolytic protease L (ClpL; hsp100) (Nguyen *et al.*, 2014a). The functions of streptococcal HSPs are summarized in Fig. 3.

Our previous study indicated that pneumococcal ClpL and ClpP were significantly induced after heat shock and modulated a variety of virulence-associated genes, such as *cbpA*, *cps2A*, *ply*, and *psaA* (Kwon *et al.*, 2003). Furthermore, ClpL is a hsp100, possesses nucleotide phosphohydrolase (NTPase) activity, and functions as a molecular chaperone without the cooperative chaperone DnaK system or interactions with adaptor proteins (Park *et al.*, 2015). DnaK, the major pneumococcal hsp70, is an abundant, constitutively expressed, and stress-inducible chaperone. ClpC also acts as a chaperone protein, remodeling choline-binding proteins and negatively regulating pneumococcal growth at higher temperatures (Charpentier *et al.*, 2000). In addition to functioning as chaperones, some HSPs have other novel functions. DnaJ (hsp40) and GroEL (hsp60) have been identified as potential antigens that elicit protection against nasopharyngeal colonization and invasive infection (Cui *et al.*, 2011). Pneumococcal ClpL can repress actin rearrangement, which provides filopodia for pneumococci to adhere to host cell surfaces (Nguyen *et al.*, 2014a). Pneumococcal ClpL has been shown to stabilize the expression of *pbp2x*, to interact with PBP2x, a key protein in the cell wall synthesis process for antibiotic resistance, and to facilitate the translocation of PBP2x (Tran *et al.*, 2011). Thus, HSPs seem to enhance pneumococcal virulence via the upregulation of virulence factors.

S. pyogenes is an important human pathogen and encodes both the *dnaK* and *groESL* operons, which are significantly induced during infection (Lemos *et al.*, 2000). These chaperones may have functions in transcriptional control or RNA stabilization, thus protecting *S. pyogenes* from heat-shock stress during infection (Woodbury and Haldenwang, 2003). In addition, *S. mutans*-encoded GroEL and DnaK act as negative regulators of surface protein antigen C (PAc), which has an important role in the interaction between bacterial cells and acquired pellicles on the tooth surface (Ishibashi *et al.*, 2010). Moreover, *S. agalactiae*-encoded GroEL and DnaK differentially regulate the expression of key streptococcal virulence factors, including those involved in the formation of biofilms and acid tolerance (Lemos *et al.*, 2007). The serine protease ClpP plays a key role in the regulation of bacterial growth and increases bacterial survival under stress conditions, particularly heat-shock conditions in *S. agalactiae* (Nair *et al.*, 2003) and *S. mutans* (Lemos and Burne, 2002). However, some non-HSPs are also important for heat-shock tolerance in *Streptococcus* species. In *S. pyogenes*, a member of the multiple drug resistance (MDR1) family of ATP-binding cassette (ABC) transporters (RscA) is required for growth at 40°C (Dalton *et al.*, 2006).

Responses of streptococci to macrophage-derived stress

Oxidative stress: During streptococcal infection, chemokines recruit host macrophages and neutrophils to the infection

