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Review

Life in a diverse oral community – Strategies for oxidative stress survival

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ABSTRACT

Background: While the oral cavity harbors more than 680 bacterial species, the interaction and association of selected bacterial species play a role in periodontal diseases. Bacterial species including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, a consortium previously designated as the '*red complex*' is now being expanded to include other new emerging pathogens that are significantly associated with periodontal disease. *Highlight*: In addition to novel mechanisms for oxidative resistance of individual species, community dynamics may lead to an overall strategy for survival in the inflammatory environment of the periodontal pocket. Complex systems controlled by response regulators protect against oxidative and nitrosative stress. *Conclusion*: The combination of these multifaceted strategies would provide a comprehensive defense and support system against the repetitive host immune response to promote microbial persistence and disease.

Contents

1. Background

Periodontal diseases are polymicrobial inflammatory-associated infectious diseases that can lead to the destruction of periodontal ligaments and adjacent supportive alveolar bone. The oral cavity harbors more than 680 bacterial species [1–3], and some of these microorganisms have been shown to be responsible for the initiation and progression of periodontal diseases [4,5]. Multiple methods including DNA–DNA hybridization, microarrays and next generation sequencing have shown that certain bacterial complexes associate with each other and that some were more likely to potentiate disease [6]. Bacterial species including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, a consortium previously designated as the *red complex*, has been shown to have the highest association with the severity of periodontal disease [7]. Additionally, *Fusobacterium nucleatum*, *Prevotella* species, *Eikenella corrodens*, *Parvimonas micra*

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(formerly *Peptostreptococcus micros*), and *Campylobacter rectus* have an increased abundance in deep periodontal pockets and are also implicated as periodontopathogens [4,5,7,8]. Recent microbiome studies of healthy and periodontal disease patients in conjunction with microbial pathogenesis evaluation, have demonstrated that emerging new pathogens such as *Filofacter alocis* may play an increasingly significant role in periodontal disease [9–12]. In this review, '*red complex*' terminology is redefined as a representation of the original characterized periodontal pathogens and the new emerging bacteria. These bacteria are not usually found alone, but in combination, in the periodontal pocket, suggesting that some bacteria may cause destruction of the periodontal tissue in a cooperative manner [13,14]. Furthermore, coaggregation, nutrient effects, and modulation of virulence factors by periodontopathogens or by interspecies interactions between periodontopathogenic and nonpathogenic organisms have been reported to contribute to oral microbial pathogenesis (Fig. 1) [15].

The primary factor that affects survival of organisms in the oral cavity is oxidative stress. Oxidative stress can be defined as an excess of pro-oxidants, such as reactive oxygen species (ROS), in the cell. Reactive oxygen species, such as the superoxide radical ($O_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2) and oxidant nitric oxide (NO), pose a significant threat to cellular integrity [16]. The damage produced by intracellular ROS induces mutagenesis resulting in the generation of a wide spectrum of oxidative DNA lesions [17] many of which are toxic and/or mutagenic [18]. Thus, mutation prevention or avoidance is of utmost priority.

Additionally, exposure of periodontal pathogens to air can give rise to the metabolic conversion of atmospheric oxygen to ROS inside bacterial cells [17] and ROS are also produced by macrophages and neutrophils during the immune inflammatory response mediated by a process called the oxidative burst [19]. Thus, it is crucial that these organisms utilize an arsenal of mechanisms to either prevent or fix oxidative damage resulting from ROS in order to survive in a hostile environment.

This paper focuses on the pattern of microbial synergy exhibited by members of the expanded *red complex* and other bacterial species, which enables them to survive cooperatively and individually in the oxidatively stressed environment of the periodontal pocket.

2. Sensory response

Numerous studies have shown that the formation of biofilms is controlled by cell-to-cell signaling mechanisms and that gene regulation during biofilm growth is due to the accumulation of signal molecules [20]. These signal molecules encapsulate what is known as the quorum sensing (QS) mechanism, which is defined as cell-density dependent bacterial intercellular communication [20,21]. In general, bacteria behave as single cellular organisms at low cell densities; but may shift their behavior to a 'multicellular' type as their population density reaches a threshold level during the formation of a biofilm [22]. As the cells sense the change in population density, they are able to communicate through small signaling molecules. This results in bacteria within the biofilm being able to express genes for different phenotypes, in particular, those that function in virulence [20,22]. QS also influences gene expression which can affect outcomes in invasion, defense, spread, and resistance to stress conditions in bacterial pathogens [23].