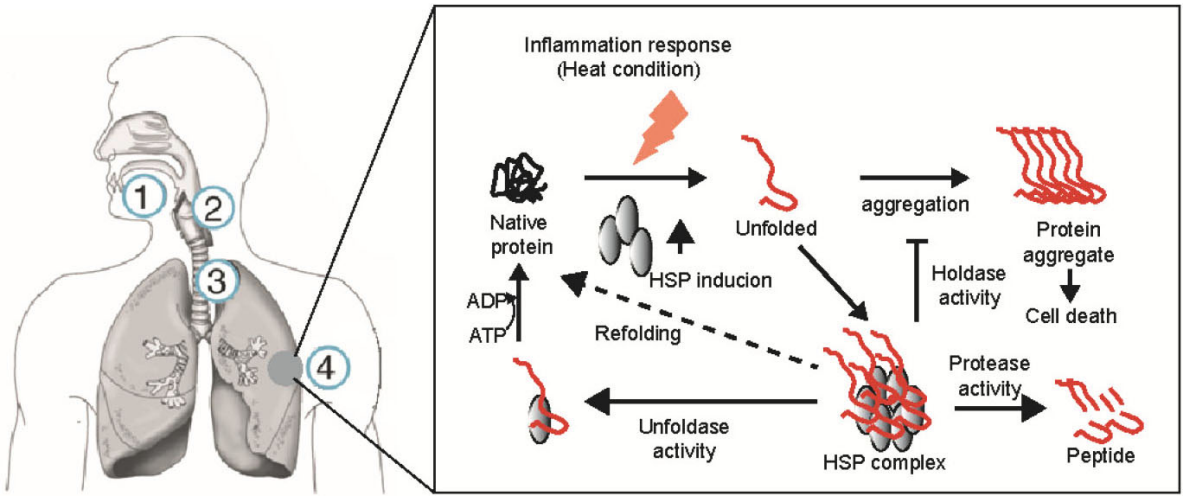

Fig. 3. Role of *S. pneumoniae* heat-shock proteins (HSPs) during infection. In the first stages of pneumococcal infection (1 and 2), the temperature is 30°C–32°C, and this increases to 37°C–37.5°C at the sites of (3) and (4). Thus, *S. pneumoniae* could encounter heat-shock stress within the infected host. Under these conditions, cellular proteins can be denatured, and the accumulation of denatured proteins then activates cell-death signals. To overcome these events, *S. pneumoniae* induces HSPs that act as chaperones or proteases. These chaperones refold denatured proteins back into their native structures. However, if the denatured proteins cannot be repaired, these proteins are cleaved.

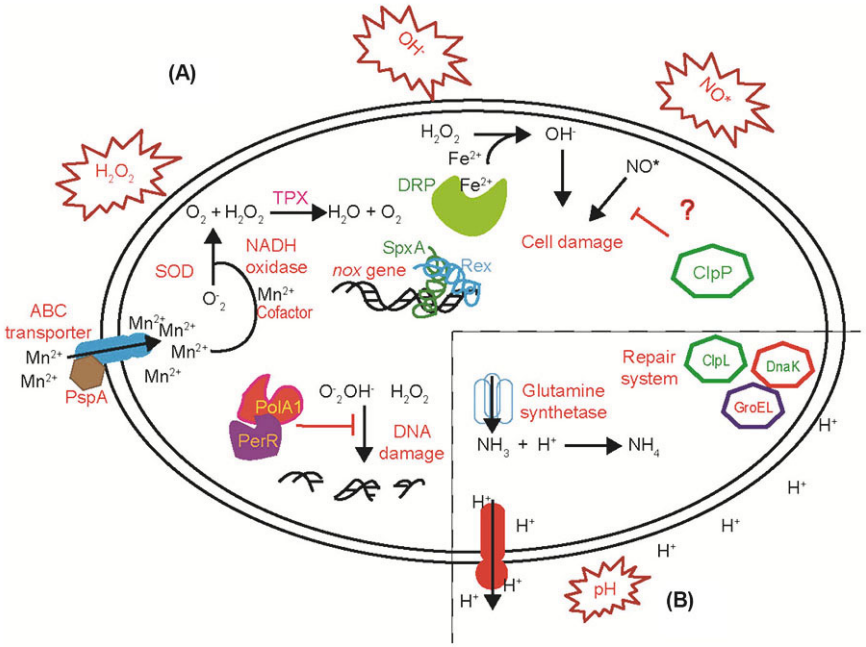

Fig. 4. Responses of *S. pneumoniae* to oxidative and pH stress. In the phagosome, *S. pneumoniae* encounters oxidative (A) and low-pH (B) environments. (A) Thiol peroxidases, nicotinamide adenine dinucleotide (NADH) oxidases, and superoxide dismutase (SOD) enzymes are utilized to neutralize reactive oxygen species (ROS)-induced oxidative stress. The ATP-binding cassette transporter and PspA protein complex transport Mn^{2+}, a cofactor for the NADH oxidase. Rex and SpxA regulate the expression of the *nox* gene, which encodes an NADH oxidase. A putative DNA polymerase I (PolA1) coordinates the peroxide stress response regulator (PerR) to reduce ROS-induced DNA damage via the upregulation of DNA repair systems. One product of the Fenton reaction (H_2O_2 and Fe^{2+}) is a highly reactive hydroxyl radical that causes bacterial toxicity; however, the Drp protein chelates the Fe^{2+} ion to inhibit this reaction. ClpL suppresses NO-induced cell damage via unknown mechanisms. (B) Under low-pH stress conditions, *S. pneumoniae* utilizes the F_1F_0 ATPase to pump protons (H^+) out of the cytosol. Chaperones (DnaK, GroEL, ClpL, and HtrA) act as protein repair systems under acidic conditions. Glutamine synthetase produces H^+ that reacts with NH_3 to form NH_4^+, which is non-toxic to the cell.

site in order to kill the bacteria. Macrophages and neutrophils bind to streptococci via specific surface receptors and engulf the bacteria. In the phagosome, lysosomes produce several reactive oxygen species (e.g. superoxide anions [O_2^-], hydrogen peroxide, hydroxyl radicals) and reactive nitrogen species (e.g. nitric oxide [NO], nitric dioxide). These free radicals generate oxidative stress and play a crucial role in the killing of bacteria (Hassett and Cohen, 1989). In general, free radicals damage bacterial components such as DNA, proteins, lipids, and cell membranes, causing mutagenesis or cell death of the bacteria. To survive in these harsh conditions, bacteria have developed enzymatic and non-enzymatic mechanisms to protect themselves from oxidative stress (Fig. 4).

Hydrogen peroxide (H_2O_2) reacts with bacterial cell walls and cellular components, inducing cell damage. Additionally, H_2O_2 can interact with Fe^{2+} ions and generate highly reactive hydroxyl radicals (·OH) which cause cellular toxicity. In bacteria, catalases play key roles in protecting the bacteria from H_2O_2-induced oxidative stress. Although *S. pneumoniae* does not produce catalase, thiol peroxidase is synthesized and utilized to eliminate cellular H_2O_2 (Hajaj *et al.*, 2012). *S. pneumoniae* contains two kinds of superoxide dismutase (SOD), MnSOD and FeSOD, which play critical roles in the protection against oxidative stress during pneumococcal infection (Yesilkaya *et al.*, 2000). Furthermore, the *S. pneumoniae nox* gene encodes a nicotinamide adenine dinucleotide (NADH) oxidase that converts O_2^- to H_2O (Yu *et al.*, 2001). *nox* mutants have been shown to be susceptible to oxidative stress (Yu *et al.*, 2001). Pyruvate oxidase (SpxB), the enzyme responsible for endogenous H_2O_2 production in *S. pneumoniae*, is necessary for resistance to H_2O_2-induced oxidative stress (Pericone *et al.*, 2003).

In the non-enzymatic mechanism, pneumococci produce several proteins (e.g. ferritin, PsaA, the glutathione transporter [GshT]) that bind to metal ions (e.g. Mn^{2+}, Fe^{2+}), thus protecting cells from oxidative stress (Hua *et al.*, 2014; Bajaj *et al.*, 2015). Several studies have indicated that *psaA* mutants are highly sensitive to H_2O_2-induced oxidative stress (Tseng *et al.*, 2002; Johnston *et al.*, 2004); thus, PsaA seems to regulate the expression of oxidative-stress response enzymes (SOD and NADH oxidase) (Tseng *et al.*, 2002). PsaA also acts as a high-affinity substrate-binding protein, facilitating the acquisition of Mn^{2+}, which plays a crucial role in protecting *S. pneumoniae* from reactive oxygen species (Bajaj *et al.*, 2015). Additionally, GshT imports extracellular glutathione into the cells. Thus, *gshT* mutants are more susceptible to oxidative stress than wild-type bacteria are, indicating that GshT is important for increasing the survival of pneumococci during oxidative stress conditions (Potter *et al.*, 2012). Moreover, *dpr* encodes a non-heme Fe^{2+}-containing ferritin that plays an important role in H_2O_2-induced oxidative stress resistance by chelating free iron (Hua *et al.*, 2014).

ClpP, a member of the hsp100/Clp family, provides resistance to NO- and H_2O_2-induced oxidative stress in macrophages; thus, *S. pneumoniae* can resist phagocytosis via the modulation of ClpP (Ibrahim *et al.*, 2005; Park *et al.*, 2010). In *S. mutans*, *clpP* mutants are sensitive to several stresses, including acid, cold, heat, and oxidative stress (Hou *et al.*, 2014). The transcriptional regulator Rex is a highly conserved protein in *Streptococcus* species and in other gram-positive bacteria (Bitoun and Wen, 2015). During oxidative stress, Rex and SpxA bind to the promoter of the *nox* gene and regulate the expression of NADH oxidase (Bitoun and Wen, 2015). Interestingly, a putative DNA polymerase I (PolA1) coordinates with the peroxide stress RR (PerR) to protect *S. pyogenes* from oxidative stress via DNA damage repair mechanisms (Toukoki and Gryllos, 2013). The *S. mutans* LytST system regulates the expression of the LrgA/B proteins, which have an effect on biofilm formation and oxidative stress tolerance (Ahn *et al.*, 2012). The expression of the *gtfB*, *gtfC*, and *gtfD* genes is responsible for biofilm formation in *S. mutans*,