QS may be used in bacteria for intraspecies or interspecies communication, a feat that is achieved through two types of QS systems, each mediated by distinct classes of autoinducers; N-acylated-l-homoserine lactones (AHLs) and autoinducer AI-2, respectively [24]. AI-2 is thought to be a non-species-specific autoinducer that mediates intra- and interspecies communication among Gram-negative and Gram-positive bacteria [25]. The AI-2 and its synthase LuxS have been shown to correlate with pathogenicity in a variety of organisms [26,27]. For our purposes, the AI-2 system is of particular importance, since it is proposed to be a universal language for interspecies communication, and may provide insights into how periodontal pathogens are able to combat oxidative stress within the periodontal pocket.

The enzyme LuxS is responsible for AI-2 biosynthesis. It is the product of the gene *luxS*, and is widely conserved throughout the bacterial kingdom [24]. LuxS synthesizes 4,5-dihydroxy-2,3-pentanedione (DPD), which undergoes spontaneous rearrangements to form a variety of DPD derivatives, known as the AI-2 pool [28]. It is important to note that the chemical nature of the active signaling molecule from aforementioned AI-2 pool varies between species, as does the nature of the AI-2 receptor for these signals [24].

It was previously shown that *P. gingivalis* possesses a gene that encodes a peptide that has 29% identity with LuxS of *Vibrio harveyi*. An insertional *luxS* mutation failed to induce luciferase activity in *V. harveyi* while wild type *P. gingivalis* ATCC 33277 induced luciferase expression [21]. Based on these findings, it has been proposed that *P. gingivalis* uses a LuxS protein in its AI-2 signaling system [21,29]. In *P. gingivalis*, QS has been implicated in the control of genes responsible for the acquisition of hemin [21] as well as promoting survival during host-induced stresses such as temperature, H_2O_2, and pH [30]. QS in the other *red complex* bacteria, including *T. forsythia* and *T. denticola*, has not been extensively studied, however previous

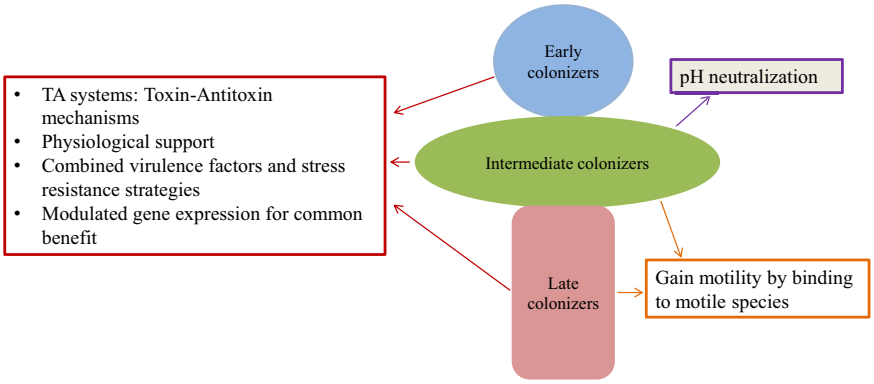

Fig. 1. General strategies displayed by interacting oral biofilm microbial species to promote survival. Diagram shows survival strategies emerging from the interaction and cohabitation of oral biofilm species. The purple arrow indicates that species from the intermediate colonizer group uses acidic pH neutralization to promote the establishment of late colonizers. The orange arrows show that species from the intermediate and late colonizer groups employ attachment to late colonizer motile species to acquire motility for deeper colonization. The red arrows demonstrate that species from early, intermediate and late colonizers use the strategies of common physiological support, toxin–antitoxin systems and modulation of gene expression to promote community-living, while combining their stress and virulence resistance mechanisms to survive host repetitive immune attacks.