which provides tolerance to oxidative stress (Senadheera *et al.*, 2005).

pH stress: Macrophages contain acidic lysosomes that are involved in the digestion and clearance of invading streptococci, and the low pH within these lysosomes causes the death of the engulfed bacteria. In low-pH environments, *Streptococcus* species have developed acid resistance mechanisms that are critical for their survival (Fig. 4). Under low-pH stress conditions, streptococci upregulate the expression of the F_1F_0 ATPase, which uses the energy from the hydrolysis of ATP to pump protons (H^+) out of the cytosol (Baker *et al.*, 2015; Cusumano and Caparon, 2015). In *S. pneumoniae*, the ATR system and the F_1F_0 ATPases are necessary for the intracellular survival of the bacteria in macrophages (Cortes *et al.*, 2015). As we mentioned previously, ComD/E and CiaR/H are important regulators of the ATR mechanism (Cortes *et al.*, 2015). Up until now, a set of genes that contribute to the ATR mechanism have been identified in *S. mutans*, including those involved in membrane composition, proton extrusion, and DNA repair (Cortes *et al.*, 2015). The synthesis of metabolic products would be beneficial for *Streptococcus* species in low-pH environments. In *S. mutans*, NH_3 is a product of branched-chain amino acid synthesis pathways involving glutamine synthetase (Len *et al.*, 2004), and reacts with H^+ to form NH_4^+. Thus, the utilization of glutamine synthetase is considered a mechanism to reduce H^+ accumulation in streptococci. Moreover, $NADP^+$ is a secondary product of branched-chain amino acid synthesis pathways that can reduce H^+ concentrations (Len *et al.*, 2004). In addition, the CovS/R system of *S. pyogenes* regulates changes in the cell wall and capsule composition in response to acidic conditions, which may increase the bacterial intracellular survival during host infection (Cumley *et al.*, 2012). *gidA* is known to be regulator in transfer-RNA modification and plays an important role in increasing the survival of *S. mutans* under low pH stress conditions (Li *et al.*, 2014).

Responses of streptococci to other stresses

Antibiotic stress: In the treatment of streptococcal diseases, the use of antibiotics is one of the most efficient therapeutic strategies. However, some *Streptococcus* species possess factors that provide them with antibiotic tolerance. Penicillin-binding proteins (PBPs) are associated with β-lactam antibiotic resistance in *S. pneumoniae* (Jensen *et al.*, 2015), and six high molecular-weight PBPs have been identified in this species (PBP1a, 1b, 2a, 2b, 2x, and 3) (Jensen *et al.*, 2015). Moreover, our studies have shown that ClpL binds to PBP2x, which subsequently enhances the antibiotic tolerance in *S. pneumoniae* by increasing cell wall thickness (Tran *et al.*, 2011). We also found that VncS/R, a member of the pneumococcal TCS, plays an important role in the regulation of pneumococcal virulence and vancomycin tolerance (unpublished data). Furthermore, ClpP has been shown to protect *S. mutans* from cell-wall-damaging antibiotics (Chattoraj *et al.*, 2010).

Osmotic stress: When streptococci invade other organs during infection, the bacteria experience changes in the osmolality of their environment. Therefore, osmoadaptation would be an important phenotype for the successful pathogenesis of streptococci. To adapt to osmotic stress, *Streptococcus* species must express several genes that maintain homeostatic concentrations of carbohydrates, salts, and organic solutes. PrtM is a protease maturation lipoprotein that is involved in K^+ utilization; thus, PrtM is essential for the osmoadaptation of *S. mutans* (Kunii *et al.*, 2014). System analyses have identified that *opcA*, *opuA*, and *piaABCD* encode ABC transporters, which are required for osmotic stress responses in *Streptococcus* species (Brown *et al.*, 2004; Abranches *et al.*, 2006).

Starvation stress: Nutrients are essential for streptococcal survival during infection; however, streptococci may encounter nutrient depletion in the host environment. To survive in these conditions, *S. pyogenes* has to enter a quiescent state that is similar to the stationary phase of *in vitro* cultures (Trainor *et al.*, 1999). Although the mechanisms by which the quiescent state helps *S. pyogenes* to survive in nutrient starvation are unknown, it is possible that *S. pyogenes* reduces any unnecessary metabolism to save the energy necessary for survival. Under iron starvation conditions, *S. pyogenes* CovS/R mediates the expression of ABC transporter operons that allow the bacteria to remain viable during infection (Froehlich *et al.*, 2009). Furthermore, *S. pyogenes* upregulates the expression of oligopeptide (*opp*) and dipeptide (*dpp*) permeases, an intracellular oligopeptidase (*pepB*), and virulence factors (*covS/R*) in response to amino acid starvation (Steiner and Malke, 2001).

Competition for streptococci in niches: The human oral cavity contains more than 700 bacterial species; thus, *Streptococcus* species must compete for nutrients and niches for their survival. Interestingly, *S. oligofermentans* inhibits the growth of *S. mutans* by producing H_2O_2, which is produced by pyruvate oxidases, lactate oxidases, and l-amino acid oxidases (Bao *et al.*, 2015). Moreover, streptococci produce H_2O_2 to kill competitive pathogens, such as *Haemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Uehara *et al.*, 2001; Regev-Yochay *et al.*, 2006; Whiley *et al.*, 2015). Genomic studies have shown that competition also occurs between *Streptococcus* species. For example, bacteriocins (produced by *S. mutans*) and H_2O_2 (produced by *S. sanguinis*) are used to inhibit each other (Kreth *et al.*, 2005). Moreover, more than 90 serotypes of *S. pneumoniae* co-colonize the same niches; thus, they must also compete with each other to survive and cause further infection (Flasche *et al.*, 2013).

Stress responses of the host to streptococcal infection

Streptococcal infection triggers a variety of stress responses in the host to defend against this infection. Endoplasmic reticulum (ER) stress is involved in this host response. During infection, the H_2O_2 produced by *S. pneumoniae* induces ER and oxidative stress and activates mitogen-associated protein kinase (MAPK) signaling pathways that regulate inflammatory responses to the infection (Loose *et al.*, 2015). During adherence to the host cells, *S. pyogenes*-produced streptolysin toxins also trigger ER stress (Baruch *et al.*, 2014). Moreover, pneumolysin, a major *S. pneumoniae* virulence factor, stimulates activating transcription factor (ATF)-3, which positively regulates cytokine production and protects against *S. pneumoniae* infection (Nguyen *et al.*, 2014b). ClpL, a major

pneumococcal HSP, has been shown to repress the activation of cofilin-2, which regulates cellular cytoskeleton and actin rearrangements in response to infection (Nguyen *et al.*, 2014a).

Since the host stress responses to streptococcal infection remained unclear, we sought to elucidate the underlying mechanisms of these responses by employing genomics, proteomics, interactomics, and system biology approaches. The Finlay group (from The University of British Columbia) previously identified several host stress-related genes using the human transcriptome during non-typhoid *Salmonella* and human immunodeficiency virus coinfection (Schreiber *et al.*, 2011). Moreover, metabolomic analyses have shown that host membrane lipids are required for the survival of *Salmonella* species during infection (Antunes *et al.*, 2011).

Conclusions

During infection, streptococci encounter several types of stress conditions within the host, such as thermal, oxidative, and acidic stresses. Over time, *Streptococcus* species have developed mechanisms that allow them to survive in the harsh host milieu. In the early stages of infection, TCSs are used to sense stress signals and turn on stress-related genes. Members of the HSP family play critical roles in coping with heat shock. However, the underlying mechanism acting against oxidative stress appears to be more complicated than that against heat shock because it requires many enzymes and protein or non-protein factors that work in synergy to protect the bacteria from this stress.

Although we understand how streptococci respond to stress during infection, most of our understanding of streptococcal stress response has been gained from *in vitro* studies. Since it is unclear whether *in vitro* results are representative of those *in vivo*, further *in vivo* studies should be performed to provide new insights into the responses of streptococci to the host environment.

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